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SO2-Alcohol-Water Fractionation of Sugarcane Straw

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Climate change resulting from fossil fuels combustion is motivating researchers to explore feasible routes to convert renewable biomass into biofuels and biochemicals for a sustainable society. Typically, biofuel is produced from corn or sugarcane but both feedstocks compete with human food supply. Thus, lignocellulosics as renewable feedstock represent a more ethical and ecofriendly approach. Sugarcane straw (SCS) is a cheap and abundantly available feedstock which potentially can be used for biofuels/biochemicals production. It can also help to mitigate environmental and health problems resulting from conventional practice of SCS burning in the fields.

There are different biomass conversion technologies for production of biofuels/biochemicals. The biochemical route offers advantages of high selectivity and conversion and can also produce widely different products. Prior to fermentation, it requires a fractionation process that can produce monomeric hemicellulose sugars and a cellulosic solid residue that is easily accessible to enzymes. The SO_2-ethanol-water (SEW) or AVAP® process meets these requirements. However, the viability of this process is highly dependent on efficient solvent recovery and full utilization of sugars. The SEW process produces a spent liquor stream that has only about 50% sugars as monomers. Only ethanol has been used in this process as solvent so far, and no data is available regarding alcoholysis reactions that consume solvent and potentially decrease the monomeric sugar yield.

In the present thesis, the SEW process is evaluated for SCS fractionation and associated potential losses of carbohydrates and ethanol as alkyl pyranosides and by lignin alkylation. This study also investigates the effect of two other alcohols, methanol and isopropanol besides ethanol on the fractionation potential of SCS. It is the first time that a secondary alcohol is being used in the SO_2-Alcohol-Water (SAW) process. The fractionation efficiency is poor using

methanol but it generates methyl xylosides at a high yield. The fractionation potential of isopropanol is comparable to ethanol, however less health and safety regulations and its low process pressure make isopropanol more attractive. The kinetics of alkyl pyranoside hydrolysis in SAW liquor after solvent evaporation are measured to establish the conditions for full sugar and solvent recovery.

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$$[Lignin] = 0.134 \times \text{kappa} \times Pulp\ Yield \quad (1)$$

$$-\frac{d[Lig]}{dt} = k'_{Lig}\,(T)\,[Lig]^a\,[H_3O^+]^b = k_{Lig}\,(T)\,[Lig] \quad (2)$$

$$\delta = \sqrt{\left[\frac{(\Delta H - RT)\rho}{MW}\right]} \quad (3)$$

$$Xylan = Xylose\left(\frac{132}{150}\right) + UA\,(0.6)\left(\frac{132}{176}\right) \quad (4)$$

$$-\frac{d[Xyl]}{dt} = k'_{Xyl}\,(T)\,[Xyl]^a\,[H_3O^+]^b = k_{Xyl}\,(T)\,[Xyl] \quad (5)$$

$$DP_v = \left(\frac{1.65[\eta] - 116[Hemi]_{pulp}}{[Cel]_{pulp}}\right)^{1.111} \quad (6)$$

$$\frac{1}{DP} - \frac{1}{DP_0} = k'_{Cel}\,(T)\;[H_3\,O^+]t = k_{Cel}\,(T)\,t \quad (7)$$

$$\text{CSF} = \log\left\{\text{t (min)} * e^{\frac{\text{T (°C) -100}}{14.7}}\right\} - \text{pH} \quad (8)$$

$$\text{Anhydro-pentose} = \text{Pentose x } (132/150) \quad (9)$$

$$\text{Anhydro-hexose} = \text{Hexose x } (162/180) \quad (10)$$

$$H_2O_2 + SO_2 \rightarrow H_2SO_4 \quad (11)$$

$$C_7H_{14}O_5 + H_2O \leftrightarrow C_2H_5OH + C_5H_{10}O_5 \quad (12)$$

$$C_8H_{16}O_5 + H_2O \leftrightarrow C_3H_7OH + C_5H_{10}O_5 \quad (13)$$

Chapter 1

INTRODUCTION

1.1 Motivation

The interest in the synthesis of bio-based products and biofuels from lignocellulosic biomass is increasing continuously. This interest stems from the fact that fossil fuels are non-renewable and their combustion releases greenhouse gases which negatively impact the air quality. On the other hand, the lignocellulosic materials are renewable, abundant and minimize the impact on climate change caused by greenhouse gases. Lignocellulosic biomass includes wood, agriculture residues, water plants, grasses and other plant substances (Rowell, et al., 2000). Bioethanol is the world's most produced biofuel so far from lignocellulosic biomass (Chovau, et al., 2013). Typically, it is produced from corn or sugarcane (Goldemberg, 2007) but both renewable feedstocks compete with human food supply because they require prime agricultural land. On the other hand, trees can be grown on marginal land, thus eliminating the conflict with food production when considering woody biomass as a feedstock for biofuels. However, presently the trees are mostly used for lumber and other construction materials as well as for pulp and paper production (FAO, 2014). Pulp and paper demand varies depending upon the degree of development of the region. However, during the last decade pulp demand in developed countries is stagnant or decreasing, while demand is still increasing in developing nations. The latter results in a shortage of wood and gradual deforestation in developing countries. For this reason, non-woody materials are being considered as feedstock for pulping and paper making products in those regions (Moryia, et al., 2007).

Agricultural residues are one of the non-woody biomass that is abundantly available in many countries. The high growth rate and adaptability to various soil types makes agriculture fibers a good alternative to wood as raw material for pulping/fractionation (Rocero, et al., 2003). Sugarcane straw (SCS) is one of the abundantly available agriculture residues. The biomass that is removed before sugarcane is crushed is known as SCS (Moryia, et al., 2007; Saad, et al., 2008). It consists of fresh and dried leaves including the tip of the plant (Saad, et al., 2008; Neto, 2005). Food and agriculture organization (FAO) statistics shows that the annual production of sugarcane worldwide in 2014 is nearly 1900 MMT (Million Metric Ton) (FAO, 2014). Since 140-150 kg of SCS is produced from one ton of cultivated sugarcane (Saad, et al., 2008), nearly 285 MMT SCS is being produced annually around the world. Brazil is the foremost sugarcane producing country with 84 MMT of SCS annual production (Canilha, et al., 2012).

Traditionally it is burnt in the field, thereby destroying a potential lignocellulosic feedstock as well as causing environmental pollution. Nowadays, in Brazil, burning of SCS is strictly controlled by a specific federal law (Decree 2661/98) and in the state of Sao Paulo by an even more restrictive law (State law 11.241/02) (Chagas, 2014). Therefore, it is logical to consider SCS for production of renewable products such as pulps, biofuels and renewable chemicals.

Although SCS is a cheap raw material to produce bio-based products, successful commercialization is highly dependent on the choice of biomass conversion technology. The fundamental difference between conversion technologies is the primary catalysis system (Foust, et al., 2008). Thermochemical conversion processes rely on heat and physical catalyst to produce an intermediate gas or liquid stream followed by conversion to biofuel. In contrast, biochemical processes use biocatalysts such as enzyme or other microbes to convert a mixed sugar stream, produced from biomass fractionation, into biofuels (Foust, et al., 2009). High selectivity and conversion efficiencies are clear advantages of biochemical processes (Himmel, et al., 2007), while thermochemical processes can handle different types as well as all components of biomass feedstocks (Hallen, et al., 1998). Foust and his team (Foust, et al., 2009) show that both conversion technologies are very similar within marginal error in terms of overall cost, efficiency and environmental impact. However, biochemical processes seem to be well suited for herbaceous biomass and pose serious challenges for thermochemical processes due to high inorganic (ash) content. Separation of lignocellulosic biomass, including SCS, into its basic components (cellulose, hemicellulose and lignin) is the first step in biochemical conversion processes. This step is frequently referred to as fractionation. However, alternative terms such as pretreatment and pulping, are used to describe the same phenomenon in this thesis.

Fractionation/pulping of lignocellulosic biomass, including SCS, is achieved employing different chemical and mechanical processes. Kraft (sulfate) and AS are the two well-known chemical pulping processes. However, organosolv pretreatments are considered environmentally friendly because they use relatively benign solvents and does not produce odorous pollutants as in the kraft pulping process. Typically, such fractionation/pretreatment processes use highly volatile organic compounds (methanol, ethanol, acetone, ethyl acetate, etc.) in view of their simple recovery by distillation (Rodríguez & Jiménez, 2008). The most commonly used solvents in organosolv processes are low molecular weight aliphatic alcohols. Methanol and ethanol are of particular interest for commercial processes because of their low cost (Oliet, et al., 2002). Alcell (ethanol+ water), Organocell (methanol/ethanol+alkali)

and ASAM (alkaline+sulfite+anthraquinone+methanol) are three well known organosolv processes tested at commercial or demonstration scale that use either methanol or ethanol (Pye & Lora, 1991; Young, 1992; Black, 1991). Methanol has advantages of very low cost and simple recovery because of its low boiling point and formation of some methanol (1020-1330 mg L^{-1}) from the biomass during the fractionation process (Zhu, et al., 1999). However, its higher toxicity and flammability make it less desirable. Ethanol in comparison is more expensive but less toxic and volatile. A disadvantage of both methanol and ethanol is the high process pressure during fractionation leading to high capital cost (Aziz & Sarkanen, 1989). The use of isopropanol is not very common in pulping but a few previous studies show that addition of isopropanol to cooking sulfite liquor improves the pulping process. For example, the addition of 40-50% isopropanol to magnesium bisulfite liquor improves pulp strength, increases pulp yield and increases the delignification rate (Sakai, et al., 1983; Sakai & Uprichard, 1987). The explanation given is that addition of isopropanol reduces the dissolution of hemicelluloses due to reduction in acidity, while it increases the solubility of high molecular weight lignin fragments. The advantage of isopropanol is its lower volatility and fewer regulations compared to ethanol.

High solvent loss is another major obstacle for full scale implementation of organosolv processes. These losses are related to chemical reaction of alcohol with dissolved carbohydrates (Hu, et al., 2012; Drouet, et al., 1994; Grisel, et al., 2014; Hu, et al., 2014), lignin (Bauer, et al., 2012; Lancefield, et al., 2017) and formed acids (Bublitz & Wilson, 1983) in addition to mechanical or vapors losses associated with washing of the produced pulp fibers. It is also worth mentioning that organosolv processes are not effective for fractionating softwood feedstocks (Paszner & Cho, 1989; Foust, et al., 2009).

A variant of organosolv pulping, the so called SO_2–Alcohol-Water (SAW) process was recently investigated in much detail at Aalto University in Finland (Iakovlev, 2011a; Yamamoto, 2014a; Sklavounos, 2014; You, et al., 2016). The process was referred as SO_2-Ethanol-Water (SEW) because ethanol was used in fractionation liquor. It can efficiently fractionate all type of lignocellulosic biomass including softwood feedstocks. Successful fractionation of spruce, beech and wheat straw was achieved with SEW (Iakovlev, et al., 2011b). Yamamoto (2014a) evaluated the enzymatic hydrolysis potential of SEW pulps produced from hardwood and softwood forest biomass. In two parallel studies Sklavounos (2014) and Jurgens et al. (2012) determined the recovery pathway and ABE fermentation potential respectively of SEW spent liquors. Most recently the SEW process was applied to SCS using a relatively low

ethanol/water mass ratio of 22.5/65.5 (You, et al., 2016). However, no clear delignification kinetics were presented because of high data scatter. Overall this process has distinct advantages of a short cooking time, lower fractionation temperature than kraft pulping, less sulfur consumption than acid sulfite (AS) cooking, simple recovery of the solvent and SO_2 by evaporation, and reduced formation of sticky lignin (Primakov, et al., 1979; Iakovlev, 2011a). However, toxicity and flammability of ethanol, high fractionation pressure and release of SO_2 to the atmosphere are some important safety concerns. Solvent losses during mechanical handling of fractionated biomass and chemical reactions of the solvent with lignin and carbohydrates are still crucial hurdles for implementation of this technology.

All above mentioned SAW studies focus mostly on using wood materials and ethanol with SO_2 (Iakovlev, 2011a; Sklavounos, 2014; Yamamoto, 2014a). Efficient fractionation of spruce (Iakovlev, 2011a), production of enzymatically digestible pulp from SW and HW biomass (Yamamoto, 2014a), spent liquor conditioning for ABE fermentation (Sklavounos, 2014) and effect of ethanol concentration on SCS fractionation (You, et al., 2016) were main issues addressed in these studies. Only ethanol was used in all these studies and the process (SEW) was not evaluated for alcoholysis reactions and solvent recovery. Therefore, the present study is undertaken to evaluate the process for different solvents and their interactions in SCS fractionation. Two other fully water miscible alcohols, methanol and isopropanol in comparison to ethanol are employed to investigate the fractionation potential of abundantly available agriculture residue SCS using the SAW process. SO_2–Methanol-Water, SO_2-Ethanol-Water and SO_2–isopropanol-Water fractionation systems are designated as SMW, SEW and SPW in this study. Methanol and ethanol are used because of above mentioned advantages while isopropanol is considered because of its low health and safety regulations. Low fractionation pressure is another positive aspect of isopropanol. Another interesting aspect of isopropanol is that it will show how SAW fractionation systems behave when a secondary alcohol rather than primary alcohols (methanol and ethanol) are used. Complete water miscibility and high solubility of SO_2 are two other important parameters which were considered in the selection of alcohols in the present study.

Chapter 2

LITERATURE REVIEW

2.1 Woody biomass and global demands

Global population level, food demand, consumption of lignocellulosic biomass and fossil fuels are very closely interlinked. The rapidly increasing world population leads to increasing demand of food and textile fibers (Pimentel, 2007; Nellemann, et al., 2009). On the other hand, rising crude oil prices and environmental concerns calls for the replacement of fossil fuels by biofuels (Agbogbo & Coward-Kelly, 2008; IEA, 2012). Many authors have reported on the production of ethanol, butanol and isopropanol from lignocellulosic biomass (Survase, et al., 2013; Nigam, 2001; Agbogbo & Coward-Kelly, 2008; Canilha, et al., 2010; Jurgens, et al., 2012; Moryia, et al., 2007; Silva, et al., 2010; Survase, et al., 2011; Biswas, et al., 2013). Forests have been cleared for cultivation of crops to meet the increasing food requirements. But at the same time the food supply chain needs large scale paper and board production from lignocellulosics. Recent FAO data (FAO, 2014) show that wrapping and packaging accounted for about half of the total paper and pulp production in 2013.

Lignocellulosics consist of different biomass species including wood, agriculture residues, water plants, grasses and other plant substances (Rowell, et al., 2000). Conventionally wood has been used to produce a wide variety of biomass products as well as functions as an energy source (FAO, 2014). The enormous increase in the use of fossil fuels over the last 100 years has not only changed the world economics but also affected air quality. At the same time, many forests have been cleared out for agriculture land and timber production (WWF, 2017). The Forest Resource Assessment (FRA) of the FAO (FAO, 2015) has documented a significant decrease in forest area from 1990-2015 as shown in Table 2.1. There has been a large loss in forest area in Africa and South America in the last two decades (1990-2000, 2001-2010), although the net forest area loss in the second decade is slowing down in these regions. Deforestation, including uncontrolled conversion of forests to agriculture land, seems to be major factor for this loss. The loss in Oceania, Asia, North and Central America regions is comparatively much smaller especially in North America, while the forest area changed from a loss to a large increase in Asia in the second decade due to new forest plantations that make 7% of forest area (or 264 million ha) (FAO, 2010).

Table 2.1: Trends in global forest area

		Annual net change		
Year	**Forest (million ha)**	**Period**	**Area (million ha)**	**% Change**
1990	4128			
2000	4056	1990-2000	-7.3	-0.18
2005	4033	2000-2005	-4.6	-0.11
2010	4016	2005-2010	-3.4	-0.08
2015	3999	2005-2015	-3.3	-0.08

2.2 Agriculture residues and their applications

The large portion of forest landmass has already turned into cultivated agricultural land and the availability of arable land is limited and even decreasing because of soil degradation (desertification, salinization) (Pimentel, 2007; Nellemann, et al., 2009). To meet the global food, fibers and fuel demands in a sustainable manner, agriculture residues can be a good option because of following reasons;

- Agriculture residues are abundantly available
- The use of agriculture residues for fuel and fibers doesn't affect food supply as for example it does when corn is used for ethanol production
- It helps with environmental protection because conventionally agriculture residues are burnt in open fields
- It helps to preserve forests

Agriculture residues include a wide variety of biomass including sugarcane bagasse (SCB), sugarcane straw (SCS), wheat straw (WS) and rice straw (RS). These materials are abundantly available throughout the world and their availability depends on the production of the respective crop and production region. FAO major crops production data (2010-2014) show that sugarcane, maize, wheat and rice are the most abundantly cultivated crops throughout the world (Figure 2.1). The world production of sugarcane has been over 1800 MMT/year since 2011. Global production of wheat and rice at about 700 MMT/y each, and maize has now surpassed 1000 MMT/y. Regarding the availability of biomass, a recent study of US Department of energy depicts that the available biomass in 2030 for industrial bioprocessing in the US would be between 1.1 and 1.6 billion tons (U.S. Department of Energy, 2011). Of this total, agriculture residues and wastes represent 404 million dry tons. Therefore, above mentioned agriculture crops can provide an enormous amount of residual biomass that can be used to produce chemicals, pulps and biofuels without affecting the food supply chain.

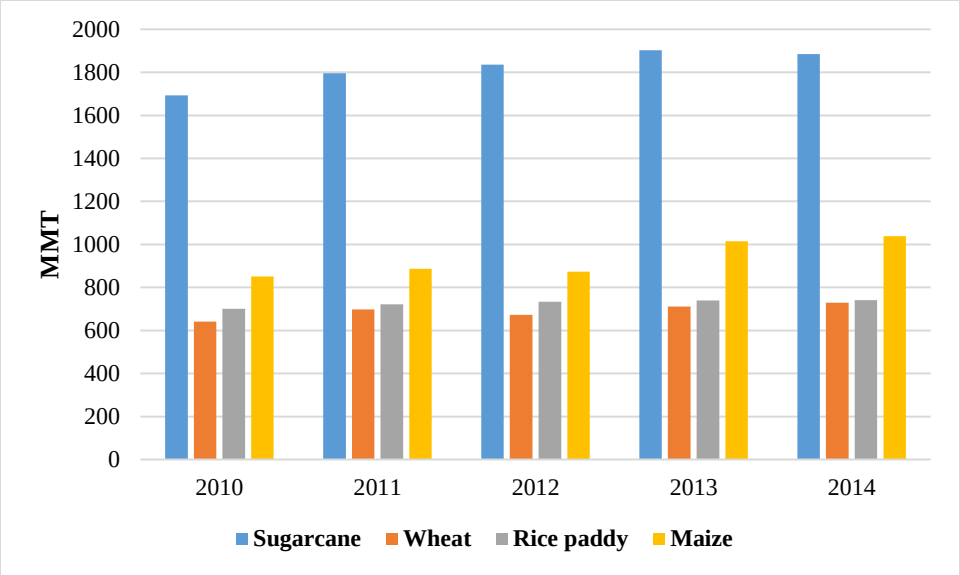

Figure 2.1: World production of major crops (FAO, 2014)

Normally most of the agriculture residues are burnt in the open field to clear the arable land. This practice is harmful for the environment, produces greenhouse gases as well as creates severe respiratory problems. Some of the conventional uses of these residues include animal feed, feedstock for the paper industry and use as organic fertilizer (Vlasenko, et al., 1997; Goncalves, et al., 2005; Ereno, 2008). The use of agricultural residues for pulp and alcohol production provides different substrates and helps to mitigate the above described pollution problems (Sun, et al., 2003). Pulp obtained from the processing of SCB is used in the manufacturing of diverse products including facial and toilet tissue, a variety of writing and printing papers, bag and wrapping papers, corrugating medium, and linerboard. These represent only a small fraction of the total SCB produced (Rowell & Keany, 1991). Pulps from SCS and RS may be used for similar products. Motivated by the availability (Figure 2.1) and high cellulose content of SCS, some researchers have focused on developing textile fibers from this residue (Costa, et al., 2013). This technology is under development and may give an innovative and non-polluting application of this feedstock. Other products for which SCS has been evaluated include lyocell fiber and ethanol (Costa, et al., 2015), enzymes (Singh & Suman, 2008), bio-oil and bio-electricity (Azad, et al., 2014), and reinforcement of polypropylene and polyurethane (Luz, et al., 2010; Miléo, et al., 2011). The use of rice straw for value added products such as ethanol and other chemicals has been widely studied. In search for feasible alternatives, California rice cultivators have considered straw as a source for liquid fuels and energy. Rice straw contains 60% carbohydrates by weight, and therefore has been considered a

significant feedstock for fermentation products (Vlasenko, et al., 1997; Sun & Cheng, 2002). In Thailand, SCB and RS are abundant agriculture residues. Biofuels and bio-based materials can be produced from cellulose and hemicelluloses obtained from these agro-residues by so called biorefinery processes (Sakdaronnarong & Jonglertjunya, 2012).

2.3 Sugarcane straw

SCS is the material that is removed from the stalk before the sugarcane is crushed (Moryia, et al., 2007; Saad, et al., 2008). It consists of the fresh and dried leaves including the tip of the plant (Neto, 2005; Saad, et al., 2008). 140-150 kg of SCS is produced from one ton of cultivated sugarcane (Saad, et al., 2008) . In a view of the total sugarcane production (Figure 2.1), especially in Brazil, enormous amounts of SCS are potentially available for pulping and biofuels production.

The composition of feedstocks is important for process development and understanding the effect of process parameters. Like all lignocellulosic biomass, SCS is composed of the three main macromolecular components: cellulose, hemicelluloses and lignin (Lu, et al., 2009). The composition of SCS depends on the material collection site, climate conditions, stage of plant development and variety (Gómez, et al., 2010; Santos, et al., 2012). Costa et al. (2015) has compared the chemical composition of SCS, reported in many earlier publications, and concludes that typically it contains 32.4-44.4% cellulose, 24.2-30.8% hemicelluloses, 12.0-36.1% lignin, 2.4-7.8% ash and 2.5-10.6% extractives. Characterization of different parts of sugarcane (stalk, straw and residual bagasse) provides important details of its structure. Several studies (Triana, et al., 1990; Neto, 2005; Canilha, et al., 2012) show large variations in the moisture content of the sugarcane material, varying from 13.5% (in dry leaves) up to 82.3% (in the tops); but the values of ash, fixed carbon, and volatile matter vary little among green leaves, dry leaves and straw, with a lower amount of ash for bagasse. Also, all components have practically the same elemental composition of carbon (~45%), hydrogen (~6%), nitrogen (0.5–1%), oxygen (~43%), and sulfur (~0.1%); mineral composition for alkalis and phosphorus shows some variation among the three components of the SCS, indicating that its content increases from the dry leaves to the tops, and is significantly higher than bagasse. Azad et al. (2014) performed proximate, ultimate and mineral analysis of three individual parts of sugarcane and found similar results.

2.4 Structural characteristics

Structural characteristics of agriculture residues, including SCS, play a crucial role in the fractionation process and end use of products obtained. Therefore, it is essential to review the structural characterization of the hemicelluloses, cellulose and lignin, the three major components of lignocellulosics

2.4.1 Cellulose

Many industrial products (paper, fibers, films, additives etc.) are made from cellulose. It is isolated from wood through a well-known process called pulping. The characteristics of pulp obtained depend on the chemicals used for pulping, operating environment (acidic, neutral, or alkaline) and operating conditions like pressure and temperature (Fengel & Wegener, 1984). Cellulose is the most abundant component in SCS (Costa, et al., 2015). The anhydroglucopyranose units join to form a molecular chain of cellulose. Therefore, cellulose can be considered as a linear glucan polymer. The anhydroglucopyranose units are linked together by β-(1→4) glycosidic linkages and thus cellulose chains have a reducing and non-reducing end. The behavior of these end groups during pulping is determined by their chemical properties. The –OH group on C_1 position is an aldehyde hydrate group deriving from ring formation by an intramolecular hemiacetal linkage. This group is identified by its reducing properties, while the –OH group on the C_4 position is an alcoholic hydroxyl and shows non-reducing behavior (Fengel & Wegener, 1984).

In cellulose, molecules form long linear chains (10,000-15,000 DP) and have a strong tendency to form intra and intermolecular hydrogen bonds. These bonds result in the formation of microfibrils that are building blocks of the cell wall. Microfibrils form both highly crystalline regions, as well as amorphous regions. The high crystallinity of cellulose imparts high tensile strength to cellulose and makes it insoluble in water and many other solvents. This high crystalline nature is also responsible for its highly recalcitrant nature towards many chemicals during pulping (Sjöström, 1993; Alén, 2000).

2.4.2 Hemicellulose

Hemicelluloses are also called polyoses, which differ from cellulose in three distinct ways (i) by composition of different sugar units, (ii) by much shorter molecular chains, and (iii) by branching of the chain molecules. Polyoses are the basis of a variety of commercial and industrial important products such as ethanol, yeast, xylitol, mannitol, emulsifiers, resin polymers, acrylates, esters, polyurethanes, nylon, etc. (Fengel & Wegener, 1984). Hemicelluloses are heteropolysaccharides with either linear or branched chains. They consist of hexose (D-glucose, D-galactose and

D-mannose) as well as pentose (D-xylose and D-/L-arabinose) sugar units. These units are linked together at different molar ratios. Moreover, xylans contain uronic acids as a side chain group. Hemicelluloses are not crystalline and their DP is much lower (100-200) as compared to cellulose. The low crystallinity is responsible for their low thermal and chemical stability as well as easy dissolution in pulping/pretreatment processes (Alén, 2000).

The composition of hemicellulose varies from species to species. Softwood (SW) contains more mannose and galactose while hardwood (HW) is rich in xylan and acetylated hydroxyl groups (Koch, 2006). Xylan is also the dominant anhydrous sugar unit in agriculture residues. HWs have normally somewhat higher hemicellulose (30-35%) content than SWs (25-30%). SW xylan contains no acetyl groups while there are about 6% acetyl groups in galactoglucomannan of SW. Glucomannan of HW is not acetylated while the amount of acetyl groups in glucuronoxylan varies from 8 to 17% (Alén, 2000). Some hemicelluloses, such as arabinogalactans in larch dissolve in water easily and most of them are easily hydrolyzed (Sjöström, 1993; Alén, 2000).

2.4.3 Lignin

Lignin is a polymer that consists of repeating units of phenylpropane (Sjöström, 1981). In contrast to other natural polymers, such as cellulose and protein, lignin is a complex three-dimensional polymer (Buranov & Mazza, 2008). Various investigations show that p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol are the major precursors and building units of lignins (Freudenberg, 1965; Bartuska, et al., 1980; Fengel & Wegener, 1984). These lignin precursors lead to formation of so-called H (p-hydroxyphenyl), G (guaiacyl) and S (syringyl) phenylpropanoid units. These units show different abundancies depending on their origin (Hage, et al., 2009). The β-aryl ether bond (β-O-4) is the most abundant inter unit linkage in all lignins (Alder, 1977). Breaking this linkage is also the central aspect of industrial processes, such as kraft pulping, and of many analytical methods because it is most easily cleaved chemically. There are five other linkages called β-5, β-β, 5-5, 5-O-4 and β-1. They are comparatively more resistant to chemical degradation. In acid sulfite pulping α-O-4 is the most important linkage which is hydrolyzed. These bonds represent only 6-8% of total polymeric linkages in lignin but their breakage and sulfonation is sufficient for lignin dissolution in the aqueous pulping solution. Lignin is so complex that its structure is not yet completely understood. Also, it has no structural regularity. In contrast to most of other natural polymers, which have a single inter-monomeric linkage, lignin contains many different carbon-to-carbon and ether linkages. It is almost impossible to isolate lignin

in pure form because of strong physical and chemical linkages between lignin and the cell wall polysaccharides (Holtmam, et al., 2003).

The composition of lignin differs from species to species. HW lignin consist mainly of guaiacyl and syringyl units in nearly equally amounts, while, guaiacyl is the major unit in SW. Grass lignins contain p-coumaryl alcohol derived units along with sinapyl and coniferyl (Alén, 2011). The lignin content varies greatly between species. Generally, SWs contain 26-32% and HWs 20-28% lignin (Sjöström, 1981), while the lignin content of agricultural residues is comparable to that of HW. Also, substantial structural differences exist between SW and HW lignins. For example, SW lignin is more branched, cross-linked and condensed and has a higher molecular weight and higher share of C-C bonds (Sjöström, 1981; Achyuthan, et al., 2010). These differences are important from a delignification point of view.

2.4.4 Extractives

Extractives are non-cell wall components, and can be easily removed from biomass using solvents such as water, acetone, ethanol and benzene. However, they pose problems in pulping of lignocellulosic biomass. Extractives include resin acids, fats, terpenes, tannins and wide variety of phenolic compounds (Alén, 2000). Phenolic compounds tend to form cross-links with lignin and cause impaired delignification in acid sulfite pulping. Long storage of biomass decreases and changes the composition of extractives and in this way, their adverse effects in pulping process can be minimized (Sjöström, 1981). Polyphenolic substances are very soluble in alkaline solutions (Fengel & Wegener, 1984) but not soluble in common organic solvents (Jensen, et al., 1963; Hergert, et al., 1965; Dietrichs, et al., 1978).

2.4.5 Inorganics

Potassium, calcium and magnesium are the most common inorganic elements in HW and SW. Some inorganics are very important for plant growth and thus critical from fertilization and soil conservation point of view. On the other hand, they are harmful in pulping and energy production processes because of scaling, reaction with pulping chemicals, and bleaching problems. Forest residues and bark contain more inorganics as compared to wood (Fengel & Wegener, 1984). Sometimes harvest and logging procedures result in entrainment of materials such as sand and increase the amount of inorganics in the biomass (Jurgens, et al., 2012). Silicon is the predominant inorganic compound in SCS ash. SCS contains up to 5% (by weight) of sand and debris as impurities. This is one of the major

reasons that alkaline pulping processes, such as soda and kraft, cannot handle agriculture residues. These impurities are the result of different transport and harvesting procedures of sugarcane (Gómez, et al., 2010).

2.5 Fractionation of SCS and challenges

Fractionating is also known as pre-treatment because it involves the separation of key components of lignocellulosics, prior to other treatments such as enzymatic hydrolysis for biofuels production. The production of paper and pulp, and biofuels (via fermentation) is greatly facilitated if the principal biomass constituents are cleanly separated into cellulose, hemicellulose and lignin. The complexity of biomass makes this task difficult. In lignocellulosic biomass cellulose is imbedded tightly in a composite structure of lignin and hemicellulose, where lignin and hemicellulose are covalently bound to each other (Fengel & Wegener, 1984; Jurgens, et al., 2012). This association of lignin with carbohydrates, especially with hemicelluloses, at α-carbon and C_4, is called lignin-carbohydrate complexes (LCC). The LCCs in herbaceous plants are different from those of woody plants and are called "lignin/phenolic-carbohydrate" complexes (Lapierre & Monties, 1989) as shown in Figure 2.2. In non-woody plants, hydroxycinnamic acids (*p*-coumaric and ferulic acids) make a lignin/phenolic-carbohydrate complex between hemicelluloses and lignin via ester and ether bonds (Baucher, et al., 1998; Sun & Tomkinson, 2002). Therefore, it is not possible to isolate lignin in pure form. Lignin is associated with hemicelluloses not only through chemical bonding but also as physical admixtures (Sarkanen & Ludwig, 1971).

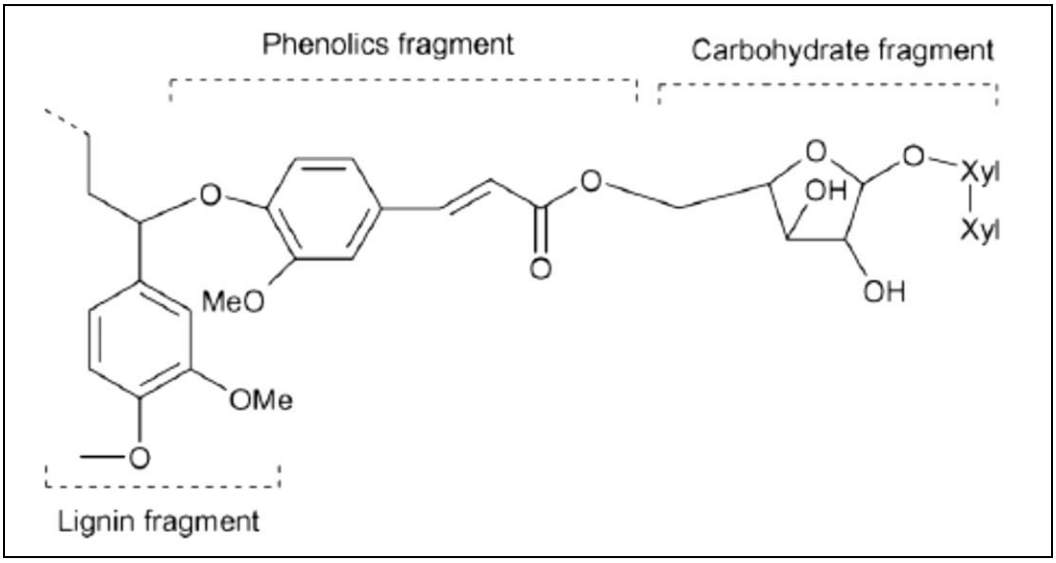

Figure 2.2: Lignin/phenolics-carbohydrate complex in wheat straw [adapted from (Sun, et al., 1997)]

There is almost 5% ferulic acid in straw lignins and a large portion of ferulic acid residues ester-linked to polysaccharides can also form an ether bond with phenylpropane units creating a bridge between wall polysaccharides and lignin, reducing the carbohydrate availability. Lignin and ferulic acid residue are released when alkali attacks the ester linkages of such bridges, producing a small amount (1-4.3%) of ferulic acid (Lam, et al., 1992; Sun & Tomkinson, 2002). The three main macromolecular components of lignocellulosic materials (cellulose, hemicelluloses and lignin) can be isolated by chemical, physical or biological treatment. Pulp and paper industries use mostly chemical processes to accomplish the task (Smook, 1992; Biermann & Christopher, 1996; Rocha, 2000).

2.6 Fractionation methods

Pulping processes disintegrate the lignocellulosic biomass into its principal components by removing, solubilizing and/or fragmenting the lignin. An ideal delignification achieves 100% isolation of lignin without chemical disruption of polysaccharides. Unfortunately, no delignification procedure meets this requirement. The isolation of lignin in unaltered form and its exact structure determination is not possible yet because of its complex molecular structure and localization within cell wall. All methods of lignin removal either result in changing its native structure or provide only partial isolation in pure form (Fengel & Wegener, 1984). Biopulping is an alternative for fractionation of biomass that focusses on minimizing the problems associated with chemical (pollution, high cost, and low yield) and mechanical (high energy consumption, low mechanical strength) pulping processes (Akhtar, et al., 1998; Messner, et al., 1998; Ferraz, et al., 2008). The basic objective in chemical pulping is to separate hemicelluloses and lignin from the lignocellulosics material while leaving cellulose intact. This is a very difficult task and compromises must be made because 100% separation is not possible (Bajpai, 2012). Chemical pulping methods can be alkaline, acidic or organosolv.

2.6.1 Alkaline fractionation

The alkaline Kraft pulping process, also known as the sulfate pulping process, is globally the most dominant pulping process. The process derives its name from the makeup chemical sodium sulfate which is added in the recovery cycle to compensate for chemical losses. In this process, white liquor (cooking liquor) consists of sodium hydroxide (NaOH) and sodium sulfide (Na_2S). One major advantage of alkaline pulping is that it can be applied to HWs as well as SWs, while the soda process that uses only sodium hydroxide is limited to HWs (Bajpai, 2012). In alkaline pulping carbohydrates undergo severe degradation and result in the formation of hydroxycarboxylic acids which are difficult

to recover (Carvalheiro, et al., 2008). Complex process of alkali recovery, production of pulps with a relative high lignin content, synthesis of foul smelling chemicals, scaling during evaporation of black liquor and carbohydrates degradation are very common problems associated with alkaline pulping. Sometimes alkaline pulping is modified by addition of anthraquinone (AQ) to limit carbohydrate degradation and improve delignification (Blain, 1993). For example, improved SCS fractionation have been reported employing 0.15% AQ with 16% Na_2O (straw to liquor ratio of 1:12 m/v%) in the soda/AQ process (Luz & Gonçalves, 2001).

2.6.2 Acidic fractionation

In acid fractionation processes, acidic conditions are provided by addition of an acid to cooking liquor and/or by formation of acids during cooking. One advantage of acid addition is reduced cooking temperature, but it is accompanied by serious corrosion problems (Iakovlev, 2011a). The main sulfite fractionation processes are acid sulfite (AS), bisulfite (magnefite), neutral sulfite semi chemical (NSSC) and alkaline sulfite. Acid sulfite (AS) is the most well-known acid pulping process. It employs SO_2 with $M(HSO_3)_n$ where M is monovalent or divalent cation (sodium, ammonium, calcium, magnesium) and n=1 or 2. Contrary to the Kraft process, the sulfite process can be operated at any pH by changing the amount and composition of chemicals (Biermann & Christopher, 1996; Smook, 1992). A major drawback of the AS process is its high overall cooking time (around 12 hours) due to very slow impregnation of the cooking chemicals (Rydholm, 1965; Fengel & Wegener, 1989; Sixta, et al., 2006). Also 10-20% of the carbohydrates are converted to aldonic acids. Ethanol, xylitol, acetic acid and lignosulfonic acids are some valuable byproducts of the AS process (Sixta, et al., 2006). The pulp produced by the sulfite process has poorer tear strength properties compared to Kraft and organosolv pulps (Johansson, et al., 1987) but this pulp can be used for specialty grade paper, tissue paper and especially dissolving pulp (Fengel & Wegener, 1989). At present AS fractionation of softwoods is the only commercially operated fractionation process which utilizes dissolved polyoses for ethanol production (Jurgens, et al., 2012).

2.6.3 Organosolv fractionation

Organosolv fractionation processes commonly use alcohol-water (for example the Alcell process) or carboxylic acid-water mixtures such as acetic acid and formic acid. Typically, organosolv processes use low boiling point organic solvents (acetone, ethanol, methanol, dioxane, ethyl acetate etc.) so that they can be easily recovered by distillation (Rodríguez & Jiménez, 2008). Alcohol based fractionation was first introduced in 1931 (Kleinert &

Tayenthal, 1931). The presence of alcohol (ethanol) in water-alcohol mixture results in fast impregnation of lignocellulosics due to the Marangoni effect (Sternling & Scriven, 1959) and reduces the overall time of fractionation. Paszner and Cho (1989) research shows that primary alcohols give the highest fractionation efficiency among all alcohols. Moriya et al. (2007) used an ethanol/water mixture for pulping of SCS and studied the effect of temperature and time on delignification. Cooking SCS at 200ºC for 2 hour with 50% (w/w) ethanol-water mixture and 1:10 (m/v) straw to solvent ratio resulted in a weak pulp of only 3.14 cP viscosity and high lignin content (58 kappa number). In another study Goncalves et al. (2008) fractionated SCS using the acetosolv process. They used 93% (w/w) acetic acid and 0.3% (w/w) hydrochloric acid as a catalyst and straw to liquid ratio of 1:16 (m/v) with two hours cooking time at 115$\pm$5ºC. Another similar kinetic study of acetosolv fractionation of SCS was performed at varying time (0-5 h) and high acetic acid concentration (73-93%). The catalyst (HCl) concentration and temperature were maintained at 0.3 % (w/w) and 115$\pm$5ºC respectively. After one hour treatment, the observed pulp yields were 45-50% (Saad, et al., 2008). Organosolv processes also use mixtures of organic solvents to improve pulp and paper quality. For example, Jiménez et al. (2002) produced pulps from wheat straws using ethanol-acetone mixtures. The pulp produced not only had a high yield, but also higher tear strength as compared to pulps produced using acetone or ethanol separately.

Although organosolv processes do not produce malodorous compounds as in the Kraft process (Xu, et al., 2007; Zhang, et al., 2007) these processes unfortunately have limitations regarding the feedstocks they can handle. For instance, SWs pose problems for many organosolv processes (Paszner & Cho, 1989). Among organosolv processes only the MILOX (peroxyformic acid) process is capable of pulping softwoods (Sundquist, 2000).

2.6.4 SAW fractionation

SO_2-alcohol-water (SAW) processes use SO_2 dissolved in an aqueous mixture of alcohol for lignocellulosics fractionation. This process is a fusion of acid sulfite (AS) and alcohol-water pulping processes. The process was introduced for the first time by Schorning (1957). He dissolved 5.5% SO_2 in a 50% mixture of alcohol-water and conducted the experiment at 110-135ºC for 5 hours. He used mostly methanol, but also employed ethanol and n-propanol in his experiments. The process has essentially the same advantages over the Kraft process as does the AS process: higher carbohydrate yields, lower cooking temperatures, and a pulp of higher brightness and bleachability (Primakov, 1961).

SO_2-ethanol-water (SEW) is one of the SAW processes that employ ethyl alcohol. Primakov (1979) and Iakovlev (2014) identified distinct advantages of this process: it is superior to the AS process in terms of higher biomass utilization and higher operational performance. For example, the overall cooking time is significantly reduced due to fast transportation of cooking liquor to the reaction sites because it eliminates the impregnation step, low energy need due to low cooking temperature, ability to handle wide variety of biomass, absence of formation of sticky lignin, simple recovery of the pulping chemicals (SO_2 and ethanol), and ability to treat "green" as well as dry feedstocks. Moreover, ethanol proved to be better solvent for lignin and lignosulfonates as compared to water (Primakov, et al., 1979) and methanol (Puumala, 1991). In his research, Primakov (1961) demonstrated that ethanol could be easily recovered from SEW cooking.

The mechanism of SAW fractionation process is similar to that of sulfite pulping. Sulfite delignification follows a two-step mechanism. The first step is fast sulfonation of lignin at the alpha position, and the second step involves the slow hydrolysis and dissolution of the sulfonated lignin (Hägglund, 1951). In their study on extraction of lignin from sugarcane bagasse and Pinus taeda, Pasquini et al. (2005) demonstrate that for efficient lignin removal a strong nucleophilic agent plus a mixture with a high dissolution capacity of lignin fragments is needed. That's why the delignification yield with 100% ethanol is less than that with a mixture of 50% ethanol – water in the Alcell pulping process. The addition of water increases the delignification rate because of the higher effective acidity in water, but the capacity of lignin to dissolve in an aqueous solution is reduced. SO_2 is a moderately strong nucleophile (Fengel & Wegener, 1989) and this species can sulfonate lignin although at a lower rate than the much stronger nucleophile, the bisulfite anion (Eliashberg, et al., 1955). In acidic cooking liquors, like acid sulfite and SAW, the main agents responsible for lignin sulfonation are hydrates and solvates of SO_2 and HSO_3^- anions. The nature of sulfonating agent has always been a question and no consensus has been reached yet (Iakovlev, 2011a). HSO_3^- is a stronger nucleophile than SO_2 hydrates and solvates (Gierer, 1970) so a higher sulfonation rate can be expected with this species. However, in a system like SAW sulfonation is mostly carried out by SO_2 species because the concentration of bisulfite anions is not significant. The rate of lignin sulfonation by SO_2 hydrates and solvates are also assumed equal (Iakovlev & van Heiningen, 2011c).

In SAW pulping, it is reported that about half of the dissolved hemicelluloses are monomeric sugars (Iakovlev & van Heiningen, 2012a) while the remaining dissolved hemicelluloses are in oligomeric form. The high fraction of

oligomeric hemicellulose is surprising considering the high acidity (pH about 1) and high temperature (135°C), and in the present thesis it will be shown that most of the oligomers are ethylated sugar monomers. After separation of sulfonated lignin, the monomeric sugars can be used as raw material for production of ethanol/butanol or biochemicals. It is evident from SEW pulping experiments on spruce, beech and wheat straw that bulk delignification follows pseudo first order kinetics in lignin, while hemicellulose removal and cellulose degradation can be described as first order and zero order kinetics (Iakovlev, et al., 2011b). The SEW process also has potential to produce rayon grade pulps and thus might potentially replace AS pulping (Sixta, et al., 2013). Some of the issues regarding the SEW process are SO_2 losses and atmospheric emissions, flammability and losses of the employed alcohol, and corrosiveness of the cooking liquor. High process pressure and toxicity of ethanol are other major concerns from a health and safety point view. Some of these problems can be overcome by employing less volatile and toxic alcohol (like isopropanol). However, it will decrease the efficiency of solvent recovery.

2.7 SAW process parameters

2.7.1 Feedstock material

The chemical structure as well as morphology of biomass species plays a crucial rule in a pulping process especially the hydrolysis of polysaccharides. For example, it is hard to remove lignin from softwood because of its condensed nature (Sjöström, 1981; Achyuthan, et al., 2010). However, the bulk delignification rate of hardwood, softwood and straws in SEW fractionation are very close, while residual delignification is comparatively slower for hardwoods than softwoods (Iakovlev, 2011a). Moreover, at constant kappa number the lignin and ash free yield for these species increases in the order: straws < hardwood < softwood (Iakovlev, et al., 2011b). High ash content of straws leads to high overall yield of pulps (including ash and lignin) from this species when compared to hardwood and softwood species (Iakovlev, et al., 2011b). The external morphology has relatively weak effects on SAW fractionation compared to the internal morphology of lignocellulosics. For example, Iakovlev et al. (2009) have shown that pulp yield, kappa number and viscosity are not affected by particle size, which is an indication that pulping is governed by chemical reaction rather than by diffusion. Iakovlev (2011a) also fractionated spruce wood chips using the SEW process (12% SO_2, 80 min, 135ºC) and found that both green (dry matter content 48.9%) and air-dried (dry matter content 92.8%) wood chips resulted in nearly the same pulp and hydrolysate properties when the amount of cooking liquor used per unit mass of biomass was adjusted for wood chips moisture content. Iakovlev's suggested explanation for this observation was the so-called Marangoni effect.

In SAW fractionation carbohydrates undergo acid hydrolysis. Their composition in the biomass feedstock and extent of chemical linkage to other components affects the hydrolysis kinetics. The rate of hydrolysis of the glycosidic bond at the reducing end of cellulose is slightly slower than that at the non-reducing end (Feather & Harris, 1967) leading to a higher rate of monosaccharide produced than calculated based on uniform cleavage (Sjöström, 1981, p. 83). Similarly, hemicellulose chains are also hydrolyzed but the rate at which pentoses, hexoses and hexuronic acids are hydrolyzed is different. The rates are estimated from acid hydrolysis of methyl pyranosides of common biomass monosaccharides. It shows that relative rate for β-D-glucose, β-D-mannose, α-D-galactose, β-D-xylose and α-D-glucuronic acid are 1.0, 3.0, 2.7, 4.8 and 0.2 respectively (Feather & Harris, 1965). It is also worth considering that xylan is more prone to acid hydrolysis than glucomannan, and this results in relatively lower substrate yield for xylan-rich hardwood species compared to glucomannan-rich softwoods (Rydholm, 1965, p. 540; Sixta, et al., 2006, p. 110).

2.7.2 SO_2 concentration

As described earlier, the SAW fractionation mechanism is similar to that of AS pulping. The first step is fast sulfonation of lignin. SO_2 in the cooking liquor is responsible for sulfonation of lignin and the degree of sulfonation is expressed as the S/C_9 molar ratio. In SEW cooking liquor SO_2 is mostly present as hydrates and solvates because no base is added, and these species are likely responsible for sulfonation. A very small amount of SO_2 is present in HSO_3^- form because of the equilibrium reaction, and is called combined SO_2 (Iakovlev, 2011a). The applied amounts of SO_2 and HSO_3^- in acid sulfite pulping are determined by the Kaufmann diagram (Rydholm, 1965, p. 467) which shows that unacceptable condensation of lignin (leading to so called "black cooks") can be avoided in AS pulping if the SO_2 concentration is 12% (w/w) or higher irrespective of the HSO_3^- concentration. Since the effective acidity is lower in SEW pulping due to the presence of ethanol, lignin condensation is also not expected to be a significant problem in SEW pulping at moderate temperatures (135 °C) and relatively high SO_2 concentrations. Iakovlev and van Heiningen (2012b) highlight that in SEW cooking the rate of delignification increases linearly with SO_2 concentration up to 12% and then increases further at a lower rate. Sixta et al. (2006, pp. 459-460) shows that for acid sulfite cooking at constant total SO_2 charge an increase in combined SO_2 results in lower acidity and decreases the delignification rate. In contrast, at constant HSO_3^- concentration, delignification increases with increase in free SO_2. Eliashberg et al. (1960) and Primakov (1961) also demonstrated that increasing SO_2 concentration at 135ºC increases the SEW delignification. Lignin condensation is an undesirable phenomenon in pulping process because it forms non-cleavable

C-C bonds and prevent delignification. High acidity and high temperature promotes lignin condensation (Rozenberger, 1961). Therefore, it is advisable to operate the SAW process at lower temperature and high SO_2 concentrations to avoid condensation reactions.

The concentration of SO_2 also affects the hydrolysis of hemicelluloses. Iakovlev et al. (2012a) describes that increasing the concentration of SO_2 results in higher monosaccharide concentrations in the hydrolysate, likely due to higher acidity of the cooking liquid. On the other hand, at fixed SO_2 concentration, increasing cooking duration doesn't increase the dissolved monosaccharide fraction as compared to the total amount of dissolved carbohydrates. This anomaly is further addressed in the present thesis. Cellulose hydrolysis, i.e. depolymerization, increases only slightly with increasing SO_2 concentration because the acidity increase is small. Cellulose is completely retained in solid phase for SEW fractionation of spruce at 3, 6 and 12% SO_2 (Iakovlev & van Heiningen, 2012b) because the cellulose DP remains high in all cases.

2.7.3 Alcohols & their concentrations

SAW cooking liquor contains SO_2 dissolved in an alcohol water mixture. The composition of the alcohol aqueous solution as well as the amount of cooking liquor employed (liquid to feedstock ratio) have a pronounced effect on the fractionation of lignocellulosic biomass. The concentration of alcohol in cooking liquor determines its impregnation potential (Marangoni effect). Impregnation is a very important step in fractionation of biomass. Generally, in AS cooking of wood chips impregnation is a very slow process that takes around 6 hours (Rydholm, 1965, p. 444; Fengel & Wegener, 1989). A higher difference between surface tension of alcohol-water mixture and water retained in the cell walls of lignocellulosics promotes the mass flow into the chips and hence decreases the impregnation time and thus the overall cooking time. The presence of alcohol in cooking liquor eliminates the need of a separate impregnation step (Iakovlev, 2011a, p. 7). Alcohol also affects the pH of the mixture and hence has a pronounced effect on carbohydrate hydrolysis and lignin condensation. Sierra-Alvarez & Tjeerdsma (1995) showed that increasing the ethanol concentration decreases the acidity of cooking liquor, and thus at very high ethanol concentrations the pH is too high to initiate fractionation. On the other hand, at low ethanol concentration the acidity is high resulting in carbohydrates hydrolysis and lignin condensation. Therefore, a moderate level of alcohol concentration is required for effective fractionation.

The type of alcohol used in cooking liquor preparation affects the solubility of lignin. Sixta (2006) and Schuerch (1952) suggest that the solubility parameter (δ-value) and hydrogen potential of solvents can be used to describe dissolution of lignin in different solvents including alcohols. In general, polymers show the highest solubility in solvents which have a δ-value close to that of the polymer. In addition, the lignin dissolution potential increases with an increase in hydrogen bonding potential of the solvent when the solvent has the optimum δ-value. Typical lignin δ -value reported in the literature is about 28-30 $(MPa)^{1/2}$ (Ni & Hu, 1995; Quesada-Medina, et al., 2010; Wang, et al., 2011; Ye, et al., 2014). Also, lignin is better removed with less polar solvents (ethanol, dioxane) while hemicelluloses are more soluble in more polar solvents like water thereby providing further motivation to use alcohol-water mixtures of a certain optimal composition for biomass fractionation (Iakovlev, 2011a).

2.7.4 pH of cooking liquor

The pH of SAW cooking liquor is dependent on the alcohol and SO_2 concentration in the solution. SO_2 is responsible for lignin sulfonation. Analysis of S/C_9 ratio of AS and ethanol-AS solid and liquid samples indicates that lignin sulfonation is not directly dependent of ethanol presence (Iakovlev & van Heiningen, 2012b). Lignin sulfonation follows the SN_1 substitution mechanism. Figure 2.3 shows the mechanism of lignin sulfonation in acidic environment. Lignin unit can be phenolic (R=H) as well as non-phenolic (R=lignin). The attack of the hydroxonium ion on the alpha position of lignin is the rate limiting step in this mechanism (Sixta, et al., 2006). Sulfonation of lignin gives rise to formation of lignosulfonic acids. In the subsequent step lignin get dissolved in the solvent because of fragmentation and ionic nature of lignosulfonic acids. Iakovlev et al. (2012b) shows that the rate of sulfonation is controlled by the amount of bound SO_2 (HSO_3^- anion) rather than the acidity. Vishnevskaya (1981) and Primakov (1979) report that lignosulfonic acids, formed by lignin sulfonation, are more soluble in ethanol than in water. The formation of lignosulfonic acids during fractionation decreases the pH of the pulping liquor, while the presence of ethanol off sets this effect thereby reducing carbohydrates hydrolysis.

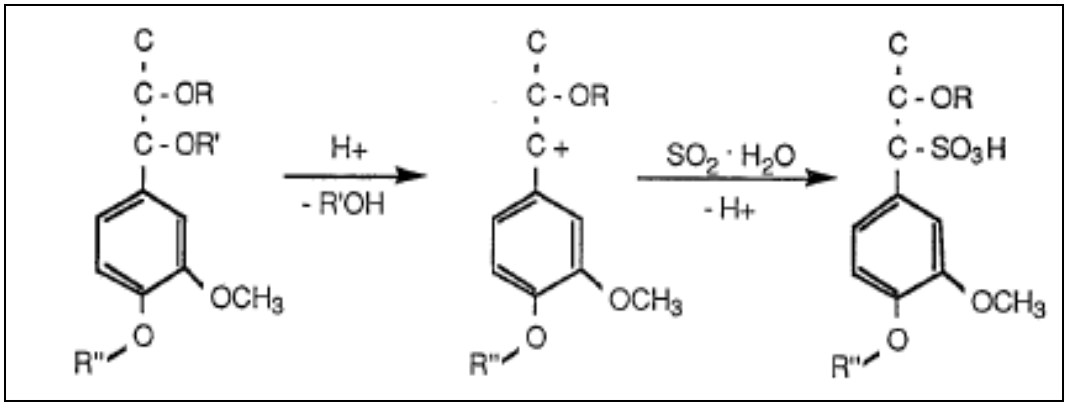

Figure 2.3: Sulfonation of lignin in acid sulfite cooking

Some of the most important monosaccharide reactions in acidic pulping systems are dehydration and oxidation. It is known that these reactions depend on temperature as well as acidity. Lower pH promotes dehydration while higher pH is responsible for oxidation reactions (Slávik, 1961; Sixta, et al., 2006, p. 419). HMF and furfural are products of dehydration reactions, with the former being further decomposed to levulinic acid and formic acid (Rydholm, 1965, p. 520). Aldonic acids are formed due to oxidation of monosaccharides, and in AS cooking HSO_3^- anions are responsible for this reaction. It is reported that oxidation of pentoses is a much faster reaction than hexoses oxidation (Larsson & Samuelson, 1969). The insignificant concentration of dehydration and oxidation products in the SEW process is related to the very low amount of bisulfite anions (due to absence of base) (Iakovlev, 2011a).

2.7.5 Liquor to feedstock ratio

Cooking liquor to feedstock ratio (L/F) is usually expressed in mL of cooking liquor/g of feedstock or L kg^{-1}. L/F is very important from an economical operation point of view. Using cooking liquor in higher quantities makes a fractionation process costly, so there is always an effort to use as little fresh cooking liquor as possible without compromising fractionation efficiency. Generally, in sulfite fractionation process a 1:3 liquid to wood ratio is used at cooking temperatures of 120-140ºC and 5-20 hours of reaction time (Sarkanen & Ludwig, 1971). Iakovlev (2011a) describes that neither the fractionation rate nor the relative composition of the resulting streams change when decreasing L/F from 6 to 3 L kg^{-1}. However, a further decrease results in somewhat slower delignification due to SO_2 depletion and/or accumulation of lignosulfonic acids (Iakovlev, 2011a, p. 106).

2.7.6 Cooking temperature & duration

Cooking temperature and cooking time are inversely related. Increasing cooking temperature in acidic pulping systems allows cooking of lignocellulosics materials in a shorter time. Also, improper increase in temperature leads to condensation of lignin. Usually in AS systems firstly the lignocellulosics are impregnated at 110-120ºC for uniform distribution of the cooking liquor in the biomass structure. If the temperature is raised before impregnation is complete, undesirable lignin condensation reactions occur. After impregnation, the actual cooking is performed at 125-150ºC (Sjöström, 1981, p. 108; Sixta, et al., 2006). Lignin condensation is truly related to pH of cooking liquor and cooking temperature and it is promoted at high temperature and lower pH (Rozenberger, 1961). In SAW, the pH of the cooking liquor is determined by the concentration of alcohol and SO_2 present in the mixture. Schorning (1957) reports slower delignification of beech due to condensation and warns not to operate at temperatures higher than 130ºC at SO_2 concentration of 5.5%. However, some contrary reports (Eliashberg, et al., 1960; Primakov, 1961) indicate that increasing cooking temperature up to 155ºC at 5-15% SO_2 considerably increases SEW delignification.

Chapter 3

MATERIAL AND METHODS

3.1 Feedstock material

SCS was obtained from the Thomaston, GA, biorefinery of API, which again obtained this material from its Brazilian partner Granbio. The material was size reduced using a Munson mill grinder with a 6.35 mm screen. Then the shredded material was screened through 1400 (sieve no. 14) and 710 microns (sieve no. 25) standard sieves on a mechanical Humboldt shaker. Straw fractions retained on each sieve and in the pan, were analyzed for yield and ash (Figure 3.1). The fraction collected in the pan was not included in the fractionation feedstock because of its high ash content. Each fraction was analyzed for Ca, Fe, K, Mg and SiO_2 on ICP-AES using EPA 200.7 method "Trace elements in water, solids and biosolids by inductively coupled plasma-atomic emission spectrometry". Elemental ash analysis revealed that the fraction retained in the pan was mostly composed of silica (Figure 3.2). SCS fractions retained on //14 and //25 were mixed (see Figure 3.2), stored in a plastic bag and later used for the fractionation experiments. The moisture content of the SCS was 6-8%. SCS in all later experiments and discussions refers to the mixture of fractions retained on //14 and //25 (Figure 3.2).

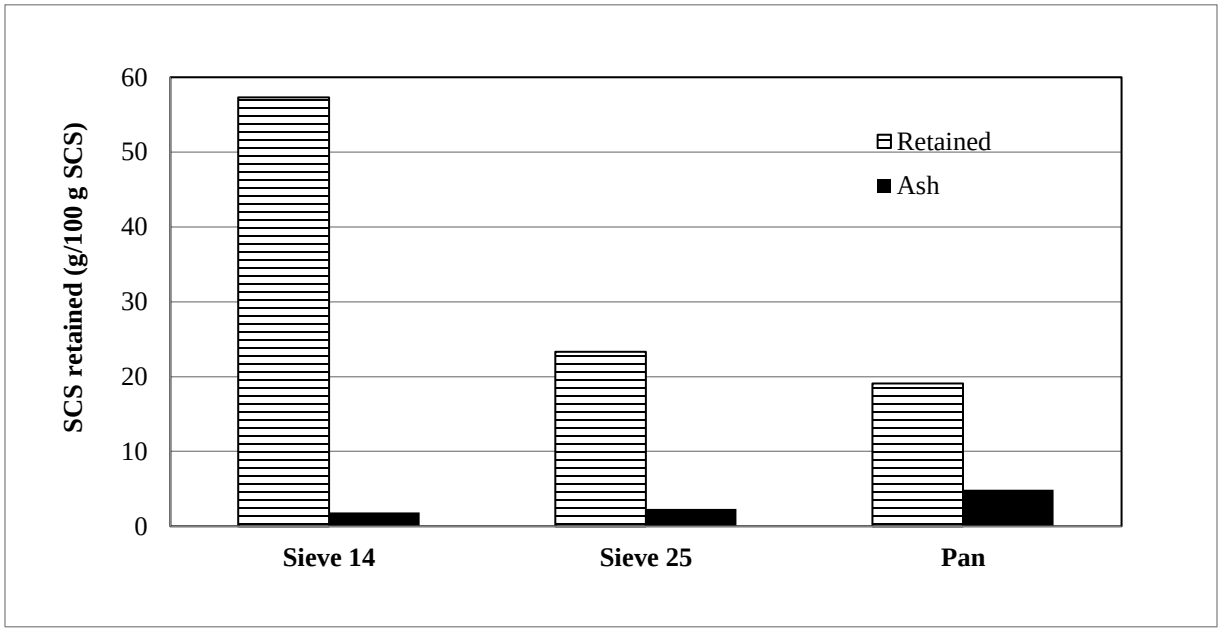

Figure 3.1: Ash in different fractions of SCS

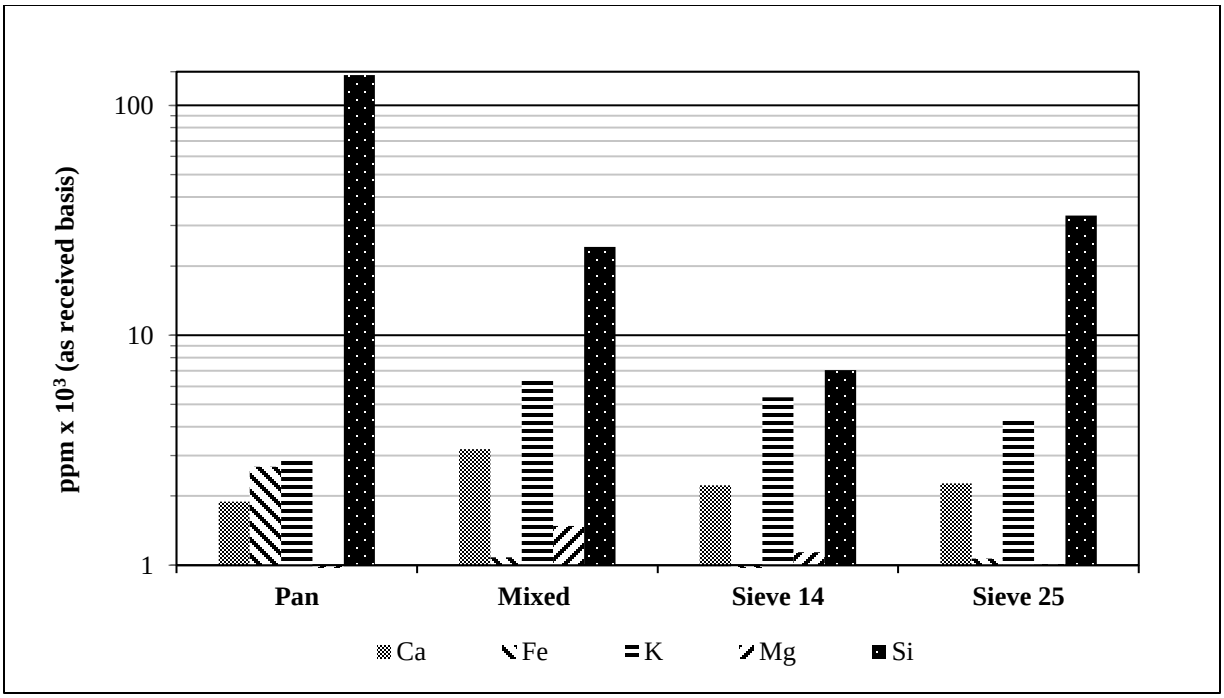

Figure 3.2: Elemental ash analysis of SCS fractions

3.2 Chemical composition of SCS

SCS was ground using a Wiley mill and analyzed for chemical composition. The acetone extractives in SCS were determined according to SCAN-CM 49:03 "Content of acetone-soluble matter". Lignin and carbohydrate content of Wiley mill ground SCS (extractive free) were determined according to NPEL/TP 510-42618 "Determination of structural carbohydrates and lignin in biomass" after double stage hydrolysis (72% H_2SO_4, 1 h, 30˚C, then 4% H_2SO_4, 1 h, 121˚C). Klason lignin was corrected for ash by burning it at 525°C for 24±6 hours in a muffle furnace. Acid soluble lignin (ASL) was determined by measuring the ultraviolet (UV) absorbance of liquor (at 205nm and extinction coefficient of 110 L/g.cm) from the hydrolyzed SCS. Scott's method was employed for uronic anhydride determination and hence for the quantification of 4-O-methylglucuronic acid (4-OMGA) (Scott, 1979). A high performance anion exchange chromatography with pulse amperometric detection (HPAEC-PAD) was used for separation of the monosugars (Davis, 1998). The instrument was equipped with Dionex CarboPac PA1 (4 x 250 mm) column (30°C), CarboPac PA1 (4 x 50 mm) guard column, IonPac NG1 (4 x 35 mm) guard column, GP50 Pump, ED40 Detector (gold electrode), and an AS50 Autosampler. The eluent flowrate was 1.0 mL min^{-1} (degassed H_2O at 0.7 mL min^{-1} + 300 mM NaOH from the post column at 0.3 mL min^{-1}). Acetic acid in the SCS hydrolysate was

determined by high performance liquid chromatography (HPLC) using a refractive index detector and BIO-RAD Aminex HPX-87K column (Bio-RAD laboratories; Hercules, CA, USA). The mobile phase used was H_2SO_4 (5 mM, 0.6 mL min^{-1}) and the oven temperature was 45ºC. SCS was also analyzed for nitrogen content by dry combustion. The protein content was determined by multiplying the nitrogen content by a factor of 5.6 (Mariotti, et al., 2008). The composition of the two mixed fractions of SCS used in pulping experiments is shown in Table 3.1.

Table 3.1: Chemical composition of sugarcane straw (SCS)

Component	Current study	(You, et al., 2016)	(Szczerbowski, et al., 2014)
Arabinan	3.0±0.2	2.8	2.57±0.64
Galactan	0.9±0.1	0.8	0.97±0.28
Glucan	35.7±0.4	35.7	32.76±0.51
Xylan	21.2±0.6	20.1	20.47±0.74
Mannan	0.6±0.2	0.4	1.01±0.03[b]
ASL	3.8±0.4	2.7	0.71±0.03
KL	19.8±0.2[c]	17.5[c]	20.57±0.19
Ash	6.1±0.2	8.5 (4.9[a])	6.23±0.20
Extractives	3.5±0.4[d]	2.1[d]	3.50±0.06[e]
Acetyl groups	2.9±0.1	-	3.26±0.07
4-OMGA	1.1±0.0	-	-
Protein	2.3[f]	-	3.72±0.11
Total	*100.9±1.1*	*90.6*	*95.77±1.18*

(a)-Acid insoluble ash, (b)-Unidentified anhydroushexose (glucans), (c)-Corrected for ash, (d)-Acetone soluble extractives, (e)-Extracted with a sequence of different organic solvents, (f)-Based on nitrogen analysis and using Jones factor of 5.6 (Mariotti, et al., 2008)

The SCS compositional analysis shows that it is rich in carbohydrates containing 61.4% anhydrous sugars. Xylan is the most abundant hemicellulose sugar, while galactan and mannan are present at less than 1%. It contains 3.8% ASL and nearly same amount of acetone soluble extractives. Like other agriculture residues it has a significant amount of ash that adds 6.1% to its dry weight. Ash free Klason lignin is 19.8% and this value likely also includes a significant amount of protein which is present in SCS at 2.24%. The composition of SCS depends on the collection site, climate conditions, stage of plant development and variety (Gómez, et al., 2010; Santos, et al., 2012). The SCS in our study is from the same source as the SCS batch III that You et al. (2016) employed in their research. It had 59.9% carbohydrates, 20.2% lignin but less extractives (2.1%) and more ash (8.5%). However, You et al. (2016) did not analyze it for protein and no information were provided regarding screening process. A comprehensive description of the composition of sugarcane residues including SCS was reported by Costa (Costa, et al., 2015). However, this reference did not contain information on protein content either. In a more recent reference it is reported that SCS

contains 3.72% proteins (Szczerbowski, et al., 2014), with the rest of the composition very similar to SCS used in current study (Table 3.1).

3.3 Fractionation of SCS

Three water miscible alcohols, namely methanol, ethanol and isopropanol, were used for fractionation of SCS. SAW cooking liquors were prepared by injecting SO_2 in alcohol-water mixtures (50 w/w %) as shown in Figure 3.3. Reagent grade methanol (purity >99%), ethanol (95 v/v. %) and isopropanol (purity >99.9%) were purchased from Fisher Scientific (Suwanee GA, USA). Compressed SO_2 was purchased from Matheson Tri-Gas (Bangor ME, USA) for cooking liquor preparation. The amount of SO_2 dissolved in aqueous alcohol mixtures was determined by monitoring the increase in weight of solution. Undissolved escaping SO_2 was captured in a vessel containing hydrogen peroxide. The concentration of SO_2 in each cooking liquor was 12% (by weight). Thus, the final composition of each fresh cooking liquor used in fractionation experiments was SO_2/Alcohol/Water = 12/44/44 (w/w %).

Stainless steel cooking digesters (220 mL) were filled with 24.4 g air dried (or 22.6 g o.d.) SCS and cooking liquor was added at a feedstock ratio of 4 L kg^{-1} which also accounts for the SCS moisture content. The digesters were immersed in a hot multi digester oil bath (MDOB) (manufactured in-house) containing polyethylene glycol as a heating media at a temperature setting of 135, 145 or 155°C (±1°C). The digesters rotated 180 degrees at 2 revolutions per minute during cooking. After completion of pulping (20-120 minutes), which included an effective heat-up time of about 10 minutes (Iakovlev, 2011a), the digesters were put into an ice bath to quench and stop further reaction. The digester content was quantitatively transferred into a nylon bag (mesh size 75) and the solid pulp was obtained after manually squeezing out the spent liquor.

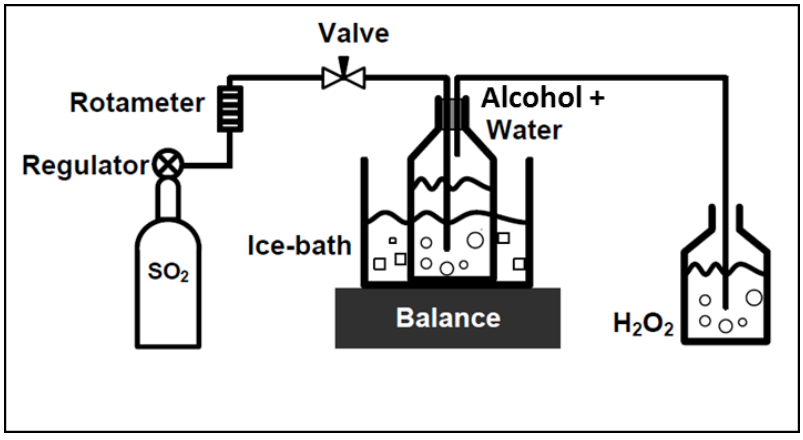

Figure 3.3: Preparation of cooking liquor

3.4 Processing of fractionated SCS

The pulp obtained from fractionated SCS was washed twice with 50% (w/w) aqueous alcohol solution (L/F = 2 L kg^{-1}) at 60°C and then washed twice with DI water (L/F = 20 L kg^{-1}) at room temperature (Figure 3.4). Finally, the pulp slurry was disintegrated, filtered through Whatman quantitative grade ashless filter paper and transformed into a pulp pad. Pulp pads were dried overnight at room temperature and then stored into plastic bags for further analysis. Methanol, ethanol and isopropanol based fractionation systems were designated as SMW, SEW and SPW respectively. The same corresponding abbreviations were used for resulting pulps, spent liquors and washings.

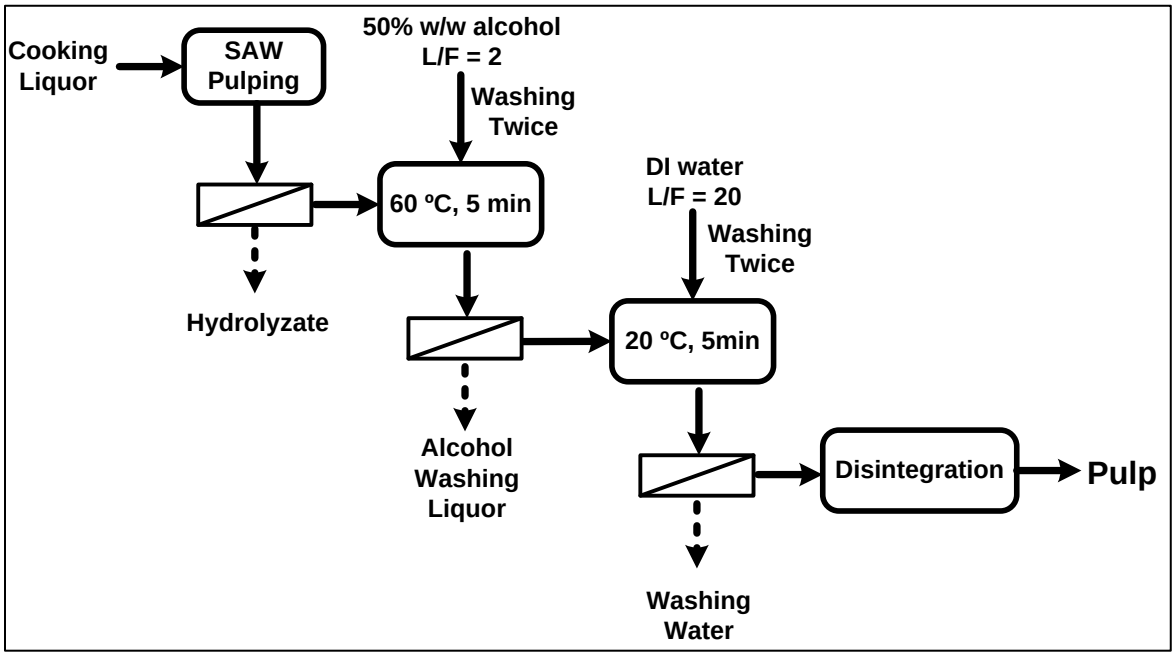

Figure 3.4: Alcohol and water washing of fractionated SCS

Chapter 4

SCS FRACTIONATION: EFFECT OF DIFFERENT ALCOHOLS

4.1 Introduction

The main goal of biomass fractionation is to efficiently separate it into the main constituents i.e. cellulose, hemicellulose and lignin. The success of fractionation was measured in terms of degree of delignification, hemicellulose removal and cellulose hydrolysis. Delignification and xylan removal kinetics were developed by quantification of residual lignin and residual xylan in treated SCS respectively using the same methods as described earlier for SCS composition analysis (see section 3.2). Pulps kappa number was determined by SCAN-C 1:00 method "Chemical pulp: Kappa number". Cellulose hydrolysis was investigated by measuring the viscosity of bleached pulps dissolved in cupriethylenediamine (CED) solution (SCAN-CM 15:99 method "Pulps: Viscosity in cupriethylenediamine solution"). All pulps were bleached with sodium chlorite following Tappi Suggested Method T230 su-66 "Viscosity of pulp (capillary viscometer method)" before viscosity determination.

4.2 Kappa-lignin correlations

SCS was subjected to fractionation at three different temperatures 135, 145 and 155°C with cooking duration ranging from 20 to 120 minutes. All cooking times include about 10 minutes for heat-up of the biomass to reach pulping temperature. Pulps produced under these conditions did not have any apparent rejects except for some of the SMW pulps with kappa >70. Acid insoluble or Klason lignin content of pulps after standard two step hydrolysis were determined, and corrected for acid insoluble ash because SCS contains a significant amount of acid insoluble inorganics, mostly silica (see section 3.1). Linear correlations between kappa number and ash free acid insoluble lignin content of SMW, SEW, and SPW pulps were obtained with intercepts of 4.64, 4.69 and 4.26 respectively at zero kappa number as shown in Figure 4.1. The results show that the lignin-kappa correlations are only a function of cooking solvent, but not of cooking temperature. The highest Klason lignin content at a fixed kappa number is seen for methanol, followed by ethanol and finally isopropanol.

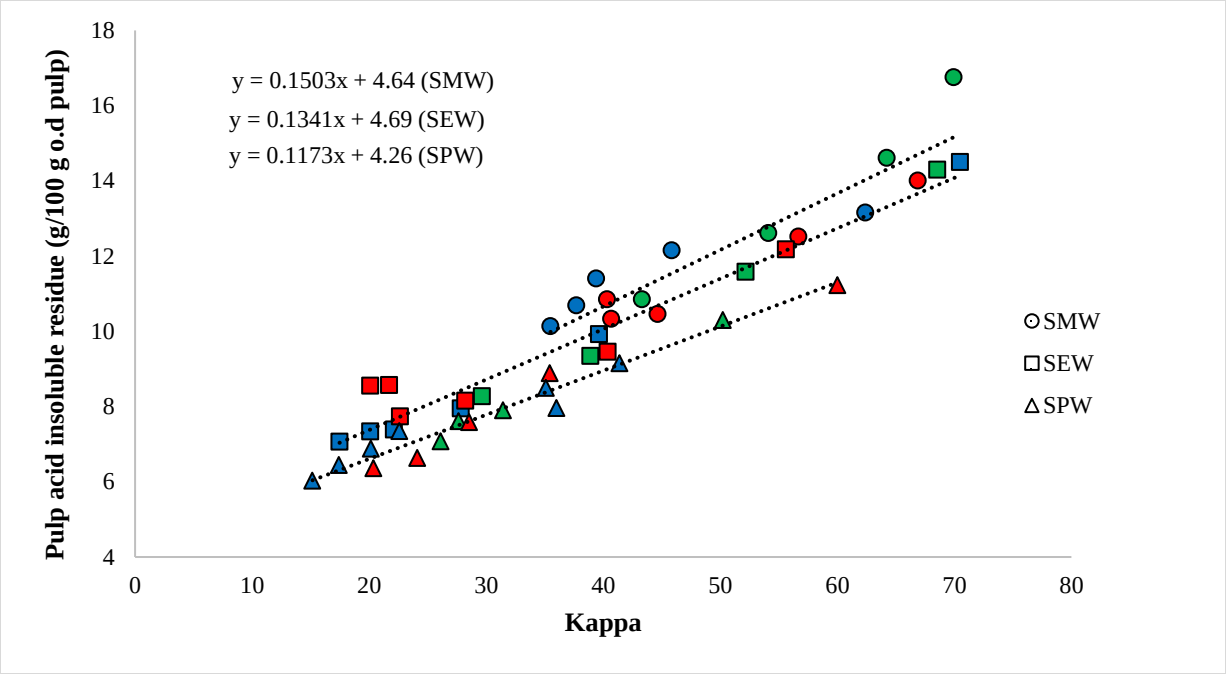

Figure 4.1: Kappa vs ash free acid insoluble lignin relations (unmodified) for SMW, SEW and SPW pulps. Color coding shows three different temperatures. Green (135°C), Blue (145°C) and Red (155°C)

Many correlations between kappa number and Klason/total lignin content of pulps have been reported in literature. However, no such correlation was ever reported for SCS. In a recent study on SEW fractionation of SCS (You, et al., 2016), the kappa number and lignin content for various pulps were reported, but no correlation was presented due to large variability of the data. Iakovlev et al. (2011c) reported that the proportionality constant in the linear kappa vs lignin correlations depends on species and fractionation process. Most of the correlations were developed for wood species, starting with Tasman and Berzins (Tasman & Berzins, 1957) in the late 50's for beech, birch, spruce and pine. In later studies (Li & Gellerstedt, 1998; Kyrklund & Strandell, 1969) slightly different proportionality constants were found depending on wood specie and pulping process, but interestingly the intercept was always found to be zero. However, for annual fiber biomass such as barley straw, a high intercept value (3.5-6.5 w/w %) was found for the correlation between Klason lignin and kappa covering a narrow range of total lignin content (Oreopoulou, 1988). The high value of the intercept in the latter study was attributed to the presence of shives, knots and permanganate non-oxidizable components in the unscreened pulp. For SEW pulping of spruce, beach and wheat straw Iakovlev et al. (2011c) also reported intercepts of the Klason lignin-kappa correlations of nearly zero for wood species, while for wheat straw it was 1.27% (w/w).

Thus, it is apparent that pretreated/pulped annual fibers are characterized by Klason lignin-kappa correlations with a high intercept even after correcting Klason lignin for acid insoluble ash present in it. This may indicate the presence of a significant amount of permanganate non-oxidizable matter in acid insoluble ash corrected Klason lignin. Recent studies show that there are 3.72% proteins (Szczerbowski, et al., 2014) and significant amounts of lipids present in SCS (del Río, et al., 2015). Such components may possibly contribute to high intercept values in Klason lignin-kappa correlations in SAW pulping of SCS. To investigate whether protein could be responsible for the permanganate non-oxidizable matter, different kappa number pulps were analyzed for protein content (A factor of 5.6 used to convert nitrogen content to protein content (Mariotti, et al., 2008). The data in Table 4.1 shows that these pulps contain a significant percentage (8.7-29.3%) of the protein present in the original SCS (2.24% on original SCS). Pulps with higher kappa number retain comparatively more protein. This suggests that a significant amount of protein ends up in the Klason lignin, and may contribute (in part) to the permanganate non-oxidizable matter in SAW pulps. Lipids (not determined in this study) are another possible contributing factor to high intercepts in lignin-kappa correlations.

Table 4.1: Protein in fractionated SCS

	SMW		**SEW**		**SPW**	
Pulp Kappa	45.8	44.7	27.8	22.6	22.6	20.4
Nitrogen (% Pulp)	0.26	0.22	0.21	0.11	0.12	0.09
Protein* (% Pulp)	1.46	1.24	1.15	0.62	0.66	0.51
Pulp Yield (%SCS)	45.1	41.1	42.3	38.3	41.4	37.9
Protein (% SCS)	0.66	0.51	0.49	0.24	0.27	0.19
Protein retained in Pulp, %	29.3	22.8	21.7	10.6	12.2	8.7

*A factor of 5.6 was used to calculate protein content based on (Mariotti, et al., 2008)

Consequently, we corrected the acid insoluble lignin content of pulps not only for ash but also for permanganate non-oxidizable matter such as lipids and proteins. This was done by subtracting the intercept values at zero kappa in Figure 4.1 from ash free Klason lignin for each corresponding pulp. The Klason lignin content corrected for ash and permanganate non-oxidizable matter for all pulps is plotted against Kappa number in Figure 4.2. It shows that the lignin content vs kappa relationship is not affected much by the type of solvent or pulping temperature. As expected, the intercepts of the modified correlations are now close to zero, like the Klason lignin-kappa correlations for wood species (Kyrklund & Strandell, 1969; Li & Gellerstedt, 1998; Iakovlev & van Heiningen, 2011c). Hence this suggests that the ash and permanganate non-oxidizable matter (including protein) corrected Klason lignin represent the real amount of lignin in pulps. Considering the scatter in the data, and that the correlations in Figure 4.2 for the SMW, SEW and SPW pulps do not differ much, it was considered appropriate to represent all the lignin-kappa data

by a single straight-line correlation which passes through the origin with a slope of 0.134. This overall correlation of the data is also seen in Figure 4.2.

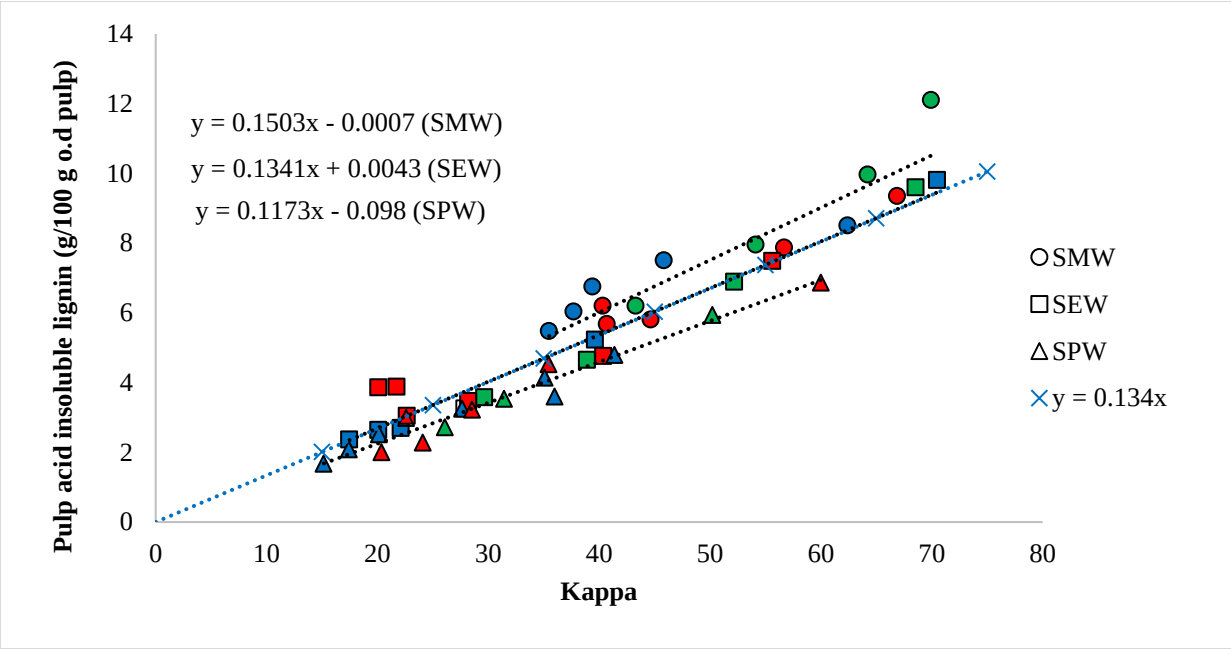

Figure 4.2: Kappa vs lignin relations (modified) for SMW, SEW and SPW pulps. Color coding shows different temperatures. Green (135°C), Blue (145°C) and Red (155°C). Blue dotted line represents the average of the SMW, SEW and SPW correlations

4.3 Delignification kinetics

The delignification kinetics were determined for the three SAW pulping series with temperature (135-155°C) and time as variables. The lignin content of the pulps based on original SCS was calculated from the kappa number using the following equation

$$[Lignin] = 0.134 \times \text{kappa} \times Pulp\ Yield \quad (1)$$

Shown in Figures 4.3-4.5 are the thus calculated [lignin] expressed in % on original SCS plotted against cooking time for pulping with methanol (SMW), ethanol (SEW) and isopropanol (SPW) respectively. In accordance with first order delignification kinetics, a logarithmic scale is used for [lignin] on the vertical axis, while pulping time on the horizontal axis is a linear scale. This plot is selected based on pseudo first order delignification kinetics found for SEW cooking (Iakovlev, et al., 2009) because the proton concentration is approximately constant during delignification as:

$$-\frac{d[Lig]}{dt} = k'_{Lig}\,(T)\,[Lig]^a\,[H_3O^+]^b = \; k_{Lig}\,(T)\,[Lig] \qquad (2)$$

Only the first 3-4 data points were used to determine the first order delignification rate constant because these data points could reasonably be represented by straight lines of increasing slopes at increasing higher temperature. The choice of 3 or 4 initial data points is somewhat arbitrary, and therefore the rate constants obtained are subject to some uncertainty. The argumentation for not including data points obtained at longer cooking times and higher temperatures is that they are indicative of slower delignification during the residual kinetic phase. The onset of the residual delignification phase occurs at shorter cooking time at increasing temperature for all three SAW systems.

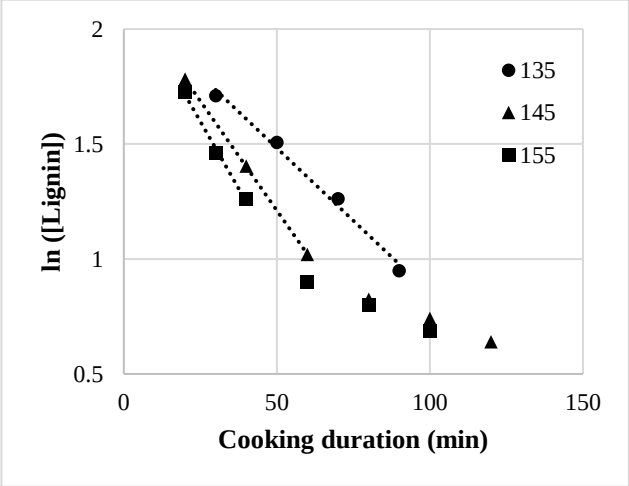

Figure 4.3: Natural logarithm of residual lignin (g/100 g SCS) vs cooking duration for SMW pulping

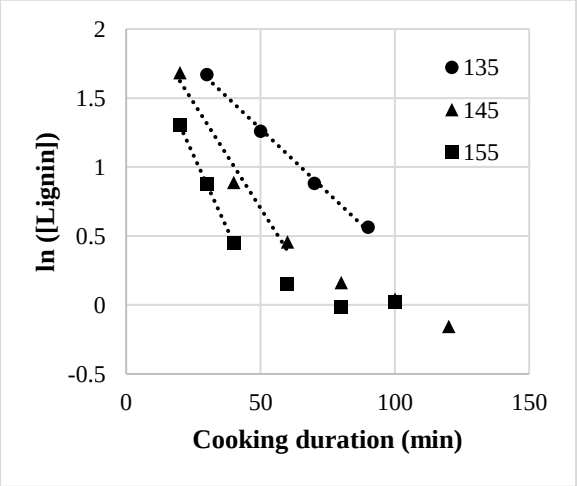

Figure 4.4: Natural logarithm of residual lignin (g/100 g SCS) vs cooking duration for SEW pulping

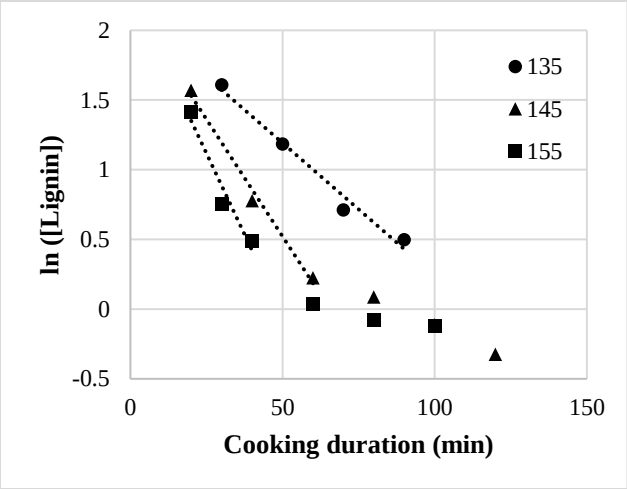

Figure 4.5: Natural logarithm of residual lignin (g/100 g SCS) vs cooking duration for SPW pulping

Since the (ash and protein corrected) [lignin] in SCS is 17.6% while the extrapolated [lignin] at 10 minutes cooking time (i.e. after heat-up) is 7-9% on original SCS, almost 50-60% of the SCS lignin is dissolved during the heat-up time for SMW, SEW and SPW. You et al. (2016) also reported the same behavior during the initial phase of SCS delignification using SEW but at a different pulping liquor composition (12:22.5:65.5 w/w %) versus the present SAW composition of 12:44:44 w/w%. Iakovlev et al. (2011b) identified two phases of delignification (bulk and residual phase) without an initial phase when pulping spruce, beech and wheat straw using SEW. In Kraft and SEW delignification of wood species, most lignin is removed in the bulk phase (Iakovlev, 2011a, p. 22). However, for SCS delignification most lignin is removed during the initial phase. This can be related to the high percentage of total lignin in the middle lamella of annual plants than for wood fibers (Zhai & Lee, 1989). As it is easier to remove lignin from middle lamella (being amorphous and more porous) than S2 layer so lignin is mostly removed in the initial phase for annual plants. On the other hand, for wood species most of the lignin is in S2 layer so very little amount is removed in the initial phase but most of the lignin is removed in the bulk phase. Also, the structure of annual fibers is more porous than that of wood species and these characteristics may explain the faster delignification rate during the initial stage of cooking of annual fibers (Huang, et al., 2007).

The delignification indicated in Figures 4.3-4.5 by dotted lines is called bulk delignification. It follows pseudo first order kinetics as seen by the straight-line relationship between ln[lignin] and pulping time. At 135°C the

bulk phase extends to 90 minutes, while at higher temperatures (145 and 155°C) it lasts shorter before transition to the residual phase of delignification which is characterized by a slower delignification rate. The residual phase for SEW and SPW starts when the lignin content based on original SCS reaches 1.5%, while for SMW it starts at about 3% lignin. At 145 and 155°C the residual phase begins after 60 and 40 minutes of cooking (includes 10 min heat-up time) both for SEW and SPW. The onset of the residual delignification phase for SMW occurs after 60 and 50 minutes at 145 and 155°C, respectively. Iakovlev et al. (2011b) showed that for beech and spruce the SEW residual delignification phase appears when the lignin content decreases below 1% (on original wood), while the threshold for wheat straw is around 2.3% (kappa 30), the latter value without correcting for lipid/protein content. The onset of the residual phase at higher lignin content in SCS is not clear, but several differences between lignin structure in annual plants and wood could be responsible for the effect.

The amount of lignin at the onset of residual delignification phase is higher for SMW than SEW and SPW (see Figure 4.3, 4.5 and 4.6). The higher amount of lignin at the onset of residual delignification for SMW may be explained by the lower solubility of lignin in 50% methanol compared to that in 50% ethanol and isopropanol based on their solubility parameters (δ values). Puumala (Puumala, 1991) also reported that ethanol provides better delignification than methanol. We also observed that SMW pulps were darker in color than corresponding SEW and SPW pulps indicating their high lignin content and possibly more condensation because of less solubility in methanol.

Bulk delignification rate constants were determined from the slopes of the straight lines in Figure 4.3, 4.4 and 4.5 are shown in Table 4.2. The delignification rate constants for SPW are highest followed by SEW and SMW at each temperature. The rate constants for each solvent system increases with increase in temperature. A 20°C increase in temperature (135°C to 155°C) results in 1.8, 2.3 and 2.4 times increase in delignification rate for SMW, SEW and SPW respectively. The maximum rate constant value achieved was 46.7±2.8 x 10^{-3} min^{-1} at 155°C for SPW delignification.

Table 4.2: Bulk delignification rate constants and activation energies

	Rate Constant k_{Lig} x 10^3 (min^{-1})			**Activation Energy (kJ mol^{-1})**
	135°C	**145°C**	**155°C**	
SMW	12.6±0.1	19.0±0.0	23.3±0.2	44.7±1.4
SEW	18.5±0.2	30.7±0.9	42.5±0.0	60.5±0.7
SPW	19.0±0.4	33.6±0.3	46.5±2.8	65.1±1.3

At similar pulping conditions Iakovlev et al. (2011a, p. 60) reported bulk delignification rate constants for three biomass species (at 135°C 30.9 x 10^{-3} min^{-1} for spruce, 39.9 x 10^{-3} min^{-1} for beech and 31.1 x 10^{-3} min^{-1} for wheat straw; and at 145°C and 155 °C the values are 64 x10^{-3} min^{-1} and 135 x10^{-3} min^{-1} for spruce respectively. At 135°C the present SEW rate constants for SCS are a factor 1.7 lower than those of spruce and wheat straw while at 155 °C the difference increases to a factor of 3.2. The low delignification rate constants in SAW pulping of SCS might be related to the high ash content in SCS (Table 3.1) which lowers the acidity of the pulping system.

The bulk delignification rate constants of the pulping solvents follow the sequence SPW > SEW > SMW. This sequence agrees with the lignin dissolution properties of cooking liquor of SPW > SEW > SMW. The lignin dissolution capacity of the cooking liquors may be estimated from the Hildebrand solubility parameter (δ) given by following equation (Schuerch, 1952):

$$\delta = \sqrt{\left[\frac{(\Delta H - RT)\rho}{MW}\right]} \qquad (3)$$

where ΔH is heat of vaporization in J mol^{-1}, T is normal boiling point in Kelvin, MW is molecular weight in kg mol^{-1} and ρ is density in kg m^{-3}. Based on the above equation the δ-values of fresh SMW, SEW and SPW cooking solvents were calculated as 37.03, 36.46 and 34.60 $(MPa)^{1/2}$ respectively. The presence of SO_2 in the cooking liquor had an insignificant effect on the δ-value. The δ-value of lignin can be determined based on knowledge of the atomic and functional group contributions in the phenylpropane unit. δ-values of 27.73, 29.12 and 36.49 $(MPa)^{1/2}$ were obtained for typical structures of G, S and H phenylpropane units respectively (Figure 4.6). Thus, the weighted average solubility parameter of SCS lignin based on G:S:H of 68:28:4 (del Río, et al., 2015) was 28.47 $(MPa)^{1/2}$. According to the Hildebrand solubility parameter theory the closer the δ-values of the polymer and solvent are, the greater will be the polymer solubility in that solvent (Hildebrand & Scott, 1950). By comparing δ-values of the cooking liquors with δ-value of SCS lignin (28.47), it can be concluded that the SPW liquor is the best SCS lignin solvent, while SMW cooking liquor is the poorest solvent. It also shows that the H lignin units have a poorer solubility in the cooking liquors than the G and S units.

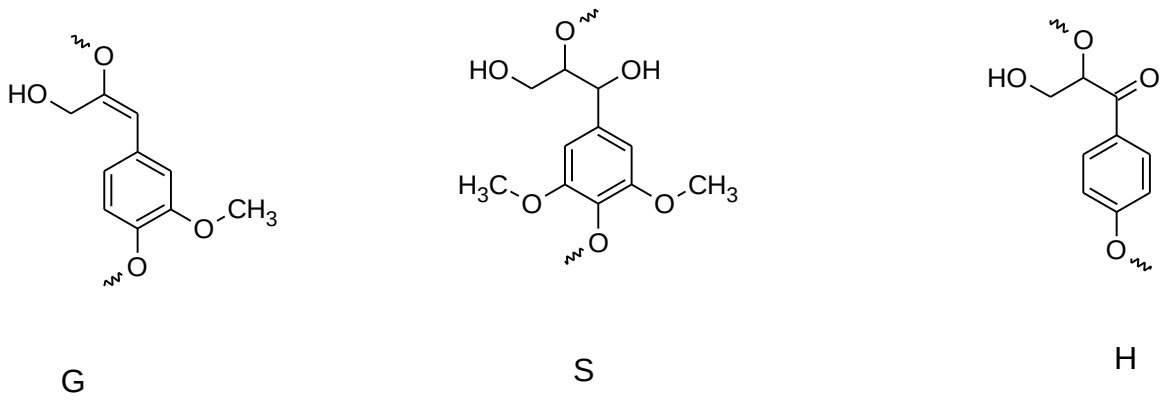

Figure 4.6: Typical structures of lignin units

Rate constants at 135°C, 145°C and 155°C from Table 4.2 were used to determine activation energies using the Arrhenius plot shown in Figure 4.7. The activation energy values for SAW delignification (see Table 4.2) are much lower than those for SEW pulping of wood species like spruce (108 kJ mol^{-1}) and beech (102 kJ mol^{-1}) (Iakovlev, et al., 2011b). Many other researchers have also found low activation energies of delignification for different straws at different pulping conditions compared to those for wood species (Huang, et al., 2007; Huang & Shi, 1986; El-Sakhawy, et al., 1996; Huang, et al., 2006; Ho, et al., 2011). The lower activation energies may be explained by the high porosity of SCS where lignin is more easily accessible as compared to hardwood and softwood species.

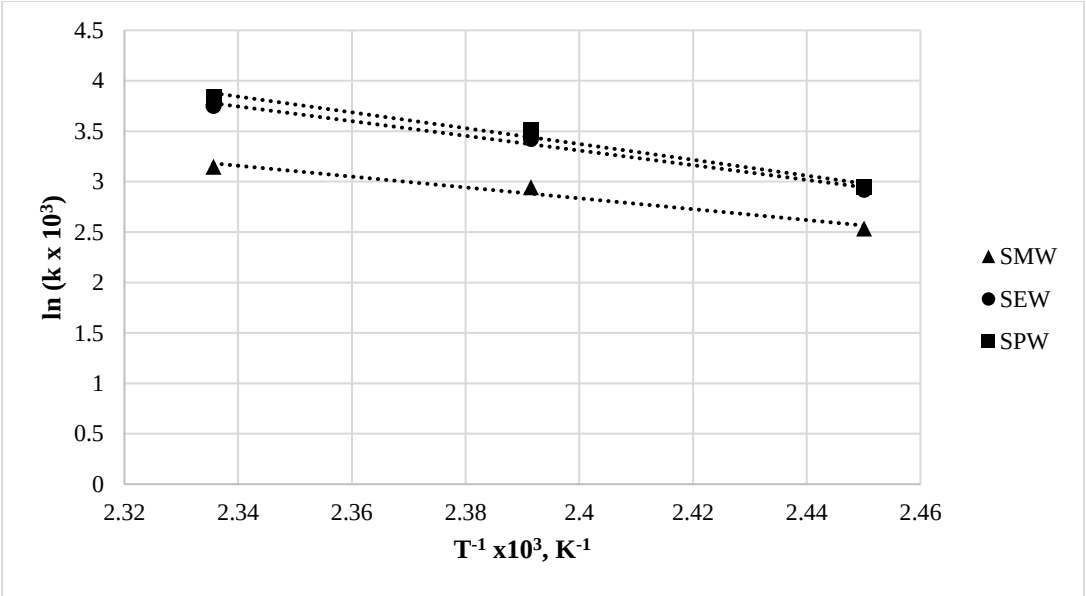

Figure 4.7: Arrhenius plot (delignification kinetics) for SMW, SEW and SPW

4.4 Xylan removal kinetics

Xylan is the most abundant hemicellulose in SCS constituting nearly 22% of its total weight (Table 3.1). Galactan and mannan are present in very small amount, 0.9 and 0.6% respectively. SCS also contains 3% arabinan, but these units are quickly removed during the initial cooking phase because of high reactivity of furanosidic bonds in an acidic environment (Shafizadeh, 1963). Iakovlev (2011a, p. 72) shows that 70 and 100% dissolution of arabinan takes place during heat-up period at 135 and 165°C respectively in SEW pulping. Therefore, we addressed in our study only the kinetics of xylan removal.

Two phases of xylan removal were observed in SMW, SEW and SPW pulping of SCS, initial xylan dissolution followed by first order xylan removal, as can be seen in Figures 4.8, 4.9 and 4.10 respectively. By comparison of the xylan content obtained by extrapolation to the heat-up time at 10 minute with the xylan content in fresh SCS, it can be seen that about 50% of xylan is removed in the first phase called "initial phase". You et al. (2016) reported that 2/3 of SCS xylan was removed in the initial phase. The higher percentage xylan removal by You et al. (2016) can be explained by the more acidic environment used due to a lower ethanol concentration with SO_2/ethanol/water of 12/22.5/65.5 (w/w%). As 50-60% of the SCS lignin was also removed during the initial phase, it may be suggested that most of the xylan and lignin were removed covalently bound to each other as lignin-carbohydrate complex (LCC). The xylan content of the solid residue was calculated by considering the uronic anhydride (UA) content just as is done for hardwoods (Genco, et al., 1990) as:

$$Xylan = Xylose\left(\frac{132}{150}\right) + UA\ (0.6)\left(\frac{132}{176}\right) \tag{4}$$

Because xylan dissolution occurs after acid hydrolysis following SN_1 substitution, the kinetics of xylan can be described by following equation (Iakovlev, 2011a):

$$-\frac{d[Xyl]}{dt} = k'_{Xyl}\ (T)\ [Xyl]^a\ [H_3O^+]^b = \ k_{Xyl}\ (T)\ [Xyl] \tag{5}$$

where [Xyl] is xylan content of pulp (% SCS); k'_{Xyl} and k_{Xyl} real and composite rate constants for xylan removal; $[H_3O^+]$ hydronium ion concentration; T is reaction temperature; a and b are reaction orders in xylan and hydronium ion concentration.

When the hydronium concentration is constant, and the reaction is first order in xylan, the above formula implies a linear relationship between the natural logarithm of [Xyl] (%SCS) and cooking duration. This is seen for SMW, SEW and SPW pulps in Figure 4.8, 4.9 and 4.10 respectively.

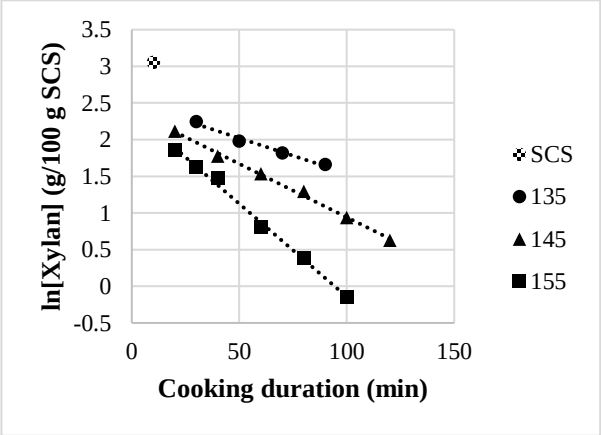

Figure 4.8: Natural logarithm of residual xylan vs cooking time for SMW pulping of SCS

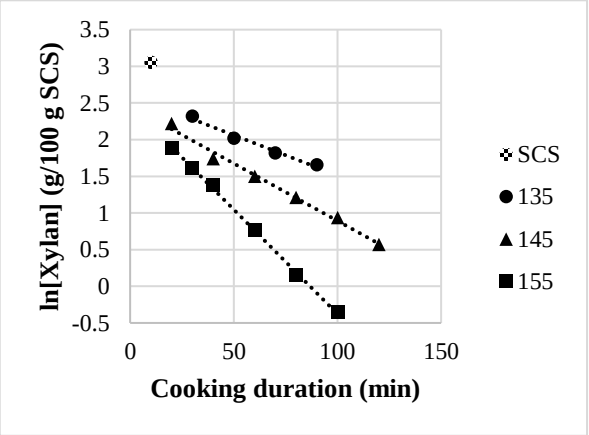

Figure 4.9: Natural logarithm of residual xylan vs cooking time for SEW pulping of SCS

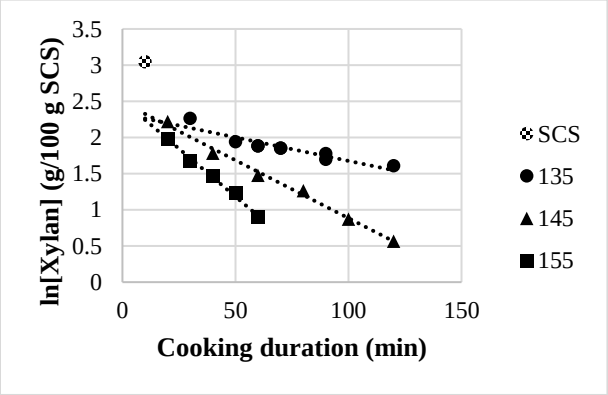

Figure 4.10: Natural logarithm of residual xylan vs cooking time for SPW pulping of SCS

The rate of xylan removal in the 2nd phase called bulk phase is indicated by straight dotted lines in Figures 4.8-4.10. Pseudo first order rate constants determined from their slopes are listed in Table 4.3. All the rate constants are nearly identical, indicating the same xylan removal rate for the three solvent systems at 145˚C. This implies that the hydronium ion concentration in each pulping system is nearly the same, as was confirmed experimentally with

spent liquors pH values ranging from 0.9 to 1.2. The maximum difference in pH values at similar pulping conditions was only 0.05 units. The present rate constant value at 135°C for SEW of 10.9 x 10^{-3} min^{-1} agrees with that of 11.6 x 10^{-3} min^{-1} determined by Iakovlev et al. (2011b) for SEW pulping of beech at the same temperature and fresh cooking liquor composition. This confirms the similar nature of xylan in SCS and hardwood species. Rate constant values for xylan removal were reported by You et al. (2016) for SEW pulping of SCS at a lower ethanol content (12:22.5:65.5 for S:E:W). The authors reported 8.74 x 10^{-3} min^{-1}, 20.5 x 10^{-3} min^{-1} and 39.8 x 10^{-3} min^{-1} xylan removal rate constants at 135°C, 145°C and 155°C respectively. The higher rate constants at the two higher temperatures may be related to the higher effective acidity of the cooking liquor in the You et al. (2016) study. It indicates that type of alcohol used in SAW fractionation and its concentration change the proton activity and hence the hydrolysis rate. This will be addressed further below.

Table 4.3: Xylan removal (bulk phase) rate constants with activation energy data

	Rate Constant k_{xyl} x 10^3 (min^{-1})			**Activation Energy (kJ mol^{-1})**
	135°C	**145°C**	**155°C**	
SMW	9.6±0.2	14.6±0.1	25.4±0.3	70.6±0.7
SEW	10.9±0.2	15.6±0.2	28.5±0.3	69.6±1.7
SPW	6.5±0.8	16.0±0.2	26.5±0.3	102.3±2.2

The rate constants increase with temperature, and were used to determine the activation energy through an Arrhenius plot seen in Figure 4.11. The activation energies based on Arrhenius plot are listed in Table 4.3 with rate constants. It can be seen from Table 4.3 that activation energy values of SMW and SEW are nearly same (70 kJ mol^{-1}). However, the SPW pulping system has a significantly higher activation energy of xylan removal (102 kJ mol^{-1}). Alcoholysis reactions are anticipated based on this activation energy data. Iakovlev et al. (2011a, p. 79) reported an activation energy of 110 kJ mol^{-1} for xylan removal from spruce in SEW pulping. No activation energy data of xylan removal from beech and wheat straw was reported in that study. The high activation energy for spruce species may be related to the fact that softwood xylan contains twice the amount of 4-O-methylglucuronic acid (4-OMGA) units per xylose unit than that in hardwood xylan (Sjöström, 1981, pp. 61-63) and contains arabinose side chains (Fengel & Wegener, 1984). It is known that the presence of 4-OMGA partially stabilizes against xylan hydrolysis (Meier, 1962) at lower temperatures. You et al. (2016) reported a high activation energy of 106.6 kJ mol^{-1} for SEW pulping of SCS (plotted in Figure 4.11). The low activation energy for SEW (69.6 kJ mol^{-1}) in our case is related to the high ethanol concentration (22.5% vs 44%) which affects the effective acidity of the pulping system and thus reaction rate. The

rate increase is higher using comparatively less polar solvent (isopropanol). Therefore, higher xylan removal rate is anticipated with isopropanol if same concentration is employed as You et al. (2016). Similar effects are seen for cellulose hydrolysis in the next section.

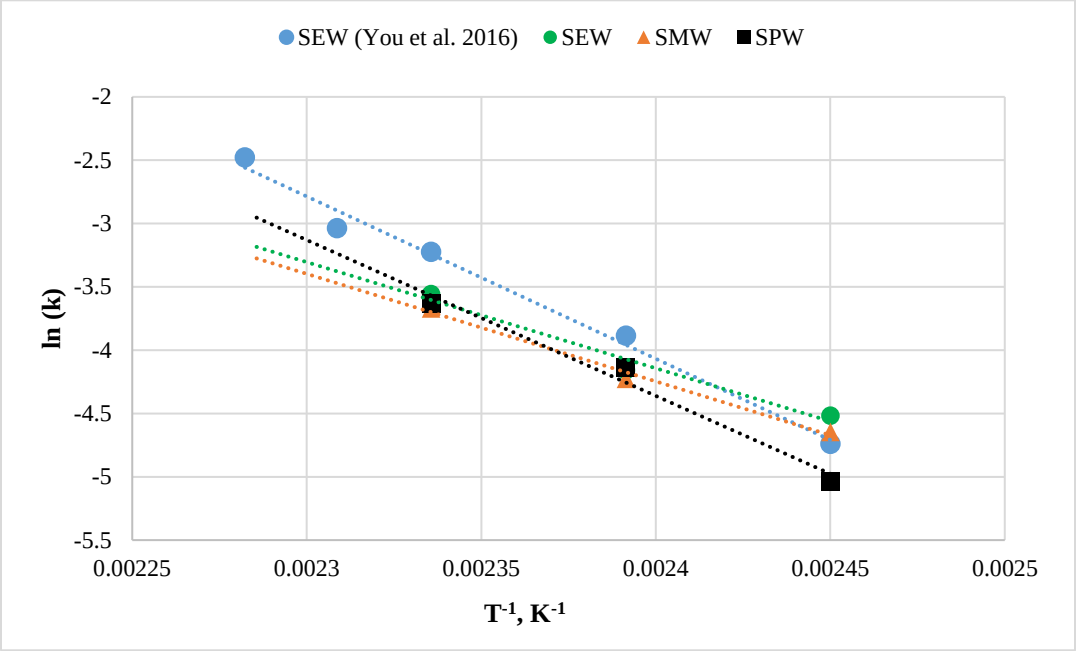

Figure 4.11: Arrhenius plot (xylan removal kinetics) for SMW, SEW and SPW

The ratio of the reaction rate constants for delignification and xylan removal are listed in Table 4.4. It shows that the delignification/xylose removal selectivity is highest for the SPW pulping system, and that the highest selectivity and thus pulp yield at the same lignin content or kappa number can be obtained at low temperatures. This agrees with the finding of Iakovlev et al. (2011a, pp. 76-80).

Table 4.4: Delignification selectivity over xylan removal

	k_{Lig}/k_{Xyl}		
	135°C	**145°C**	**155°C**
SMW	1.3	1.3	0.9
SEW	1.7	2.0	1.5
SPW	2.9	2.1	1.8

4.5 Cellulose hydrolysis kinetics

Cellulose DP was calculated by measuring the intrinsic viscosity of pulp in cupriethylenediamine (CED) solution. The viscosity average DP of cellulose is nearly equal to the weight average DP (Evans & Wallis, 1989). In the current study, we used the pulp viscosity ([η]), hemicellulose content ([Hemi]) and cellulose content ([Cel]) in the following equation to determine the cellulose DP (da Silva Perez & van Heininen, 2015).

$$DP_v = \left(\frac{1.65[\eta] - 116[Hemi]_{pulp}}{[Cel]_{pulp}}\right)^{1.111} \quad (6)$$

Kappa vs viscosity relationships for SMW, SEW and SPW pulps are shown in Figure 4.12, 4.13 and 4.14 at 135, 145 and 155 °C respectively. It can be observed that the intrinsic viscosity remained high (>800 mL g^{-1}) at 135°C down to a kappa number of 30 for SEW and SPW (see Figure 4.12). At 145°C in Figure 4.13, however, the viscosity drops rapidly for SMW pulping below kappa 45, while the same occurs for SEW and SPW below kappa 25. At 155 °C these sharp drops occur at even higher kappa numbers of 50 and 30 respectively (see Figure 4.14). Hence at lower kappa numbers SMW pulp shows the lowest viscosity while for SEW and SPW the values are nearly equal. As xylan removal rate is about the same in three solvent systems (at 145°C), it indicates that cellulose hydrolysis is more sensitive to hemicellulose removal than delignification. Thus, SEW and/or SPW fractionation of SCS at 145˚C for 60 minutes can produce a paper grade quality pulp with 800-850 cP viscosity and kappa number of about 25 while SMW pulps have a much higher lignin content in this viscosity range.

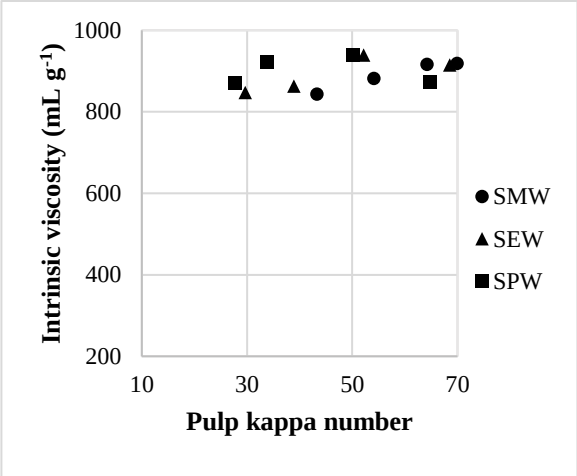

Figure 4.12: Methanol (SMW), ethanol (SEW) and isopropanol (SPW) pulps viscosity at 135°C

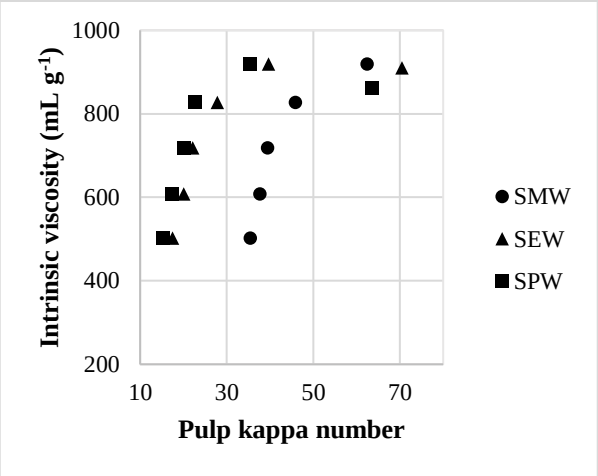

Figure 4.13: Methanol (SMW), ethanol (SEW) and isopropanol (SPW) pulps viscosity at 145°C

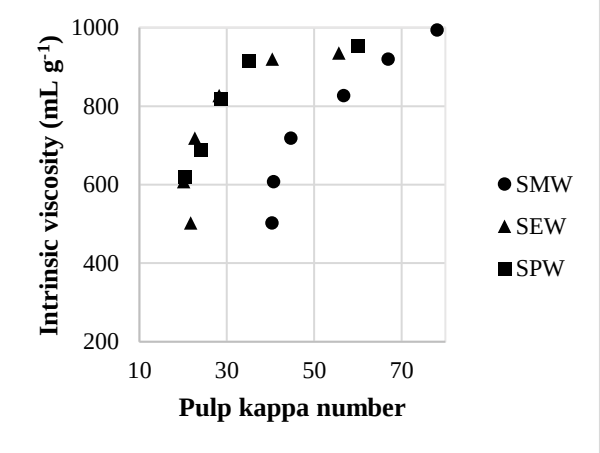

Figure 4.14: Methanol (SMW), ethanol (SEW) and isopropanol (SPW) pulps viscosity at 155°C

The inverse of DP was plotted against cooking time to evaluate the rate of cellulose hydrolysis in the three different fractionation systems (see Figure 4.15, 4.16 and 4.17). The linear relationship between DP^{-1} and time shows that cellulose hydrolysis is zero order in cellulose and obeys the following equation under the assumption of constant hydronium ion concentration.

$$\frac{1}{DP} - \frac{1}{DP_0} = k'_{Cel}\,(T)\;\;[H_3\,O^+]t = \;k_{Cel}\,(T)\,t \qquad (7)$$

Rate constants for cellulose hydrolysis were calculated from the slopes of the straight lines and are shown in Table 4.5. The rate constants for SEW pulping are 1.14 x 10^{-6} min^{-1}, 2.99 x 10^{-6} min^{-1} and 3.84 x 10^{-6} min^{-1} at 135°C, 145°C and 155°C respectively (see Table 4.5). The corresponding rate constants reported in You et al. (2016) for SCS at a lower ethanol concentration than in the present study (22.5% vs 44%) are 1.59 x 10^{-6} min^{-1}, 5.86 x 10^{-6} min^{-1} and 17.0 x 10^{-6} min^{-1} respectively, i.e cellulose hydrolysis rate constants which are 1.4, 2.0 and 4.4 times higher respectively. Again this shows that the effective acidity is higher at lower ethanol concentrations, and that effect becomes more pronounced at higher temperatures. Iakovlev et al. (2011a, p. 86) who employed SEW liquor of essentially the same composition as the present study (12:43.5:44.5 vs 12:44:44) for pulping of spruce and beech, found rate constants of 1.51 x 10^{-6} min^{-1} (135°C), 4.76 x 10^{-6} min^{-1} (145°C) and 15.4 x 10^{-6} min^{-1} (155°C) for spruce and 1.04 x 10^{-6} min^{-1} (125°C), 2.75 x 10^{-6} min^{-1} (135°C) and 7.80 x 10^{-6} min^{-1} (145°C) for beech.. The mostly lower rate constants in the present SCS study compared to those obtained for wood species may be related to the presence of a significant amount of ash in SCS (Table 3.1) which decreases the effective acidity of the pulping system.

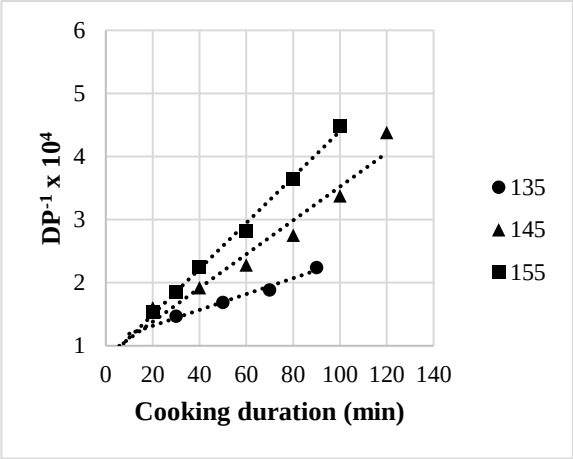

Figure 4.15: Cellulose hydrolysis kinetics in SMW pulping system

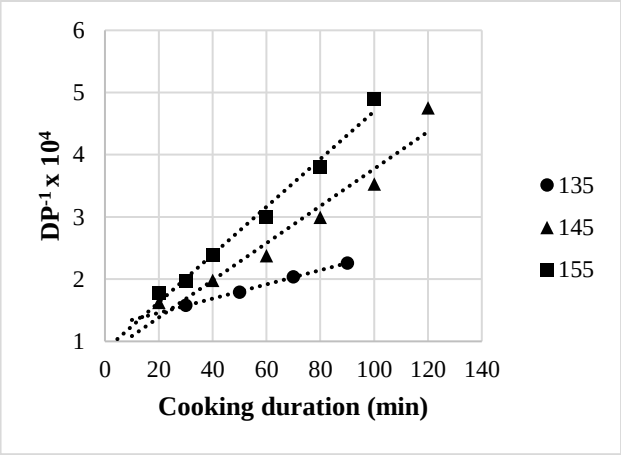

Figure 4.16: Cellulose hydrolysis kinetics in SEW pulping system

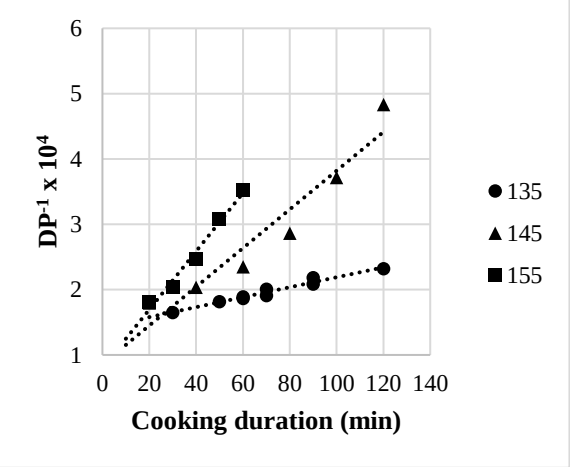

Figure 4.17: Cellulose hydrolysis kinetics in SPW pulping system

The Arrhenius equation was applied to determine the sensitivity of the rate constants to reaction temperature (Figure 4.18). Activation energy of cellulose hydrolysis for SMW, SEW and SPW were found to be 77±4, 88±8 and 130±10 kJ mol^{-1} respectively (Table 4.5). These activation energies are similar to many other agricultural materials used for pulping however much lower than for wood species (160-180 kJ mol^{-1}) reported in the literature.

Table 4.5: Rate constant and activation energy values for cellulose hydrolysis

	Rate Constant k_{Cel} x 10^6 (min^{-1})			Activation Energy (kJ/mol)
	135°C	**145°C**	**155°C**	
SMW	1.26±0.03	2.68±0.13	3.64±0.04	77±4
SEW	1.14±0.01	2.99±0.15	3.84±0.08	88±8
SPW	0.76±0.03	2.96±0.24	4.46±0.09	130±10

Generally, the low activation energies of SCS cellulose hydrolysis may be related to differences in the physical structure of biomass; i.e. degree of polymerization and crystallinity. Crystallinity values have been reported which vary from 73% for cotton linter to 23% for corn maize (Aberuagba, 1997; Ghose, 1977). Millett et al. (1954) shows that wood cellulose hydrolyzes twice as quickly as cotton cellulose. Low activation energies of cellulose hydrolysis for agricultural biomass have been reported in literature which range from 34 kJ mol^{-1} for rice husk (Ajani, et al., 2011) to 80.34 kJ mol^{-1} for corn cobs (Eken-Saracoglu, et al., 1998). For SEW pulping of spruce (Iakovlev, et al., 2009) and beech (Iakovlev, et al., 2011b) activation energy values of 166 kJ mol^{-1} and 135 kJ mol^{-1} were reported respectively. You et al. (2016) also found an activation energy of 166 kJ mol^{-1} for SCS cellulose hydrolysis. Since the ethanol concentration in our cooking liquor (SEW) is 44% (w/w), which is almost twice that of You et al. (2016) (22.5%), it appears that doubling the ethanol concentration reduces the activation energy for cellulose hydrolysis by about half (Figure 4.18).

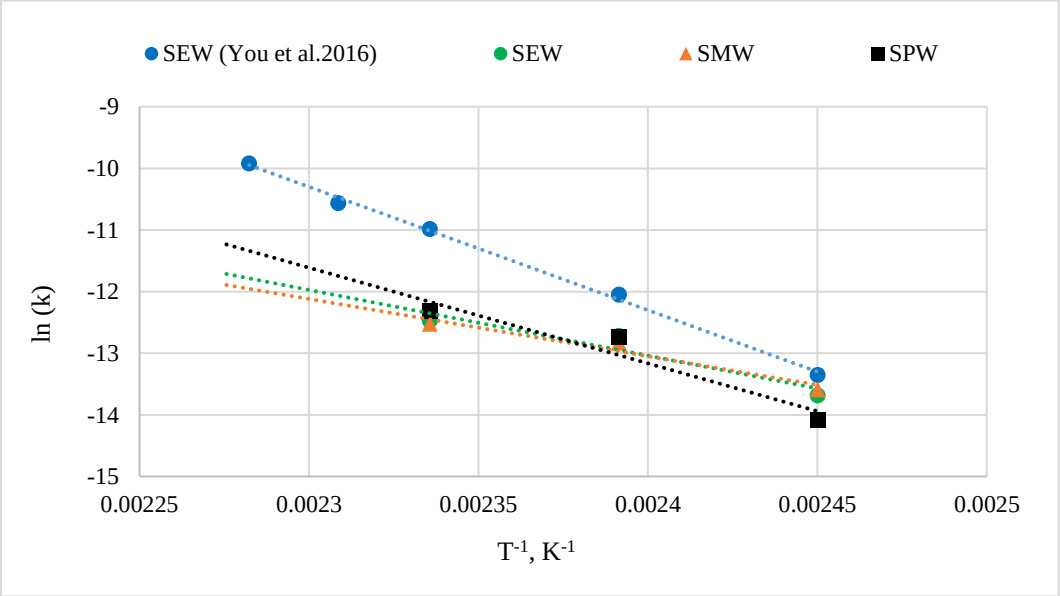

Figure 4.18: Arrhenius plot (cellulose hydrolysis kinetics) for SMW, SEW and SPW

SPW pulping has a higher activation energy for cellulose hydrolysis compared to SMW and SEW (Figure 4.18 and Table 4.5). This may be explained in terms of the effect of solvent on the proton activity which is responsible for the cellulose hydrolysis. Earlier it was reported (Philipp, et al., 1979; Lai, 2000) that the addition of ethanol, n-propanol or methylethylketone (MEK) to water increases the cellulose degradation by decreasing the proton hydration or cellulose swelling. Similarly, it may be expected that the proton hydration becomes weaker employing less polar solvents. As isopropanol is less polar in comparison to methanol and ethanol, it results in high rate increase in cellulose hydrolysis with increasing temperature (Figure 4.18). The hydrolysis rate increase is about same for methanol and ethanol. Comparing these results with You et al. (You, et al., 2016) indicates that ethanol effects are more pronounced at high temperatures. Therefore, two factor are responsible for cellulose hydrolysis: 1. Higher alcohol/water ratio decreases the hydrolysis rate due to decrease in effective acidity (You, et al., 2016); 2. Less polar solvent (i.e. isopropanol) increases the rate faster because of less swelling properties/increasing proton activity. This suggests that effective acidity increase with temperature is higher for less polar solvents.

The delignification selectivity expressed as the ratio of the delignification and cellulose degradation rate constants, k_{Lig}/k_{Cel}, is listed in Table 4.6. It shows that pulping with isopropanol in the SAW process at low temperature provides the highest selectivity among the three employed solvents.

Table 4.6: Delignification selectivity over cellulose hydrolysis

	(k_{Lig}/k_{Cel}) x 10^3		
	135°C	**145°C**	**155°C**
SMW	10.0	7.1	6.4
SEW	16.2	10.3	11.1
SPW	25.0	11.4	10.4

4.6 Conclusions

SCS was fractionated into cellulose, hemicellulose and lignin by the SAW process using methanol, ethanol or isopropanol. Delignification kinetics for SCS were developed for the first time by correcting Klason lignin for ash (mostly silica) and permanganate non-oxidizable material (proteins identified as one of the contributing factors). In terms of delignification, SPW is the most effective pulping system because of its highest lignin dissolution capacity, while SMW has the lowest delignification rate. The low activation energy values for SCS relative to wood species indicate the ease of delignification for highly porous agricultural residues such as SCS. The residual delignification

phase for SEW and SPW starts when the lignin content reaches about 1.5% (on original SCS), while for SMW it starts at about 3%. This compares to less than 1% for beech and spruce using SEW fractionation. Thus, the residual lignin content of SAW produced SCS pulp is relatively high compared to that of SEW wood pulp. The rate increase of xylan removal and cellulose hydrolysis is highest for SPW fractionation system. The xylan removal and cellulose hydrolysis rate is nearly the same for all three solvent systems at 145°C, but the activation energy is highest for the SPW pulping system. It indicates that proton activity increase with temperature is comparatively higher for less polar solvent (i.e. SPW) in SCS fractionation. Also, alcoholysis reactions in SAW fractionation are anticipated based on different activation energy values. Cellulose hydrolysis is more affected by hemicellulose removal than lignin removal. It is possible to produce a paper grade quality SCS pulp of 800-850 cP viscosity and kappa number of about 25 using SEW or SPW pulping at 145 °C for 60 minutes, while SMW pulps have a much higher lignin content in this viscosity range.

Chapter 5

CARBOHYDRATES IN SPENT LIQUORS AND SUGARS MASS BALANCE

5.1 Introduction

Spent liquors from SAW fractionation of SCS were obtained by manually squeezing the fractionated biomass in a nylon bag (75 mesh size). No more than 70% (based on volume of initially charged cooing liquor) spent liquor could be squeezed out. Spent liquors from SAW fractionation of SCS using methanol, ethanol or isopropanol were designated as SMW, SEW and SPW respectively. The effect of fractionation time and temperature on spent liquor composition will described in this chapter for the three fractionation systems.

The spent liquors were analyzed for total sugars, monosaccharides and sugar degradation products. The monomeric sugars in the fresh spent liquors were determined using HPAEC-PAD with fucose as internal standard. To prepare samples for HPAEC injection, fresh spent liquor (1 mL) was treated with NaOH (0.1 M, 10 mL) and then diluted with Milli-Q water (dilution factor = 400). To determine the total carbohydrates, spent liquors were first subjected to rotary evaporation at 40°C. Rotary evaporation was done by adding acetone (1 mL) to fresh liquor (1 mL) and then evaporating the mixture to dryness. The samples were then treated with 72% H_2SO_4 (1.5 mL) at 30˚C for one hour under continuous stirring. The hydrolyzed samples were then diluted by adding DI water (43 mL) and subjected to a 2^{nd} hydrolysis step at 121˚C for 1 hour. After the two step hydrolysis procedure the samples were centrifuged, filtered through a 0.45µm filter and finally analyzed by HPAEC-PAD using fucose as an internal standard, and reported as anhydrous sugars as originally present in SCS.

Fresh and hydrolyzed spent liquors were also analyzed for sugars degradation components (HMF and furfural) for mass balance calculations. The fresh/hydrolyzed spent liquors were diluted with deionized water (dilution factor = 42) and then analyzed on HPLC using a refractive index detector and BIO-RAD Aminex HPX-87K column. The mobile phase used was H_2SO_4 (5 mM, 0.6 mL min^{-1}) and the oven temperature was 45ºC.

5.2 pH of spent liquors

The acidity of biomass fractionation systems was indirectly estimated from the pH of spent liquors. An Oakton pH meter RS-232 with recorder output was used to determine pH at room temperature. Table 5.1 shows the pH of spent liquors from SMW, SEW and SPW fractionation of SCS at different temperatures and cooking durations. The pH of spent liquors is measured at room temperature and it does not provide information about the true acidity at fractionation temperatures (135, 145 and 155˚C). However, this pH data is important for relative analysis of three solvent systems. The pH decreases with increasing time from an initial value of about 1.2 to about 0.9 at the highest temperature and time. However, the pH values of all spent liquors are very close at the same fractionation conditions (differences of generally less than 0.1 unit). These small differences suggest the same level of acidity and hence carbohydrates hydrolysis in the SEW, SMW and SPW fractionation systems. However, high rate increase was seen for SPW fractionation system (Chapter 4) indicating increased proton activity with temperature.

Table 5.1: pH of SMW, SEW and SPW spent liquors at different fractionation conditions

Temperature	Time (min)	SMW	SEW	SPW
135˚C	30	1.20	1.20	1.22
	50	1.21	1.17	1.16
	70	1.14	1.13	1.08
	90	1.03	1.13	1.06
145˚C	20	1.04	1.23	1.16
	40	1.03	1.13	1.01
	60	1.01	1.10	0.98
	80	1.00	1.07	0.94
	100	1.00	1.04	0.93
	120	0.98	1.00	0.89
155˚C	20	1.06	1.10	1.20
	30	1.05	1.04	1.10
	40	1.06	0.99	1.04
	60	1.00	0.97	0.96
	80	0.97	0.92	-
	100	0.93	0.90	-

Note: pH is measured at room temperature

Hydrolysis of biomass carbohydrates (cellulose and hemicellulose) breaks down long chain molecules into short length oligomers and finally monomers. Hemicelluloses with a low degree of polymerization (DP) are more easily to hydrolyze and dissolve in water as compared to highly crystalline and high DP cellulose. The extent of hydrolysis is highly dependent on the acidity or more specifically effective acidity of the system which apparently increases with temperature for SMW, SEW and SPW fractionation systems as indicated by the pH values.

As will be shown in Chapter 6, the monomeric sugars in the spent liquors also react with the alcohols at the pulping conditions to form in particular alkyl/alcohol xylosides. Therefore, the spent liquors were also analyzed for alkyl furanosides and pyranosides (for details on the identification and quantification of these compounds, see chapter 6).

5.3 Carbohydrates in spent liquors

5.3.1 Combined severity factor (CSF)

Spent liquors were obtained from SCS fractionation at different temperatures (135-155°C) and varying cooking duration (20-120 minutes). To reduce the number of independent parameters and compare the three fractionation systems on the same basis a composite parameter called "Combined Severity Factor" (CSF) was introduced. The CSF is an empirical correlation that describes the severity of a reaction systems as a combined effect of temperature T (°C), cooking duration t (min) and pH. This so-called CSF parameter for SMW, SEW and SPW fractionation systems was calculated using the severity factor defined by (Overend & Chornet, 1987; Garrote, et al., 1999) which was modified to include the effect of pH (taken from Table 5.1);

$$CSF = \log\left\{t\,(\text{min}) * e^{\frac{T\,(°C)\,-100}{14.7}}\right\} - pH \qquad (8)$$

5.3.2 Monomeric and total sugars

Monomeric and total sugars in SEW spent liquors at different fractionation conditions are shown in Figure 5.1. The concentrations are strong function of fractionation temperature and duration. The monomeric and total dissolved sugars yields increase with temperature and cooking duration but decrease at severe conditions (145˚C, t>80 min and 155˚C, t>60 min). This decrease is related to further sugars conversion to HMF, furfural and humins. The same data for SEW spent liquors is replotted in Figure 5.2 using equation 8 in terms of CSF. It can be seen from Figure 5.2 that CSF approach works very well to describe monomeric and total dissolved sugars yield. Therefore, all three fractionation systems (SMW, SEW and SPW) can be compared on CSF basis. Following the same approach as for SEW spent liquor, the monomeric and dissolved sugars results for SMW and SPW are plotted in Figure 5.3 and 5.4 respectively. All the sugars were measured as monosaccharides but described here as anhydrous sugars on original SCS basis. Monomeric and total sugars in SEW, SMW and SPW spent liquors as a function of CSF are reported as anydrosugars in Figure 5.2, 5.3 and 5.4 respectively. The amount of monomeric and total sugars in each of the spent

liquor increases until CSF<= 2.4 and then decreases gradually. The highest amount of total sugars in SMW, SEW and SPW spent liquors at CSF=2.4±0.1 (average of two or three data points from varying fractionation conditions) is 25.9±0.5, 26.9±1.3 and 25.5±1.5 (based on original SCS) respectively. The corresponding monomeric sugars levels in SMW, SEW and SPW spent liquors are 9.2±0.3, 14.4±0.7 and 16.5±0.2 respectively. It shows that methanol, ethanol and isopropanol spent liquors contain nearly same amount of total sugars after two step hydrolysis, but SPW liquor has the most monomeric sugars in unhydrolyzed spent liquor, while SMW liquor has the lowest level of monomeric sugars among three spent liquors. The decrease in sugar concentration at higher severity factor is related to dehydration of pentose and hexoses as well as humins formation.

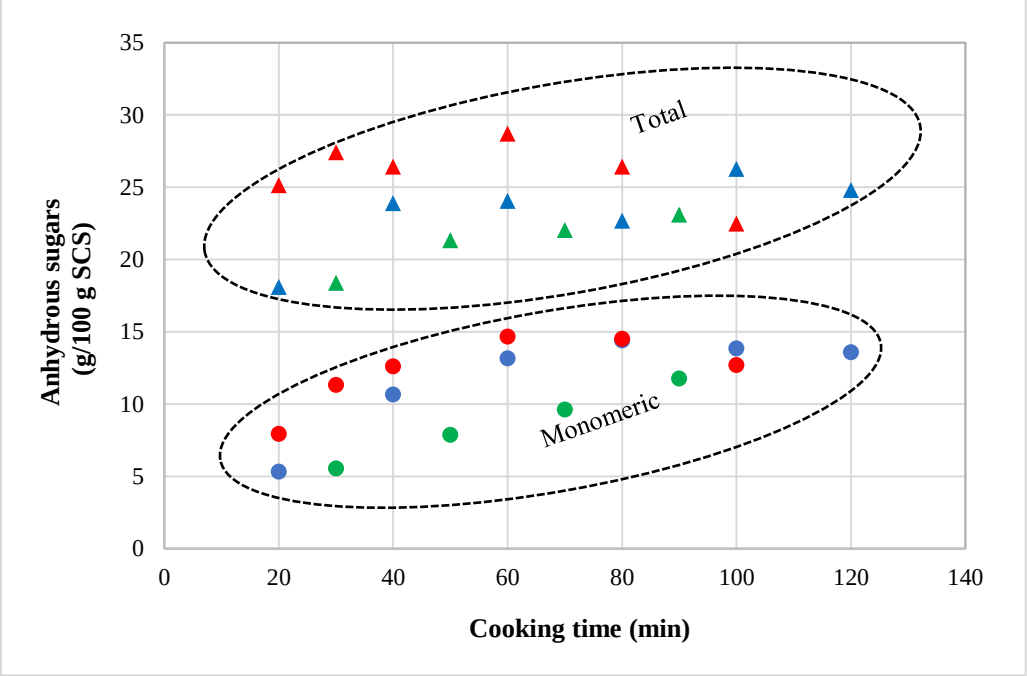

Figure 5.1: Monomeric and total dissolved sugars in SEW spent liquors as a function of temperature and time. Color coding shows different temperatures. Green (135°C), Blue (145°C) and Red (155°C)

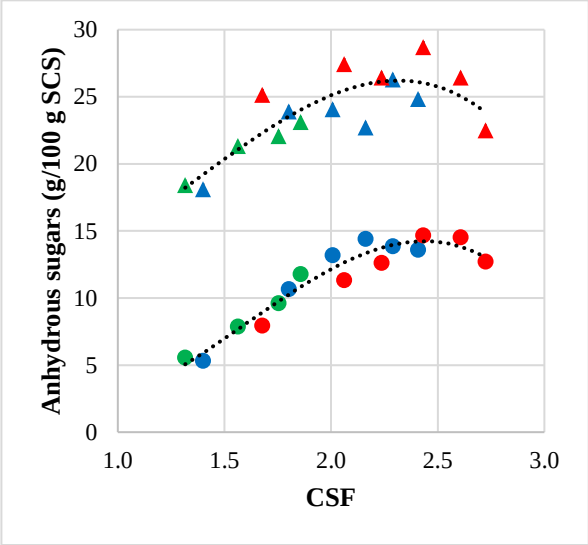

Figure 5.2: Monomeric and total sugars in SEW spent liquors

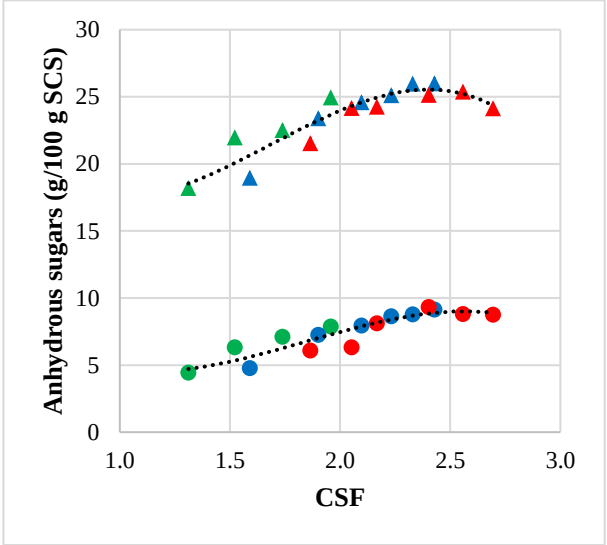

Figure 5.3: Monomeric and total sugars in SMW spent liquors

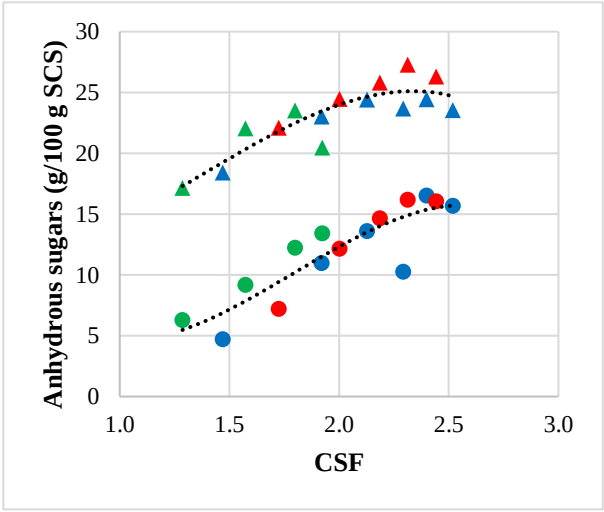

Figure 5.4: Monomeric and total sugars in SPW spent liquors

Concentrations of each individual sugar in spent liquor are determined at 2.4±0.1 CSF and shown in Table 5.2. The concentration of total sugars in each spent liquor is nearly the same at 26% on SCS or 60 g L^{-1}. Xylan is the most prominent sugar in these spent liquors at a level of approximately 16.5% on SCS or 38 g L^{-1}. The total concentration of glucan in each spent liquor is about 5.4% on SCS or 12 g L^{-1}, which is high considering that cellulose

is normally not significantly dissolved in the SEW process (Iakovlev & van Heiningen, 2012a). This implies that SCS likely still contains a significant amount of sucrose. Total galactose, mannose and arabinose are 0.9, 0.5 and 2.9% on SCS respectively which closely matches the corresponding original contents in SCS of 0.9, 0.6 and 3.0% (see Table 3.1). Thus, the hemicellulose side-groups arabinose and galactose are essentially fully cleaved and dissolved, while glucomannan is also mostly fully hydrolyzed and dissolved. The concentration of total monomeric sugars in the fresh SMW, SEW, and SPW cooking liquors is 20.8±0.7, 31.1±1.7 and 37.0±0.5 g L^{-1} respectively (Table 5.2). Hence monomeric sugars contribute 36, 54 and 64% to the total sugar content in the hydrolyzed SMW, SEW and SPW spent liquors respectively. Xylose is the major monomeric sugar in the fresh SMW, SEW and SPW spent liquors at 13.8±0.6, 21.3±1.6 and 25.2±0.3 g L^{-1} respectively. It can also be seen that the monomeric sugars concentrations in SEW are only slightly lower than that in the SPW spent liquor, while the monomeric sugars concentrations are significantly lower in the SMW spent liquor. The concentrations of sugars dehydration products (HMF and furfural) are extremely small (0.3-0.5 g L^{-1}). Therefore, sugars dehydration reactions are very unlikely at this fractionation condition for each solvent system. Sklavounos et al. (2013a) had similar observations in SEW fractionation of spruce (12% SO_2, 55 v/v% ethanol-water, L/W = 3:1, 150˚C, 30 min).

Table 5.2: Sugars and dehydration products concentrations in fresh & hydrolyzed SL at CSF=2.4±0.1

	%SCS			g L^{-1}		
Fresh SL	**SMW**	**SEW**	**SPW**	**SMW**	**SEW**	**SPW**
Ara	0.9±0.1	1.4±0.2	1.5±0.2	2.1±0.3	3.1±0.4	3.4±0.4
Gal	0.2±0.0	0.4±0.0	0.5±0.0	0.5±0.0	0.9±0.0	1.0±0.0
Glu	1.8±0.1	2.8±0.1	3.1±0.0	4.0±0.3	6.2±0.1	7.0±0.1
Xyl	6.1±0.2	9.5±0.7	11.2±0.1	13.8±0.6	21.3±1.6	25.2±0.3
Mann	0.2±0.1	0.3±0.1	0.2±0.0	0.4±0.1	0.6±0.2	0.4±0.0
Total	**9.2±0.3**	**14.4±0.7**	**16.5±0.2**	**20.8±0.7**	**31.1±1.7**	**37.0±0.5**
HMF	0.0±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.1	0.2±0.0
Furfural	0.2±0.1	0.2±0.1	0.1±0.0	0.5±0.2	0.5±0.3	0.3±0.1
Hydrolyzed SL						
Ara	2.9±0.2	2.9±0.4	2.8±0.4	6.5±0.4	6.6±0.8	6.3±0.8
Gal	0.8±0.1	0.9±0.2	0.9±0.2	1.8±0.1	2.0±0.4	2.0±0.5
Glu	5.4±0.1	5.7±0.1	5.0±0.2	12.2±0.3	12.8±0.2	11.2±0.3
Xyl	16.2±0.5	16.7±1.3	16.4±1.5	36.5±1.1	37.7±2.9	36.9±3.3
Mann	0.5±0.1	0.6±0.2	0.5±0.2	1.2±0.2	1.4±0.4	1.0±0.4
Total	**25.9±0.5**	**26.9±1.3**	**25.5±1.5**	**58.2±1.2**	**60.5±3.0**	**57.4±3.5**
HMF	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.1	0.1±0.0	0.1±0.1
Furfural	1.2±0.2	1.0±0.2	0.8±0.1	2.7±0.5	2.3±0.3	1.8±0.1

Note: Sugars in fresh and hydrolyzed SL are determined as monomeric sugars but reported as anhydrous sugars

The results above show that the total sugar concentrations after spent liquor hydrolysis are only a function of the severity of fractionation. This is understandable because the similar acidities in the three systems leads to similar

hemicellulose dissolution with increasing cooking severity as shown earlier in Chapter 4 (see section 4.4). However, the concentration of monomeric sugars in each spent liquor is dependent on the type of alcohol used for SAW cooking as well as the severity factor, with the monomeric sugars representing 36, 54 and 64% to the total sugar content in the hydrolyzed SMW, SEW and SPW spent liquors respectively. Earlier studies have also shown that the monomeric sugars concentration in spent liquor is only a fraction of the total sugars concentration. For example, Iakovlev and van Heiningen (2012a) showed that the monomeric fraction increased with the SO_2 concentration in cooking liquors of 3, 6 and 12% SO_2 from 10-25% to 35-45% and 45-50% at SEW fractionation of spruce at 135°C and 6 L kg^{-1}. In a similar study, 42.1% monosugars are reported (3% SO_2, 150°C, 120 min, 6 L Kg^{-1}) (Sklavounos, et al., 2011). In these studies, the difference between the unhydrolyzed monomeric and hydrolyzed total sugar concentration was identified as oligomeric (i.e. not fully hydrolyzed) sugars. Using the same concept, the present results may be interpreted that the oligomeric sugar fraction decreases in the order SMW>SEW>SPW. Since hydronium ions are responsible for oligomer hydrolysis these results could be interpreted that the effective acidity of the systems increase in the order SMW<SEW<SPW. The same concept is suggested based on literature (Lai, 2000; Mellmer, et al., 2014) where less polar solvents enhance the effective acidity for strong acids (like lignosulfonic acids) because of less shielding of the hydronium ion. Therefore it is more likely that the fraction of sugars is wrongly identified as oligomers but are actually monomeric sugars which have reacted with the solvent to form xylosides and glycosides, and that their yield decreases in the order of methanol>ethanol>isopropanol. This would explain the lower monomeric sugars content in SMW compared to SEW and finally SPW spent cooking liquors. Formation of glycosides and xylosides in acidic organosolv treatments have been earlier reported in literature (Grisel, et al., 2014; Zhang, et al., 2016). The presence of alkyl pyranosides (especially xylosides) in spent liquors from SAW fractionation systems was confirmed, and their quantification will be reported on in in Chapter 6.

Sugar mass balances are crucial for evaluation and practical implementation of fractionation processes. It also helps to quantify and compare the different carbohydrate reactions in the solvent systems. Sugars mass balances (as anhydrous sugars) for the SMW, SEW and SPW fractionation systems at CSF of 2.4±0.1 are shown in Table 5.3. Spent liquor sugars are measured as monomeric sugars but reported as anhydrous sugars using the following equations;

$$\text{Anhydro-pentose} = \text{Pentose} \times (132/150) \quad (9)$$

$$\text{Anhydro-hexose} = \text{Hexose} \times (162/180) \quad (10)$$

Furfural and HMF are also described as anhydro-pentose and anhydro-hexose respectively in sugars mass balance. Multiplication factors of 1.375 and 1.284 are used for furfural and HMF respectively to determine their equivalent amount of anhydrous pentose and hexose sugars. The sum of anhydrous sugars in pulps and spent liquors of SMW, SEW or SPW fractionation systems is equal to 61.0±0.7, 61.1±1.4 and 59.7±1.7 % on SCS respectively (Table 5.3). These numbers are very close to the total anhydrous sugars in the original SCS showing excellent sugars mass balance (considering standard deviations) for each of the fractionation system. The excellent mass balance of sugars shows that the presence of alcohols suppresses the formation of humins in SAW fractionation system. This is in agreement with sugars mass balance for SEW fractionation of softwood and hardwood species (Yamamoto, et al., 2014b; Iakovlev & van Heiningen, 2012a), forest biomass (Yamamoto, et al., 2011) and oil palm empty fruit bunch (Sklavounos, et al., 2013b) and spruce (Iakovlev & van Heiningen, 2012a; Sklavonous , et al., 2013a).

Table 5.3: Total sugars mass balance at CSF= 2.4±0.1

	SMW	**SEW**	**SPW**	
Pulp				**SCS**
Ara	0.0±0.0	0.0±0.0	0.0±0.0	3.0±0.2
Gal	0.0±0.0	0.0±0.0	0.0±0.0	0.9±0.1
Glu	31.1±0.1	30.4±0.3	30.6±0.8	35.7±0.4
Xyl	2.2±0.3	2.2±0.4	2.4±0.1	21.2±0.6
Man	0.1±0.0	0.1±0.0	0.1±0.0	0.6±0.2
Total in pulp	*33.4±0.3*	*32.7±0.5*	*33.1±0.8*	-
Spent Liquor				-
Ara	2.9±0.2	2.9±0.4	2.8±0.5	-
Gal	0.8±0.1	0.9±0.2	0.9±0.2	-
Glu	5.4±0.1	5.7±0.1	5.0±0.2	-
Xyl	16.2±0.5	16.7±1.3	16.4±1.6	-
Man	0.5±0.1	0.6±0.2	0.5±0.2	-
HMF[a]	0.0±0.0	0.1±0.0	0.0±0.0	-
Furfural[b]	1.7±0.3	1.4±0.2	1.1±0.1	-
Total in SL	*27.6±0.6*	*28.4±1.3*	*26.6±1.5*	-
Sum	**61.0±0.7**	**61.1±1.4**	**59.7±1.7**	**61.4±0.8**

CSF =2.4±0.1 refers to SCS fractionation at 145˚C, 100-120 min for SEW and SMW and 100 min for SPW, 4 L kg^{-1}, 12% SO_2 or 155˚C, 60 min, 4 L kg^{-1}, 12% SO_2
(a)-Anhydo-hexose, (b)-Anhydro-pentose

The total amount of arabinose, xylose, galactose, mannose, furfural and HMF in spent liquors (as anhydrous sugars, %SCS) represents the dissolution and dehydration of hemicelluloses in SAW fractionation of SCS. Table 5.3

shows that for SMW, SEW and SPW spent liquors this equals to 22.1±0.6, 22.5±1.4 and 21.7±1.7% respectively. The sum of these sugars in the original SCS produces a value of 25.7±0.6. Thus, most of the hemicellulose (>85%) are dissolved in SAW fractionation of SCS. All the three SAW pulps contain about 2.3% xylan and no arabinan indicating that arabinan units are completely cleaved from arabinoxylan of SCS. This is confirmed by arabinan units in SAW spent liquors that show 3.0% arabinan (with experimental error), an amount equals to that is in original SCS. Similarly, galactan units are also completely cleaved from galactoglucumannan while some mannan is found in the solid residues. SCS glucomannan contains 0.4% glucan (glucan = mannan/1.6) that is possibly removed with other hemicellulose units as indicated by high glucan in spent liquors (5.0-5.7%). Iakovlev (2011a, p. 85) also describes that in SEW fractionation of spruce (12% SO_2, 135-155˚C) all arabinan units are removed in 15-30 minutes of cooking. However, mannan and small amount of galactan are found in the solid residue due to their high initial content in spruce (2.19% galactan vs 1.8% mannan). Yamamoto et al. (2014b) shows 74% and 84% hemicellulose dissolution of HW and SW biomass in SEW fractionation (150˚C, L/F = 6 L kg^{-1}, S:E:W = 12:43.5:44.5, 20-30 minutes). In another study (Sklavounos, et al., 2013a), about 82 and 87% hemicellulose removal is also seen in SEW fractionation of spruce and softwood biomass respectively with no arabinan in the pulp (150˚C, L/F = 3 L kg^{-1}, SO_2 = 12%, ethanol/water = 55v/v%, 30 minutes). It can be concluded that in SAW fractionation arabinan is completely removed because of high reactivity of furanosidic bonds towards acid hydrolysis as indicated by Shafizadeh (1963). It is shown that cellulose in the SEW fractionation process is almost completely retained in the solid residue because of its high crystallinity and resistance towards acid hydrolysis (Iakovlev & van Heiningen, 2012a; Sklavounos, et al., 2013a; Yamamoto, et al., 2014b). However, in the present study a high decrease is seen in SCS glucan content (~5%) in the fractionation process. This decrease in glucan content is attributed to dissolution of sucrose from SCS.

The total amount of anhydrous sugars based on original SCS in SMW, SEW and SPW spent liquors is 27.6±0.6, 28.4±1.3 and 26.6±1.5 respectively. This account for nearly 45% of the total amount of sugars in original SCS. Iakovlev et al. (2012a) (spruce, 135˚C, 120 min, 6 L kg^{-1}, 12% SO_2) and Sklavounos et al. (2011) (spruce, 150˚ C, 120 min, 6 L kg^{-1}, 3% SO_2) describe that one third of spruce sugars are dissolved in SEW fractionation. High concentration of total sugars in the present study can be related to biomass structure and high severity conditions (145 ˚C, 120 min, 4 L kg^{-1}, 12% SO_2 or 155˚C, 60 min, 4 L kg^{-1}, 12% SO_2). The dissolution of sucrose, not present in wood species, is also a major factor of high % sugars in SCS spent liquors. In terms of total sugars distribution, SMW, SEW

and SPW spent liquors are very identical indicating negligible effect of employed alcohols on carbohydrates dissolution amount. Some pentose sugars are dehydrated to furfural in acid hydrolysis of spent liquors in sugars analysis. No significant amount of HMF is found in the hydrolyzed spent liquor indicating high resistance of hexose sugars towards acid hydrolysis.

5.4 Sugar degradation products

Furfural and HMF are two major sugar degradation products in acidic fractionation processes that affect the downstream processing of spent liquor for bioethanol production and other saccharification processes. The lowest inhibitory levels of furfural and HMF concentrations in SEW spent liquors specified for fermentation by C. acetobutylicum are 1.0 and 1.5 g L^{-1} respectively (Jurgens, et al., 2012). Furfural and HMF levels in spent liquors are shown in Table 5.2. In Table 5.2 fresh liquors refer to spent liquors from fractionation of SCS without any further hydrolysis, while hydrolyzed spent liquors are obtained after hydrolyzing (two step hydrolysis with H_2SO_4) rotary evaporated spent liquors (see section 5.1). The concentration of HMF in fresh SMW, SEW and SPW liquors are 0.1±0.0, 0.1±0.1 and 0.2±0.0 g L^{-1} respectively (Table 5.2). These results agree with previous results obtained for SEW fractionation of spruce (Iakovlev & van Heiningen, 2012a; Sklavounos, et al., 2011) and OPEFB (Sklavounos, et al., 2013b). The concentration of furfural in fresh SMW, SEW and SPW spent liquors is 0.5±0.2, 0.5±0.3 and 0.3±0.1 g L^{-1} (Table 5.2). It represents 0.2±0.1, 0.2±0.1 and 0.1±0.0% (based on SCS) furfural in SMW, SEW and SPW spent liquors respectively. The furfural concentration levels in SEW spent liquors also very like Sklavounos et al. (2011; 2013b) findings. The low furfural content (0.01%) reported by Iakovlev and van Heiningen (2012) is related to low temperature treatment (135˚C) in comparison to other studies. Sklavonounos et al. (2011) fractionated spruce wood at 150˚C using SEW cooking liquor (3% SO_2, 120 min, 6 L kg^{-1}). The comparatively high concentration of furfural versus HMF in the spent liquors indicate that the hexoses are more stable towards acid dehydration in SAW fractionation. The results in Table 5.3 show a significant increase in furfural concentration after sulfuric acid hydrolysis (4% w/w H_2SO_4, 121˚C, 1 h) while no significant increase in HMF was observed. Some HMF may be converted into levulinic and formic acid during spent liquors hydrolysis but their concentration were below detection limits. The increase in pentose sugar degradation in processed spent liquors can be related to the increased acidity in rotary evaporation step due to increased concentration of lignosulfonates and the near absence of the solvent. Comparing furfural levels in processed spent liquors indicates that pentose degradation is about same within

experimental errors. It should be kept in mind that spent liquors are hydrolyzed with H_2SO_4 only to establish mass balances but no mineral acid treatment is required in processing of liquor for biofuels production such as ABE fermentation process. Thus, the concentrations of furfural and HMF in SMW, SEW and SPW spent liquors are below the inhibitory limits as indicated by Jurgens et al. (2012) and can be successfully employed in ABE fermentation by C. acetobutylicum.

5.5 Conclusions

SMW, SEW and SPW fractionation systems successfully remove 85% hemicelluloses from SCS, dissolves any sucrose present in SCS, and leaves most of the cellulose intact. Thus, spent liquors are potentially an excellent source of dissolved sugars. The concentration of dissolved sugars increases with severity of fractionation, expressed as CSF. The amount of total dissolved sugars and monosaccharides attains a maximum value at CSF of 2.4±0.1. More severe fractionation conditions (CSF>2.4) decreases the spent liquor sugar content due to degradation reactions. SMW, SEW and SPW spent liquors contain nearly equal amount of total sugars (27.5% on original SCS basis) at CSF of 2.4±0.1 in agreement with the very similar pH in in each fractionation system. However, the fresh spent liquors of the three fractionation systems are quite different in monosaccharides composition. The concentration of monomeric sugars in SMW, SEW and SPW spent liquors are 20.8±0.7, 31.1±1.7 and 37.0±0.5 g L^{-1} respectively. This represents 36%, 54% and 64% of the total sugars in hydrolyzed SMW, SEW and SPW spent liquors respectively. The different levels of monosaccharides with nearly similar amount of total sugars in these spent liquors points to the formation of alkyl xylosides and glucosides by monosugar alcoholysis. This will be discussed in the next chapter. The excellent sugars mass balance for SMW, SEW and SPW fractionation systems shows that sugars can be fully recovered in SAW process. Spent liquors also contain HMF and furfural but their concentrations are below inhibitory limits for ABE fermentation.

Chapter 6

ALKYL PYRANOSIDES FORMATION

6.1 Introduction

Fractionation of SCS with SMW, SEW or SPW provides cellulose rich pulp and a spent liquor that is rich in sugars especially xylose. The produced spent liquor has great potential for bio-based products such as bioethanol and xylitol. However, the viability of the process highly relies on effective recovery of the solvent. Also, biochemical conversion technology requires a spent liquor stream that is rich in monomeric sugars with few sugar degradation products. In organosolv fractionation systems, alcohol is consumed in esterification reactions (for example with acetic acid) leading to solvent losses (Bublitz & Wilson, 1983). However, alcohol could also be consumed in other reactions than esterification, and this need to be quantified.

The most common reactions consuming alcohol in organosolv systems are thought to be alcoholysis based on some earlier and recent literature. These reports show that in reaction systems involving alcohol the carbohydrates/sugars undergo alcoholysis in acidic media (Drouet, et al., 1994; Hu, et al., 2012; Grisel, et al., 2014; Hu, et al., 2014; Lancefield, et al., 2017). In such systems pentose and hexose sugars react with alcohol forming alkyl pyranosides and furanosides. Organosolv (with or without acid addition) and SAW fractionation are examples of such systems where dissolved sugars may react with the employed alcohol. However, only recently it has been shown that alkyl pyranosides and furanosides are formed in 95% methanol and 94% ethanol treated using 40 mM H_2SO_4 as catalyst with the objective to generate furan derivatives (Grisel, et al., 2014), and in 95% n-butanol with 0.2 M HCl as catalyst for production of unmodified lignin (Lancefield, et al., 2017). In SAW fractionation, the dissolution of hemicellulose during fractionation results in significant concentrations of xylan oligomers/xylose and other hemicellulose sugars in the spent liquors (Iakovlev & van Heiningen, 2012a) (also see Chapter 5). No severe carbohydrate side reactions such as dehydration and oxidation are reported. However, at the prevailing acidic conditions it is likely that xylan hydrolysis proceeds together with the formation of alkyl xylosides (methyl, ethyl or isopropyl), furfural and humins as shown in the proposed scheme (Figure 6.1).

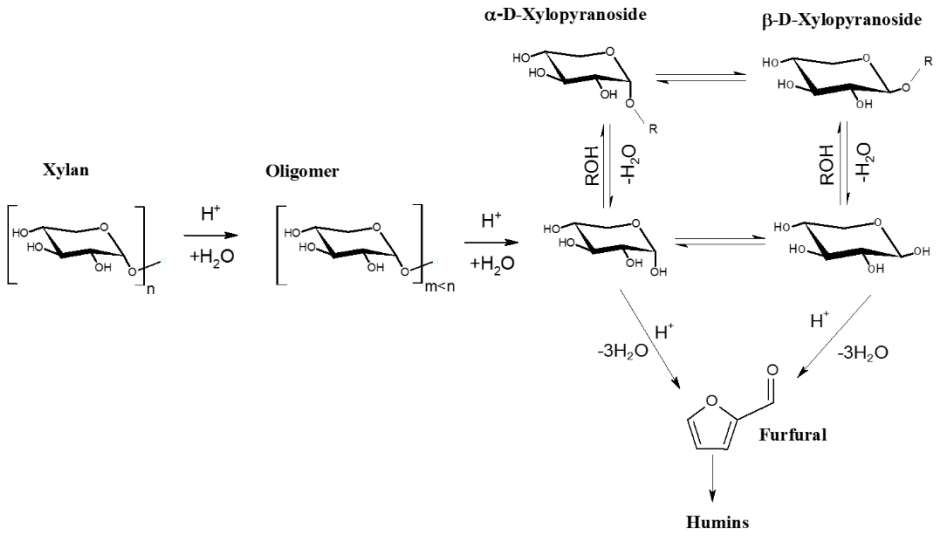

Figure 6.1: Hydrolysis of SCS xylan and formation of alcohol xylosides in acidic media (R=alcohol group i.e. CH_3O, C_2H_5O or C_3H_7O)

When formed, alkyl xylosides decrease the amount of free monomeric xylose and solvent (methanol, ethanol or isopropanol) in the spent liquor, and these must be hydrolyzed for full xylose and ethanol recovery (Chapter 7). In different SEW fractionation studies not more than about 50% (based on dissolved carbohydrates) free sugars are found in the spent liquor stream (Iakovlev & van Heiningen, 2012a; Sklavounos, et al., 2013b). In a recent review of organosolv pretreatment of plant biomass, alkyl xylosides formation has been attributed as the most likely cause for poor xylan mass balances (Zhang, et al., 2016). The formation of alkyl xylosides is also confirmed in a recent study of the solubilized hemicellulose fraction of biomass after treatment with 95% alcohol (ethanol or n-butanol) and HCl as catalyst (Lancefield, et al., 2017). However, the amount of alkyl xylosides and the effect of the severity of process conditions on alkyl xyloside formation are not quantified as is required for scale-up of a viable biochemical conversion technology.

This chapter focuses on quantifying the yield of xylan hydrolysis products such as xylan-oligomers, monomeric (free) xylose, furfural and humins, and particularly that of alkyl xylosides which are formed by reaction of alcohol with xylose during SAW fractionation at different severity conditions. Methyl xylosides, ethyl xylosides and isopropyl xylosides in SMW, SEW and SPW spent liquors are designated as MX, EX and PX respectively.

6.2 Alkyl xylosides quantification

SMW, SEW and SPW spent liquors were diluted with DI water (dilution factor 42) and then analyzed for xylosides on HPLC using a refractive index detector and BIO-RAD Aminex HPX-87K column. The mobile phase used was H_2SO_4 (5 mM, 0.6 mL min^{-1}), and the oven temperature was 45ºC. Alpha and beta anomers of xylosides were found in each spent liquor. Beta xylosides elute before the alpha xylosides on BIO-RAD Aminex HPX-87K column. The elution time of beta-D-xylosides of methanol, ethanol and IPA are 10.7, 11.3 and 11.7 minutes respectively, while corresponding elution times of the alpha anomers of these xylosides are 11.7, 12.7 and 13.7 minutes (Figure 6.2). Xylose peak appears at 9.5 minutes.

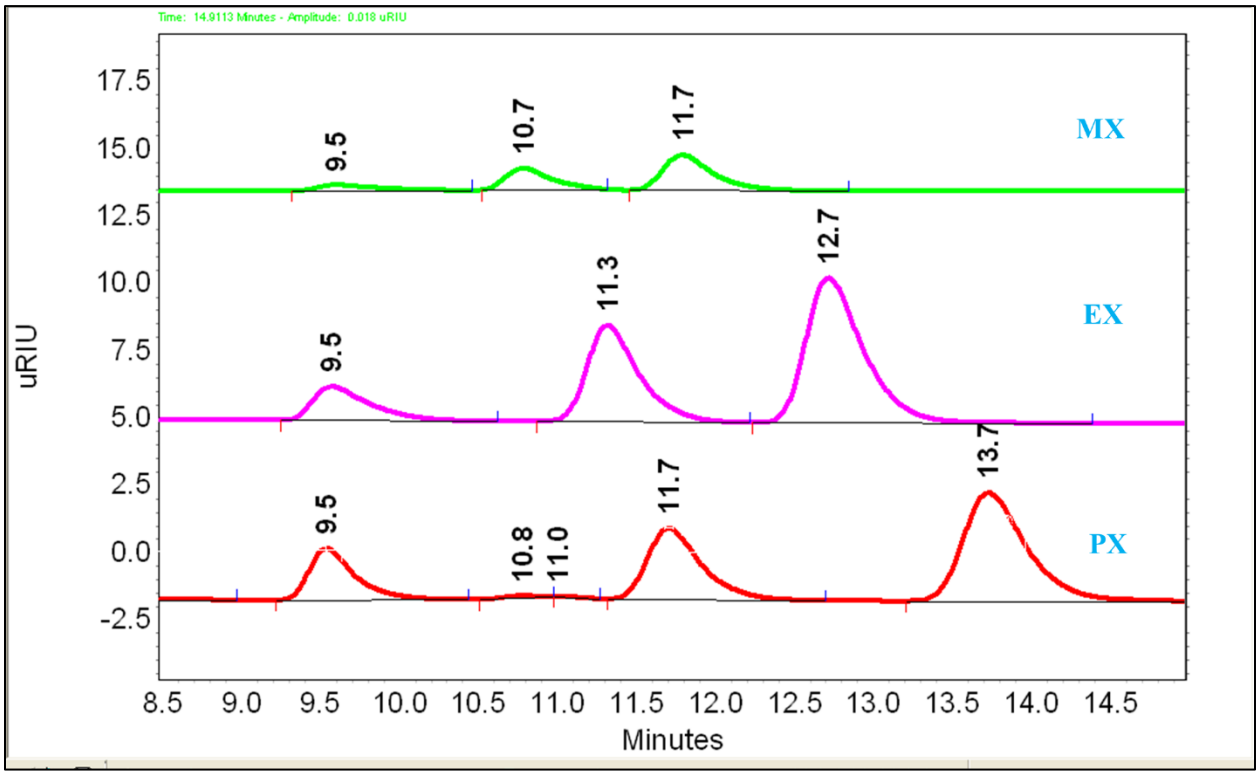

Figure 6.2: Xylosides chromatograms on BIO-RAD Aminex HPX-87K HPLC column

SO_2 in ethanol spent liquor interferes with the quantification of alpha ethyl xyloside (aEX) because it elutes at the same time as aEX on Bio-RAD Aminex HPX-87 column (i.e. 12.7 min). To eliminate SO_2 interference we added H_2O_2 (32 µL, 35% solution) to 0.2 mL of cooking liquor and then diluted with deionized water to 10 mL (dilution factor = 50) before analyzing on HPLC. The amount of H_2O_2 required was calculated from concentration of SO_2 in

the cooking liquor (120-130 g L^{-1}) and later confirmed by HPLC analysis with no SO_2 peak (see Figure 6.3). SO_2 reacts with H_2O_2 to form H_2SO_4 (Equation 11).

$$H_2O_2 + SO_2 \rightarrow H_2SO_4 \quad (11)$$

Therefore, all SEW spent liquors were treated with H_2O_2 for aEX quantification in similar manner as we did for fresh liquor however, less dilution was used for spent liquor samples (dilution factor 40). We did not measure alkyl pyranosides and furanosides of other sugars than xylose in the SAW spent liquors.

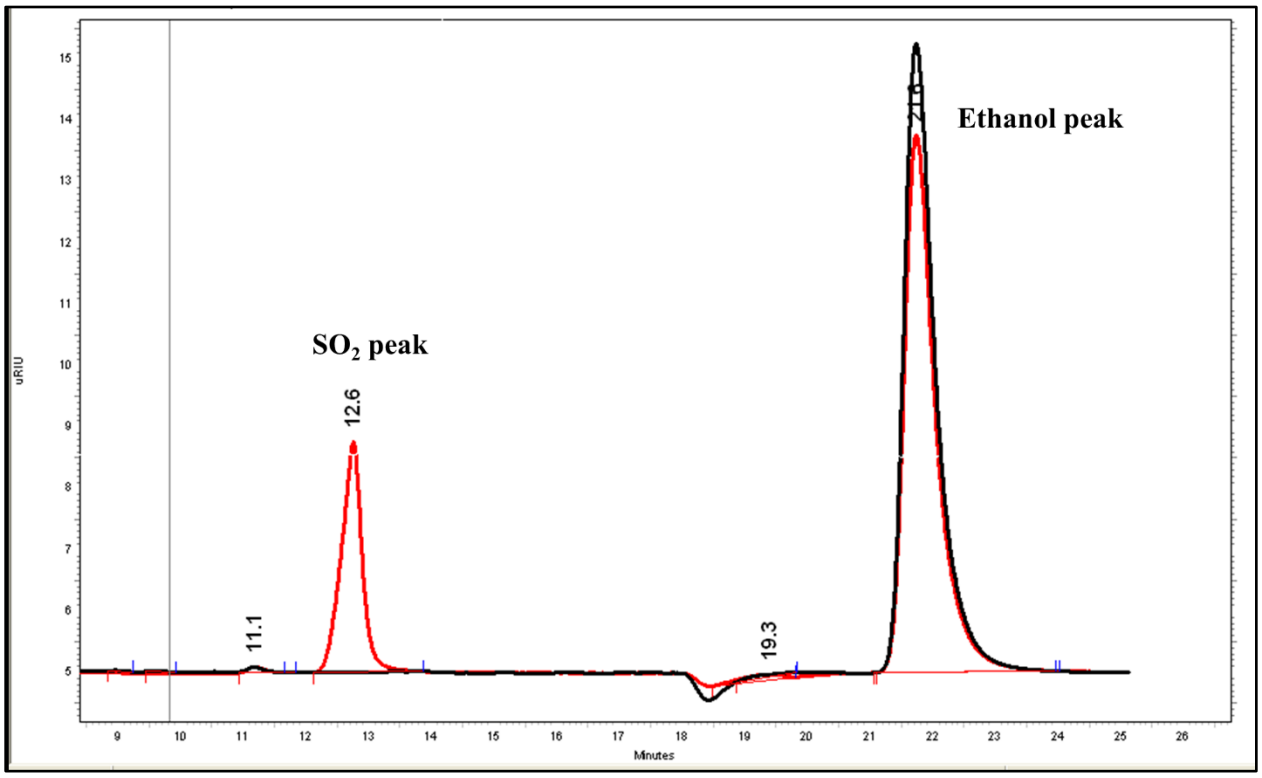

Figure 6.3: HPLC chromatograms showing the effect of adding H_2O_2 in fresh SEW cooking liquor. Red color shows chromatograms for fresh SEW cooking liquor while black after H_2O_2 addition. SO_2 elution time is 12.6 minutes

α-D-Methyl xyloside (aMX) purchased from Pfaltz & Bauer (Waterbury CT, USA) and ß-D-methylxyloside (bMX) (>99%) from Sigma Aldrich (St. Louis MO, USA) were used for quantification of methyl xylosides (MX) in SMW spent liquors. ß-D-ethyl xyloside (bEX) (>98%) purchased from Carbosynth (Berkshire, UK) was used for quantification of this monomer in SEW spent liquors. Alpha ethyl xyloside (aEX) and isopropyl xylosides (PX) are not commercially available. Therefore, we prepared these xylosides (EX and PX) by reacting about 100 mg xylose (1.6 g L^{-1}) and alcohol (725 g L^{-1}) at 121°C for one hour at acidic conditions (4% H_2SO_4). The final reaction mixture

contained unreacted xylose, unreacted alcohol, alpha xyloside, beta xyloside, furfural and humins. The amount of humins formed was estimated from two degradation experiments; one involving only xylose and H_2SO_4, and one involving xylose, methanol and H_2SO_4 at the reaction conditions given above. In both cases the amount of humins formed was determined based on a xylose mass balance before and after reaction involving xylose, furfural and methyl xylosides (for which standards were available), all expressed as xylose equivalent amount. Based on the calculated amount of humins, expressed as equivalent amount of xylose, the humins/furfural ratio was found to be 0.50 and 0.49 g/g in these two experiments respectively (see Table 6.1).

Table 6.1: Xylosides preparation from xylose-alcohol mixtures

Reaction	**Xylose inlet**	**Furfural**	**Unreacted xylose**	**Humins**	**Humins / Furfural**	**Xylosides**
Xylose + H_2SO_4	99.4	8.8	86.2	4.4[a]	0.50	NA
Xylose + H_2SO_4 + MeOH	99.8	5.7	8.1	2.8[b]	0.49	83.2
Xylose + H_2SO_4 + EtOH	99.6	6.3	10.1	3.1	0.50	80.1[c]
Xylose + H_2SO_4 + IPA	99.8	11.4	15.3	5.6	0.50	67.5[d]

All quantities are expressed as mg of xylose equivalent.
a, b, c and d are based on xylose mass balance

The HPLC calibration curves for aMX and bMX are seen in Figure 6.4. The calibration curves are almost identical. Based on the reaction scheme in Figure 6.1 and the fact that the humins/furfural ratio is not affected by the presence of methanol in the second experiment, this ratio was also assumed to be 0.50 in the two degradation experiments with ethanol and isopropanol mixed with xylose and H_2SO_4 thereby allowing the amount of humins to be estimated. Then the amount of ethyl and isopropyl xylosides formed in these respective mixtures could be calculated based on a xylose mass balance involving xylose, furfural, humins and xylosides all expressed in equivalent amount of xylose, and assuming that the HPLC response for the alpha and beta form of the ethyl and isopropyl xylosides are identical. The mass balances for these two experiments are also shown in Table 6.1. The calibration curves for the alpha and beta form of the ethyl and isopropyl xylosides based on the chromatograms and the xylose mass balances are seen in Figure 6.5 and Figure 6.6 denoted as EX-Lab and PX-Lab respectively. Figure 6.5 also includes the calibration curve obtained with the purchased ß-D-ethyl xyloside (bEX-Std), and this curve exactly coincides with the bEX-Lab curve. This further confirms the mass balance approach for establishing the calibration curves as well as that the HPLC response of the alpha and beta forms of the xylosides are the same. The experimentally

determined calibration lines for aPX and bPX are shown in Figure 6.6 and were used to determine the amount of these xylosides in SPW spent liquors.

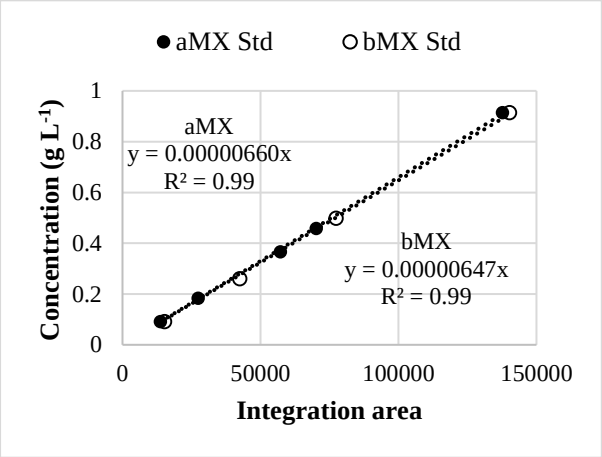

Figure 6.4: Methyl xylosides calibration curves from standards

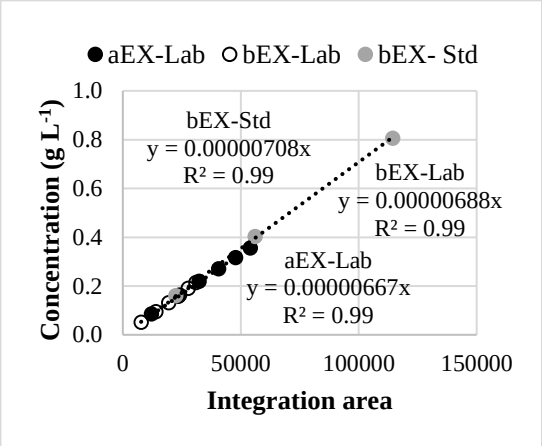

Figure 6.5: Ethyl xylosides calibration curves from standard and laboratory prepared sample

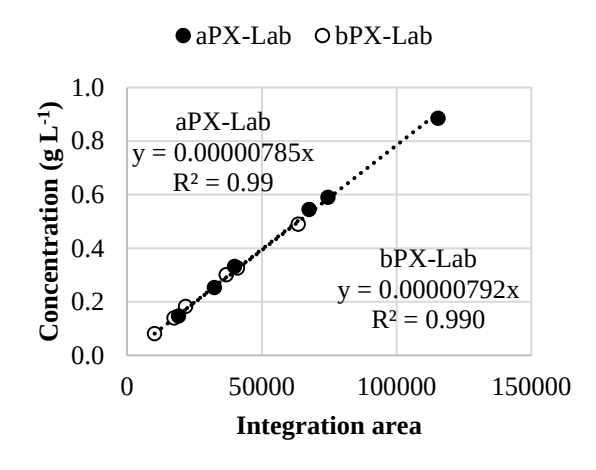

Figure 6.6: Isopropyl xylosides calibrations from laboratory prepared sample

6.3 Effect of solvent on alkyl xylosides formation

Spent liquors from SMW, SEW and SPW fractionation of sugarcane straw contain methyl, ethyl and isopropyl xylosides respectively. The yield of xylosides formation in each fractionation system depends on cooking temperature and duration. Figure 6.7 and Figure 6.8 show the concentration of aEX and bEX respectively in the SEW spent liquors

as a function of fractionation temperature and cooking duration. The concentration of aEX and bEX increase with increase in temperature but under severe fractionation conditions a gradual decrease is observed at 145 and 155°C. The maximum concentration of both anomers is achieved in 60 minutes at 155°C and in 80 minutes at 145°C. The maximum concentration of aEX and bEX in SEW spent liquor under these conditions was nearly 9 g L^{-1} and 5 g L^{-1} respectively.

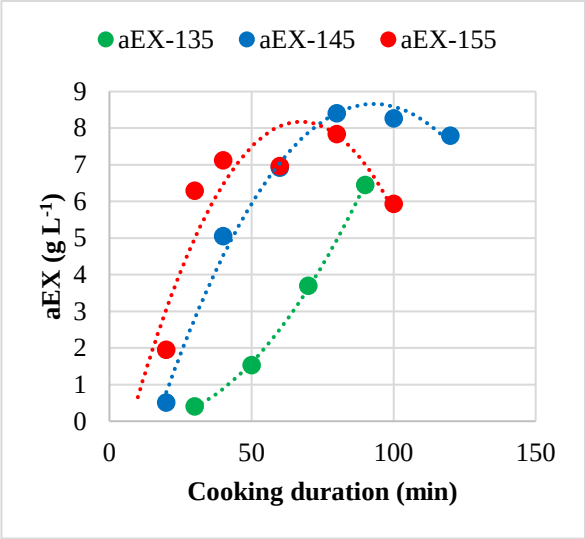

Figure 6.7: Alpha ethyl xyloside (aEX) formation in SEW fractionation of SCS

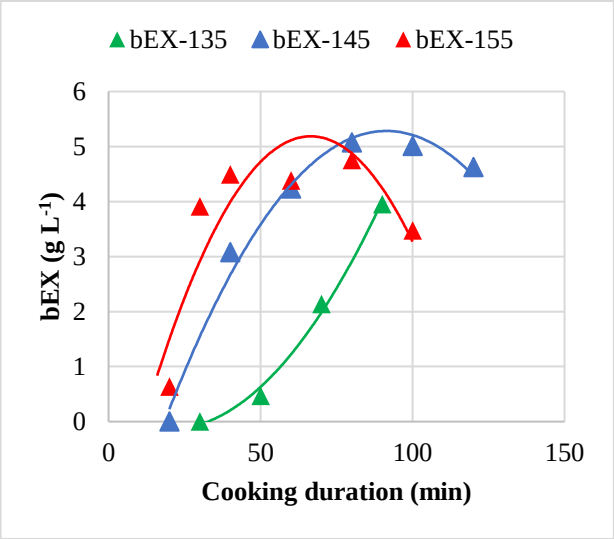

Figure 6.8: Beta ethyl xyloside (bEX) formation in SEW fractionation of SCS

Following the same kinetic approach as is used in section 5.3 using equation 8, the EX formation results are replotted versus CSF. The aEX and bEX concentrations in Figure 6.7 and Figure 6.8 are replotted against CSF on the horizontal axis in Figure 6.9 and Figure 6.10 respectively. Also, included in these figures are the alpha and beta xyloside concentrations in the methanol (SMW) and isopropanol (SPW) spent liquors. The concentration of alpha xylosides is higher than that of beta xylosides at the same CSF for each system (Figure 6.9 and Figure 6.10). For each alcohol system, the maximum concentration of alpha and beta xylosides is observed at CSF of 2.4±0.1. The maximum concentration of the alpha anomer of methyl, ethyl and isopropyl xylosides is 14.2±0.3, 7.6±0.7 and 6.3±1.5 g L^{-1} respectively, while the respective concentrations of the beta anomers are 7.8±0.5, 4.6±0.3 and 3.5±0.9 g L^{-1}. The amount of alpha xylosides in SMW, SEW and SPW spent liquors based on original SCS are 6.3±0.1, 3.4±0.3 and 2.8±0.7 g/100 g respectively. The corresponding level of beta anomers are 3.5±0.2, 2.1±0.1 and 1.5±0.4 g/100 g SCS.

This agrees with Hu et al. (2012) who also reported formation of more alpha xylopyranoside than beta xylopyranoside in acidic methanolysis of xylose.

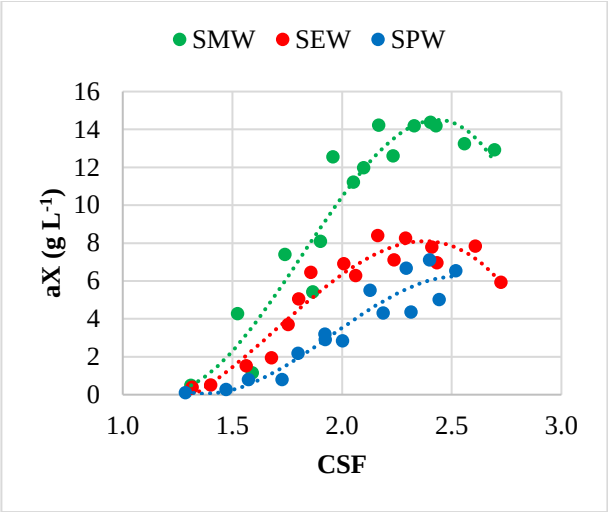

Figure 6.9: Kinetics of alpha xyloside (aX) formation in SMW, SEW and SPW fractionation

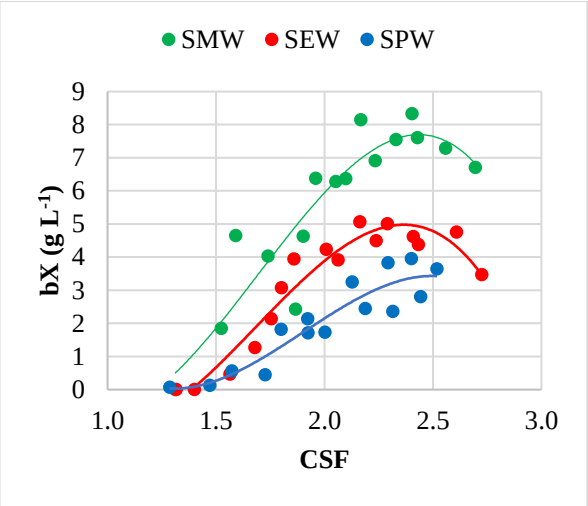

Figure 6.10: Kinetics of beta xyloside (bX) formation in SMW, SEW and SPW fractionation

To better understand the alpha and beta xyloside formation results, it is good to review the two studies by Bishop and Cooper (1962; 1963). These studies on methanolic hydrogen chloride treatment of sugars showed that D-xylose is rapidly converted to methyl xylofuranosides, followed by a two order of magnitude slower conversion of the methyl furanosides in methyl xylopyranosides. Thus, after sufficient reaction time the final products are almost solely methyl alpha and methyl beta D xylopyranosides. The methyl-alpha-D-xylopyranoside (or aMX) is in equilibrium with methyl-beta-D-xylopyranoside (bMX) with an equilibrium constant of 2.2 at 25°C (Bishop & Cooper, 1963) (Table 6.2). This value compares rather well to the aMX/bMX ratio of 1.84±0.08 (CSF > 2.0) obtained in the present study for the SMW system as can be seen in Figure 6.11. A constant ratio for ethanol and isopropanol spent liquors shows that alpha xyloside/beta xyloside equilibrium ratio (aX/bX) is independent of solvent system. A high scatter in aX/bX data at low CSF in Figure 6.11 is expected as the concentrations of xylosides at low CSF are relatively small. In the present study, we did not detect any xylofuranosides. Therefore, while it is likely that also in the present fractionation systems the alkylation of xylose proceeds through formation of the two alkyl-D-xylofuranoside anomers, these are very rapidly further converted into the corresponding alkyl-D-xylopyranoside anomers. Hu et al. (2012) also reported negligible formation of xylofuranosides in acid-catalyzed conversion of xylose in methanol-rich medium.

Table 6.2: Rate constants for D-xylose methanolysis (adapted from Bishop & Cooper, 1963)

	k_1	k_2	$k_1 + k_2$	K
1. Pentose → Furanosides	57	-	-	-
2. Furanosides: α ↔ ß	49	29	78	1.69
3. Furanosides ↔Pyranosides	0.12	0.0004	-	320
4. Pyranosides: ß ↔α	0.031	0.014	0.04	2.2

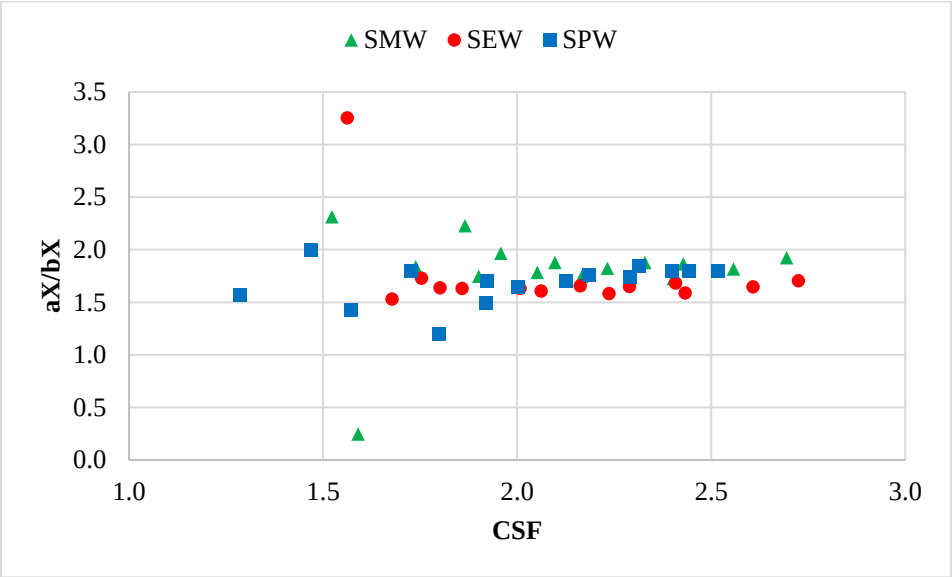

Figure 6.11: Alpha xyloside (aX) and beta xyloside (bX) equilibrium in spent liquors

The sum of the alpha and beta xylosides concentrations in the three fractionation systems follow a similar pattern (Figure 6.12). In each system, the concentration increases slowly until a maximum at a CSF of 2.4±0.1 and then decreases. Under mild fractionation conditions (CSF of 1.5±0.1) the concentration of EX and PX is nearly zero while the MX concentration is around 1.0 g L^{-1}. The decrease in xylosides concentration at severe fractionation conditions (CSF>2.4) can be explained by decomposition of xylose to furfural and humins (Hu, et al., 2012) which leads to hydrolysis of the xylosides due to its equilibrium with xylose. At the same CSF, the MX concentration is the highest among the three systems, i.e. SMW > SEW > SPW. The average highest concentration of the sum of the two anomeric xylosides occurs at 2.4±0.1 CSF, and are 22.0±0.9, 12.3±1.0, 9.7±2.4 g L^{-1} or 9.8±0.4, 5.4±0.5 and 4.3±1.1 g/100 g original SCS in SMW, SEW and SPW spent liquors respectively. These values are obtained by averaging the two or three data points in the CSF range of 2.4±0.1 at the three different temperatures (135, 145, 155°C). It shows that the maximum MX concentration is almost double that of EX and PX, with the last having the lowest concentration.

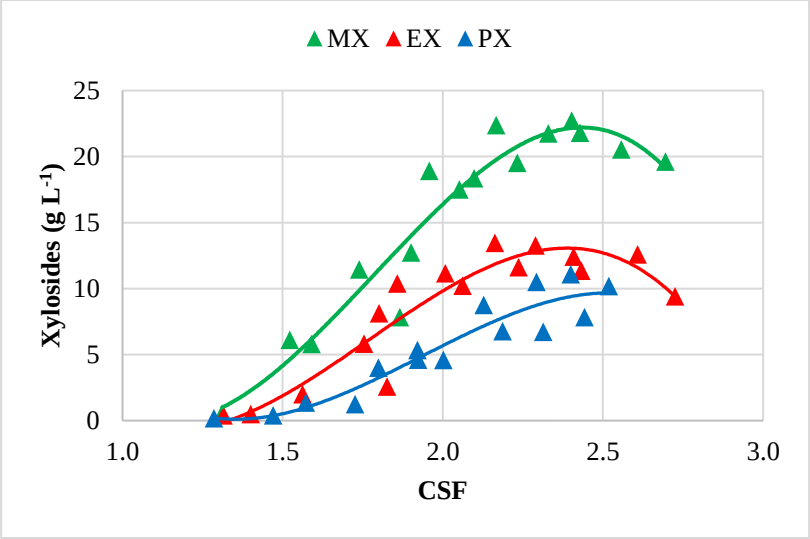

Figure 6.12: Xylosides concentration in SMW, SEW and SPW spent liquors

The present results agree with earlier findings (Drouet, et al., 1994; Grisel, et al., 2014). Drouet et al. (1994) enzymatically prepared alkyl beta-D-xylosides at 40°C using a mixture of xylose and methanol, ethanol or propanol and Trichoderma reesei beta-xylosidase. It took more than six days to reach equilibrium. Drouet et al. (1994) results show that longer chain alcohols are less likely to form alkyl xylosides. Similarly, more methyl glucosides were formed than ethyl glucosides during acid (30 mM H_2SO_4) catalyzed alcoholysis of wheat straw with methanol and ethanol (Grisel, et al., 2014). The high temperature of 200°C used in the Grisel et al. (2014) led to a final liquor pH of 1.5 after 30 minutes which corresponds to a CSF value of 2.93 (equation 8) which is significantly higher than that for the maximum xylosides formation of 2.4 found in the present study. Therefore, the xylosides in the Grisel et al. (2014) are mostly degraded to furfural and humins, while cellulose is mostly hydrolyzed and then converted into glucosides, alcoxymethylfurfural and levulinates. At much milder conditions below 130°C and with Amberlyst 70 as catalyst Hu et al. (2012) found that most of the xylose in aqueous methanol (methanol/water mass ratio of 4.5) was converted into MX with negligible humins formation, similarly to the present results in Table 6.1. However, at temperatures above 150°C dehydration of xylose resulted in significant amounts of humins together with furfural and 2-(dimethoxymethyl) furan formation. Iakovlev and van Heiningen (2012a) evaluated the spent liquors obtained from SEW fractionation of spruce at 135°C (12% SO_2). However, they did not analyze it for ethyl pyranosides and furanosides. Therefore, this is the first SAW study whereby alkyl xylosides are quantified.

6.4 Effect on monomeric xylose

Figure 6.13 shows the concentration of monomeric xylose in SMW, SEW and SPW spent liquors as a function of CSF. The concentration of monomeric xylose in each spent liquor is nearly the same (about 4 g L^{-1}) at mild fractionation conditions (CSF $\leq$ 1.5). The xylose concentration increases with increasing CSF until it reaches a maximum value at a CSF of around 2.4±0.1 for all systems similar to that found for the xylosides. Figure 6.13 shows that the maximum concentration of monomeric xylose (at CSF=2.4±0.1) in SMW, SEW and SPW spent liquors is 15.7±0.4, 23.6±0.5 and 29.7±0.1 g L^{-1} respectively that corresponds to 6.1±0.2, 9.5±0.7 and 11.2±0.1 xylan on original SCS. Thus, it appears that the concentration of monomeric xylose in SPW spent liquor is double in comparison to SMW spent liquors due to the more extensive reaction of methanol with xylose compared to that with isopropanol.

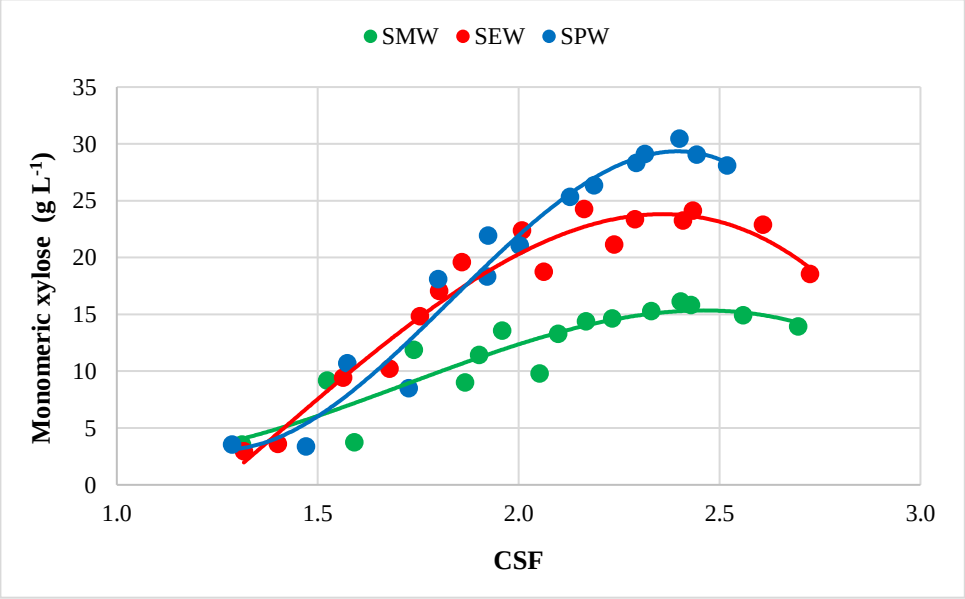

Figure 6.13: Concentration of monomeric xylose in SMW, SEW and SPW spent liquors

Similarly, to the development of the xylosides, at very severe conditions (CSF>2.4) a decrease in xylose concentration is observed in each fractionation system. This suggests that xylose and xylosides are near equilibrium concentrations at high CSF values, and the decrease in xylose concentration due to increased humins formation induces hydrolysis of the xylosides to xylose. It should also be noticed that the concentration order of the monomeric xylose curves for the different solvent systems in Figure 6.13 follows the opposite order to that of the corresponding xylosides curves in Figure 6.12. These results confirm the finding of Bishop and Cooper (1962; 1963) that the xylosides are

formed by equilibrium reactions from D-xylose, and that the equilibrium is shifted furthest towards xylosides for the SMW fractionation system followed by SEW and finally SPW. Figure 6.14 shows that ratio of xylosides to xylose as a function of CSF for the three spent liquors. The ratio increases with increasing CSF and reaches a constant, but different values for the three fractionation systems at CSF>1.8. The constant ratios are 1.4, 0.5 and 0.3 for methanol, ethanol and isopropanol fractionation respectively. This supports that at a higher severity of CSF>1.8 the xylosides concentrations are in equilibrium with the xylose concentrations, and that the equilibrium constant increases from isopropanol to ethanol to methanol.

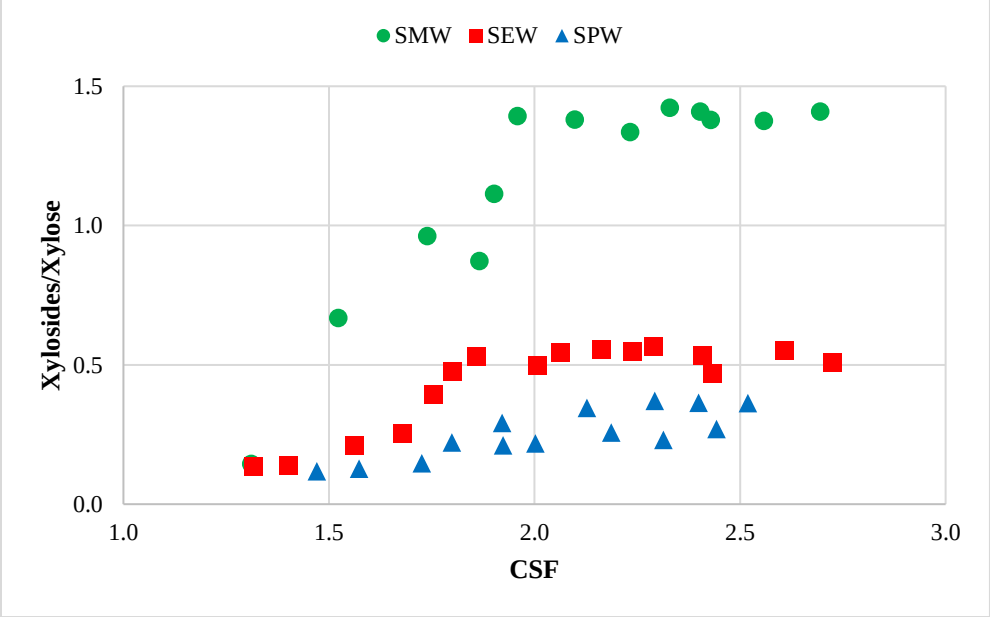

Figure 6.14: Xylosides formation by an equilibrium reaction

6.5 Effect on xylo-oligomer and humins

Table 6.3 shows the xylose mass balance for the three fractionation systems for pulps and liquors produced at a CSF of 2.4±0.1. In terms of equivalent amounts of anhydroxylose (or xylan), the sum of xylose, xylosides and furfural quantified in the fresh spent liquor add up in all three systems to about 14% based on original SCS. After hydrolysis of the spent liquor by treatment with H_2SO_4 (see section 5.1) the analyzed xylose represents approximately 16.5% xylan based on original SCS. Thus, the hydrolysis leads to a significant increase in xylose concentration due to hydrolysis of both xylo-oligomers and xylosides. It is important to notice that xylosides were not present in detectable amounts in the spent liquor after the hydrolysis step. With the addition of furfural (as anhydroxylose) in the

hydrolyzed liquor, the total amount of xylan accounted for is equal to about 18% based on original SCS. This compares well to total amount of dissolved xylan (about 19%) calculated from the difference of xylan in SCS (21.4±0.6) and in pulps. The difference of 0.9-1.3% (on original SCS) is the xylan mass balance loss due to degradation to mostly humins. It should be noted that the calculated xylan mass balance difference is generally within the experimental error, and thus confirms that partial replacement of water by alcohol suppresses humins formation (Hu, et al., 2012; Grisel, et al., 2014). Finally, the amount of xylo-oligomers is calculated as the difference between the total recovered xylan in the hydrolyzed liquor and the total amount of xylan identified in the untreated spent liquor. It shows that about 3-4% of xylan is present as oligomers in the SMW, SEW and SPW spent liquors. It accounts for 16-23% of the total xylan present in spent liquors, and is about 1.7 to 3.6 times smaller than the equivalent amount of xylan identified as monomeric xylose in the untreated spent liquor. Iakovlev and van Heiningen (2012a) reported for spent liquor from SEW fractionation of spruce (12% SO_2) that the amount of dissolved oligomeric hemicellulose was about the same as the sum of the monomeric hemicellulose compounds (i.e. mannose, xylose, etc.) independent of fractionation operating conditions. They overestimated the amount of dissolved hemicellulose oligomers because the ethyl pyranosides were accounted for as hemicellulose oligomers by calculating the oligomers from the difference in sugar concentration between the hydrolyzed and untreated spent liquor. Our results for monomeric xylose in SEW spent liquor agree with their results. It can also be seen that the amount of furfural in the spent liquor is very small (0.3-0.4% on SCS) which again is an indication that the presence of alcohol suppresses sugar degradation reactions.

Table 6.3: Xylan in pulp and its equivalent amount in different components of SL at CSF=2.4±0.1

Fractionation system	**SMW**	**SEW**	**SPW**
SCS	21.2±0.6	21.2±0.6	21.2±0.6
Pulp	2.2±0.3	2.2±0.4	2.4±0.1
Xylosides (Spent liquor)	7.9±0.3	4.0±0.3	3.0±0.7
Monomeric (Spent liquor)	6.1±0.2	9.5±0.7	11.2±0.1
Furfural (Spent liquor)	0.3±0.1	0.3±0.2	0.2±0.1
Identified in Spent Liquor	**14.3±0.4**	**13.8±0.8**	**14.4±0.7**
Xylose (after hydrolysis of spent liquor)	16.2±0.5	16.7±1.3	16.4±1.6
Furfural (after hydrolysis of spent liquor)	1.7±0.3	1.4±0.2	1.1±0.1
Total recovered (xylose + furfural)	17.9±0.6	18.1±1.3	17.5±1.6
Total Dissolved (SCS-Pulp)	**19.0 ±0.7**	**19.0 ± 0.7**	**18.8 ± 0.6**
Xylo-oligomers (Total Recovered-Identified in spent liquor)	3.6±0.7	4.3±1.5	3.1±1.7
Xylan mass balance loss (Total dissolved – Total recovered)	**1.1±0.9**	**0.9±1.5**	**1.3±1.7**

The effect of CSF on the xylan mass balance and xylo-oligomer formation for the SEW process is seen in Table 6.4. The concentration of dissolved xylose in spent liquor increases with CSF. A 4% increase in xylan dissolution is seen with 0.7 units increase in CSF. SCS contains 21.2% xylan and 20.3±0.7 xylan is dissolved in spent liquor leaving nearly 1.0% in residual pulp at CSF of 2.7±0.1. Only 0.4% xylan is reduced to furfural at this high severity factor, but the xylan mass loss is comparatively high (~4.0%). Xylan mass loss at CSF of 2.0±0.1 and 2.4±0.1 are within experimental error equal to zero, indicating a complete xylan mass balance. This indicates that under severe fractionation conditions (CSF of 2.7) xylan is converted to humins leading to significant xylan mass balance losses. Grisel (Grisel, et al., 2014) also observed high xylan losses at severe cooking conditions. In that study a loss of more than 40% was reported when wheat straw was ethylated at 2.79 CSF (175°C, 120 min, 40 mM H_2SO_4, pH 1.5 of final liquor). More severe conditions (170°C, 120 min, 50 mM H_2SO_4, CSF is around 3.1 assuming pH of final liquor 1) resulted less furfural and xylosides yields and consequently higher humins. Comparison with this acid catalyzed ethylation study of wheat straw suggests that xylan losses are significantly lower using SO_2 as catalyst in SEW fractionation of SCS but H_2SO_4 as catalyst gives higher furfural yields.

Table 6.4: Effect of CSF on xylo-oligomer and humins formation in SEW fractionation of SCS

CSF	**2.0±0.1**	**2.4±0.1**	**2.7±0.1**
SCS	21.2±0.6	21.2±0.6	21.2±0.6
Pulp	4.9±0.4	2.2±0.4	0.9±0.3
EX (Spent liquor)	3.4±0.2	4.0±0.3	3.6±0.7
Monomeric (Spent liquor)	8.0±0.6	9.5±0.7	8.9±1.3
Furfural (Spent liquor)	0.0±0.1	0.3±0.2	0.4±0.2
Identified in Spent Liquor	***11.4±0.6***	**13.8±0.8**	***12.9±1.5***
Xylose (after hydrolysis of spent liquor)	*15.8±2.1*	16.7±1.3	*14.8±1.8*
Furfural (after hydrolysis of spent liquor)	1.2±0.1	1.4±0.2	1.3±0.0
Total recovered (xylose + furfural)	*17.0±2.1*	18.1±1.3	*16.1±1.8*
Total Dissolved (SCS-Pulp)	16.3±0.7	19.0 ± 0.7	20.3±0.7
Xylo-oligomers (Total Recovered-Identified in spent liquor)	*5.6±2.2*	4.3±1.5	*3.2±2.3*
Xylan mass balance loss (Total dissolved – Total recovered)	***-0.7±2.2***	**0.9±1.5**	***4.2±1.9***

6.6 Solvent and xylose losses

The alpha and beta xylosides in the spent liquor represent a significant amount of chemically bound xylose and alcohol which could interfere with alcohol recovery and complete utilization of xylose. Figure 6.15 shows the amount of xylose and solvent bound in the form of alkyl xylosides based on original SCS. SMW spent liquor contains nearly 10 g/100 g SCS MX (alpha 6.3 g/100 g and beta 3.5 g/100 g) accounting for 1.9% methanol and 9% xylose on SCS. SEW spent liquor contains nearly 6.0 g EX /100 g SCS (3.4 g alpha /100 g SCS and 2.1 g beta /100 g SCS) or 1.4 g ethanol /100 g SCS and 4.6 g xylose/100 g SCS. Similarly, SPW spent liquor contains the smallest amount of xylosides (2.8 g alpha/100 g SCS, 1.5 g beta/100g SCS) binding 1.3 g isopropanol/100 g SCS with 3.4 g xylose/100 g SCS.

Figure 6.15: Xylose & alcohol bound as alkyl xylosides in SAW spent liquors

Considering that only a few percent (based on dry biomass feedstock) of alcohol may be lost during solvent recovery for an economic viable fractionation process, the chemically bound solvent in alkyl xylosides represents a significant loss unless the alkyl xylosides are hydrolyzed or recovered and sold as an additional product. This applies even more to xylose chemically bound in the alkyl xylosides because of its higher potential value and significant larger mass percentage based on dry biomass. It may be very interesting to separate alkyl xylosides as a byproduct of the fractionation process and find their commercial/industrial applications. In the next chapter, we described process conditions which allow hydrolysis of the alkyl xylosides so that both the alcohol and xylose can be recovered and used for production of biofuels and biochemicals. For example, the hydrolyzed spent liquor from SEW process might be most suitable for ethanol production.

6.7 Conclusions

SAW fractionation of SCS results in simultaneous hydrolysis of xylan and formation of alkyl xylosides. Xylan hydrolysis and xylosides formation is significantly influenced by the alcohol used in the acidic organosolv fractionation/SAW process. Alkyl xylosides and xylo-oligomers are dominant in SMW spent liquors while SPW spent liquors are rich in monomeric xylose. Monomeric xylose and alkyl xylosides are in equilibrium in the spent liquor stream at a combined severity factor (CSF) of >1.8 and their maximum concentrations are observed at 2.4±0.1 CSF

because of further conversion of xylose to furfural and humins. The concentration of monomeric xylose in SPW and SEW spent liquor are 3.0 and 1.9 times higher than their corresponding xylosides. On the other hand, SMW spent liquor contains 1.4 times more methyl xylosides than monomeric xylose. The amount of oligomeric xylose in the SEW spent liquor is about three times as small as the xylose concentration (both as xylan), contrary to earlier reports that these amounts were approximately the same. However, the latter reports did not consider the formation of xylosides. The very small amounts of furfural and humins in the spent liquors indicate that the presence of ethanol and SO_2 suppress the xylose degradation to furfural and humins as compared to aqueous H_2SO_4 catalyzed fractionation processes. With additional hydrolysis of the SEW spent liquors after ethanol (and SO_2) recovery, the SEW process might be most suitable for ethanol or ABE production from biomass.

Chapter 7

SUGARS AND SOLVENT RECOVERY

7.1 Introduction

Since the maximum amount of xylose in SAW spent liquor is obtained at CSF of 2.4±0.1, it is optimal to stop the fractionation treatment of SCS at this CSF value. A CSF value of 2.4±0.1 is obtained by pulping SCS for 100-120 minutes at 145˚C in the present SMW, SEW or SPW system (SAW as 12/44/44%). The same CSF can be achieved by SAW treatment of SCS at 155˚C for one hour. In this chapter the latter conditions were employed to fractionate SCS and obtain spent liquors which then were subjected to different severities of hydrolysis to study the recovery of the solvent and sugars. Liquid to feedstock ratio, alcohol and SO_2 concentration were kept the same as described earlier (see section 3.3). The only modification was a change in the water washing step of the fractionated biomass to minimize the amount of water introduced (less water needs to be evaporated in the solvent recovery step). This time the solid residue was washed three times with DI water (L/F = 2 L kg^{-1}) after alcohol washing (see for Figure 3.4 for comparison). This modification did not have a significant effect on pulp kappa number as can be seen from Table 7.1. The SMW system was not studied for solvent and sugar recovery because the high yield of methyl xylosides may make it more valuable as a byproduct. Spent liquor, alcohol washings and water washings were added together forming a dilute mixture called MSAW (Mixed SO_2-Alcohol-Water) liquor. The MSEW (Mixed SO_2-Ethanol-Water) and MSPW (Mixed SO_2-Isopropanol-Water) liquors were further investigated for alkyl or alcohol xylosides hydrolysis kinetics to study the recovery of sugars and the solvents.

Table 7.1: Effect of water washing on pulp properties

Process	**Original Washing Scheme**		**Modified Washing Scheme**	
	Pulp Yield	Pulp Kappa	Pulp Yield	Pulp Kappa
SEW	38.3	22.6	38.9±0.2	22.4±0.6
SPW	37.9	20.4	37.3±0.5	20.1±0.9

7.2 Washing and handling losses

SAW fractionation employs highly volatile solvents for easy solvent recovery. However, the high volatility also results in serious evaporation mass losses during handling of the fractionated biomass as shown in Table 7.2. An overall mass loss of 68.2 and 73% (based on SCS) was observed for the SEW and SPW fractionation system without

any washings. Even higher mass loss occurred when fractionated biomass was washed with aqueous alcohol and DI water in the subsequent processing steps (Table 7.2). All the spent liquor separations and solid residue washings were done under an exhaust hood. Therefore, these losses are attributed to evaporation of solvent and SO_2 when handling fractionated biomass in open vessels. However, such handling losses can be eliminated to a large extent by performing solid residue (pulp) separations and washings in closed containers.

Table 7.2: Evaporative handling mass losses during SAW fractionation of SCS

		SEW Fractionation		SPW Fractionation	
		No Washing	With Washings	No Washing	With Washings
Mass (in)	SCS	24.0	24.0	24.0	24.0
	Cooking liquor	86.8	86.7	85.4	84.4
	Alcohol washings	-	82.2	-	80.9
	Water washings	-	135	-	135
	Total mass in	**110.8**	**327.9**	**109.4**	**324.3**
Mass (out)	Spent liquor	67.4	67.2	65.0	64.3
	Alcohol washings	-	72.4	-	71.1
	Water washings	-	130.5	-	127.7
	Wet Pulp	28.0	25.3	27.9	25.9
	Total mass out	**95.4**	**295.4**	**92.9**	**289.0**
Loss	*% Mass loss*[a]	*68.2*	*144.8*	*73*	*156*

All values are described as mass of material in grams
(a)-Based on oven dried SCS (~22.6 g)

7.3 Alkyl pyranosides hydrolysis

7.3.1 SO_2 and alcohol removal

The addition of alcohol and water washings to the spent liquor resulted in substantial lignin precipitation. This is called precipitate I in this thesis. The precipitated lignin was separated from the MSAW liquor, vacuum dried (25˚C) and then gravimetrically quantified (Table 7.3). The amount of lignin precipitate I was corrected for covalently bound sugars (17-21%), determined by subjecting the solids to double stage hydrolysis (72% H_2SO_4, 30˚C, 1h and 4% H_2SO_4, 121˚ C, 1h), and for ash content (4-7%). Thus, precipitate I represents 34-38% of total lignin in original SCS. It should be noted that the alcohol concentration in the MSAW liquors was 25-28%. After precipitate I removal, the MSAW liquors were subjected to alcohol and SO_2 evaporation in a Heidolph rotary evaporator equipped with a Maxima vacuum dryer. The water bath temperature was set at 80°C for rotary evaporation. It took about 40-60 minutes to achieve ~65-75% mass reduction of the MSAW liquors. The removal of alcohol from MSAW liquors by rotary evaporation also formed precipitated lignin designated as precipitate II. The precipitate II amounts were comparable

to precipitate I except that their content of sugars was much lower (3-7%). The resultant liquor is called EVAP in accordance with nomenclature in earlier studies (Sklavounos et al. 2013a, You et al. 2017b). Precipitate II was separated from the EVAP liquors ($EVAP_E$ for ethanol and $EVAP_P$ for isopropanol) by centrifugation (4000 rpm, 15 min). The dissolved lignin concentration in the EVAP liquors is 66-84% lower than that in the MSAW liquors. This decrease in lignin content due to removal of precipitated lignin (I and II) is comparable to75% reduction in lignin content reported by Sklavounos et al. (2013a) who used the same treatment for spruce MSEW spent liquor. Our results also agree with You et al. (2017b) who found only 2-3% dissolved lignin in $EVAP_E$ liquor after rotary evaporation of MSEW liquors obtained from SCS fractionation (155˚C, 58 min, SEW liquor composition 12:44:44 w/w%). It indicates that lignin removal from spent liquors by precipitation depends on alcohol dilution/depletion level irrespective of lignocellulosic biomass.

Table 7.3: Rotovap and lignin precipitation

Fractionation process	**SEW**	**SPW**
Lignin in SCS[a]	19.8	19.8
Lignin in Pulp[b]	1.2	1.1
Lignin MSAW (SCS – Pulp)	18.6	18.7
Precipitate I	7.5	6.8
Dissolve I*	11.1	11.9
Lignin in EVAP		
Precipitate II	7.9	5.5
Dissolve II*	3.2	6.4

All values are based on % SCS
a-Ash free Klason lignin
b-Calculated based on equation 1 and Table 7.1
*Calculated as numerical difference "Lignin in MSAW – Precipitate I/II"

Table 7.4 shows the comparison of fresh (SAW), mixed (MSAW) and rotovap (EVAP) spent liquors obtained after SEW and SPW fractionation of SCS. A 3-4% increase in monomeric xylose content is seen while adding alcohol and DI water washings to fresh SEW and SPW spent liquors. Similarly, washing removes an additional 2.5-3.5% lignin from fractionated SCS. Some alkyl xylosides are also removed from the solid residue (or pulp) in the washing process. These findings agree with Iakovlev et al. (2009) and Sklavounos et al. (2013a) that alcohol/water washing removes a significant amount of lignin and carbohydrates from fractionated biomass. Very small changes in monomeric xylose and xylosides content (SCS basis) occur when MSAW liquors are subjected to rotary evaporation at 80°C indicating that xylosides and oligomer hydrolysis are not significant at this temperature. The small difference in free xylose content in EVAP and MSAW liquors agrees with the amount of xylose found in precipitate II (0.3-0.6%

on SCS). The concentrations of ethanol (EtOH) and isopropanol (IPA) in $EVAP_E$ and $EVAP_P$ liquors are very low at 0.1 g L^{-1} (~0.02-0.03 g/100 g SCS). Thus, more than 99% of the alcohol present in the MSAW liquors is removed in the rotovap step. However, alcohol chemically bound to carbohydrates or lignin remains in the EVAP liquor. The fresh spent liquors have a high acidity (pH ~1.0) due to the presence of lignosulfonic acids. As expected the pH increases to 1.2-1.3 due to dilution with lignosulfonic acid and SO_2 free washing liquor. The pH again slightly goes down when SO_2 and alcohol are removed from the liquors by rotary evaporation because the increase in acidity due to concentration is only partly balanced by the removal of SO_2.

Table 7.4: Effect of washings and rotary evaporation on spent liquors contents

	SEW fractionation			**SPW fractionation**		
	SEW	**MSEW**	**$EVAP_E$**	**SPW**	**MSPW**	**$EVAP_P$**
Monomeric xylose	9.9 (29.1)	12.8(10.0)	12.2 (43.6)	10.5 (32)	14.5 (11.5)	14.3 (38.1)
Xylosides	5.8 (18.5)	7.0 (5.7)	6.8 (24.6)	2.7 (9.3)	4.7 (3.9)	4.7 (12.5)
Alcohol	129 (429)	328 (272)	0.03 (0.1)	113 (385)	274 (235)	0.02 (0.1)
Lignin	15.8(52.5)	18.6(15.4)	3.2 (16.0)	15.3 (52.1)	18.7 (16.4)	6.4 (23.6)
pH	0.97	1.23	1.15	0.96	1.32	1.08

Mass concentration factor (m_i/m_f) 4.1 and 3.0 were used for SEW and SPW respectively in the calculations of EVAP (g/100 g SCS). The numbers in parenthesis shows the concentrations in g L^{-1}.

7.3.2 Heat treatment of EVAP liquors

EVAP liquors obtained after rotary evaporation and separation of precipitate lignin II were subjected to heat treatment for xylosides hydrolysis at 100, 110 and 121˚C in small vials placed in a stainless-steel vessel containing polyethylene glycol (PEG) as a heating media. For the xylosides hydrolysis experiments we placed 1 mL EVAP liquor in a 2.5 mL HPLC vial, closed it tightly and immersed in preheated PEG. The vials were weighed before and after the reaction to monitor any mass loss due to leakage. The xylosides hydrolysis duration ranged from 10-130 minutes including about 2-3 minutes for heat up of the vial content. After heat treatment, the samples were filtered (0.45 µm) and analyzed for monomeric (free) xylose on HPAEC-PAD using fucose as internal standard. HPAEC injection samples were prepared by adding fucose (1 g L^{-1}, 1 mL) to the hydrolyzed liquor samples (0.1 mL) and then diluted to 10 mL with NaOH (0.1M).

The amount of alcohol (ethanol or isopropanol) in all liquor samples was determined by HPLC. The HPLC operating conditions were the same as described earlier (see section 3.2). Samples for HPLC analysis were prepared by taking 0.1mL (0.45 µm filtered) liquor sample and diluting it with deionized water (dilution factor =10). The amount of ethyl xylosides (EX) was determined by treating the 0.2 mL liquor with H_2O_2 (6.4 µL, 35%) and diluting

it with water (DF = 4-5). Only a small amount of hydrogen peroxide was added as SO_2 is almost completely removed during rotary evaporation (Sklavounos, et al., 2011). It was experimentally confirmed that a large excess of H_2O_2 (relative to SO_2) did not affect the determination of the exact amount of EX in $EVAP_E$ and the corresponding heat treated samples. Isopropyl xylosides (PX) in $EVAP_P$ and corresponding hydrolyzed liquors were estimated using the calibration curves developed earlier (Chapter 6). However, isopropanol samples were analyzed without any H_2O_2 addition as SO_2 elution does not interfere with PX anomers analysis (see Figure 6.2 and Figure 6.3 in Chapter 6). In the EVAP and hydrolyzed spent liquor samples, all dilutions were performed with DI water at lower dilution factors (4-6) to keep the xyosides concentrations at measurable detection levels.

7.4 Ethyl pyranosides hydrolysis

$EVAP_E$ spent liquor contains 6.8% EX on SCS (Table 7.4). Figure 7.1 shows the hydrolysis of EX (sum of α and β ethyl pyranosides) during heat treatment at different temperatures. At 100°C a gradual disappearance in EX is seen over a period of about 2 hours. The rate of disappearance increases with temperature and negligible EX remains after 70 minutes at 121°C.

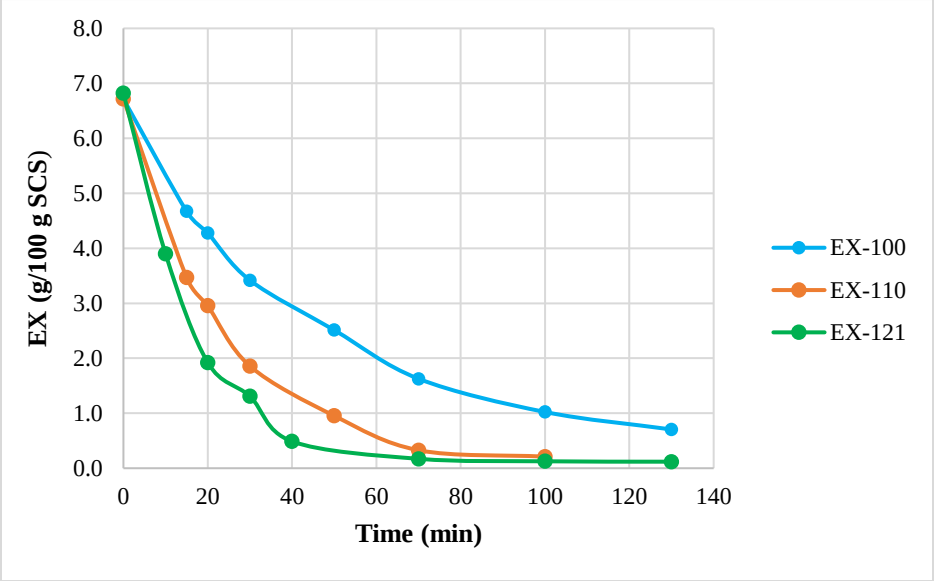

Figure 7.1: Ethyl xylosides (EX) hydrolysis at different temperatures

Plotting the natural logarithm of the EX molar concentration vs reaction time (Figure 7.2) produces straight lines. The data points after 70 minutes at 121˚C are not included in the kinetic analysis as it produces inaccurate results

because of very low EX concentration. Thus, hydrolysis follows first order kinetics in EX. The first order rate constants were determined from the slopes of the straight lines in Figure 7.2 and are listed in Table 7.5. An activation energy for EX hydrolysis of 66 kJ mol^{-1} is determined from the rate constants based on the Arrhenius equation. The rate constant data show that hydrolysis is two times faster at 110°C than at 100°C and another 1.5 times faster at 121°C. The PX hydrolysis results will be discussed later in this chapter.

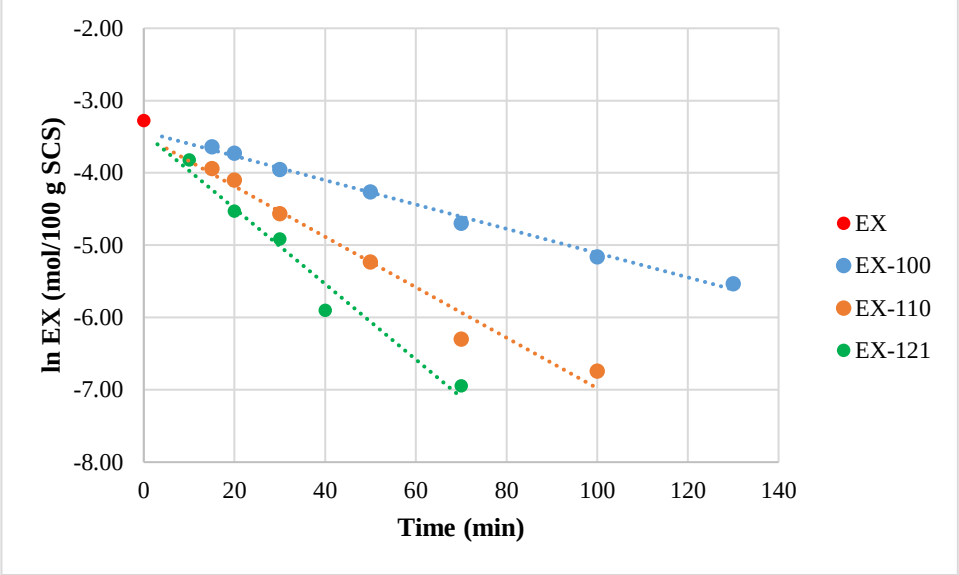

Figure 7.2: Kinetics of ethyl xylosides (EX) hydrolysis

Table 7.5: Kinetic paraments of alkyl xylosides hydrolysis

	k_{hyd} x 10^2 (min^{-1})			Activation Energy (kJ mol^{-1})
	100˚C	110˚C	121˚C	
EX	1.68	3.49	5.23	66
PX	2.13	5.97	20.06	130.7

1 mole of EX hydrolysis produces 1 mol of xylose and ethanol (see equation 12).

$$C_7H_{14}O_5 + H_2O \leftrightarrow C_2H_5OH + C_5H_{10}O_5 \quad (12)$$

The $EVAP_E$ liquor initially has 6.8 g EX (per 100 g SCS) corresponding to 38 mMol (per 100 g SCS) of EX. It also contains 12.2 g xylose (or 81.2 mMol) which is subtracted from the total xylose content measured after EX hydrolysis. The development of the molar concentration of the newly formed xylose at different hydrolysis conditions is plotted in Figure 7.3. The rate of xylose release increases dramatically as the temperature is increased from 100°C to 121˚C.

Figure 7.1 suggests that complete EX hydrolysis requires more than 2 hours at 100°C, while the same can be achieved in 70 minutes at 121°C. A similar heat treatment method is patented by Retsina and Pylkkanen (2013) to recover lignin from SEW spent liquors. In the patented process, it is claimed that heat treatment of SEW spent liquors produces water insoluble lignin while also all "oligomeric sugars" are converted to monomers. However, based on Chapter 6 findings it is clear now that the described "hydrolysis of oligomeric sugars" in the patent, involves mostly the hydrolysis of EX. In the current heat treatment of $EVAP_E$ liquors no additional acid catalyst, such as H_2SO_4, is needed for EX hydrolysis because the SAW process produces its own strong soluble acid catalyst, lignosulfonic acid. The addition of an acid catalyst promotes sugars dehydration reactions leading to reduced sugar yield as is seen in earlier studies (Grisel, et al., 2014). Grisel et al. (2014) encounter high sugar losses in organosolv (94% EtOH or 95% MeOH) treatment of wheat straw with mineral acid (H_2SO_4) as catalyst. The reported losses of more than 25% are attributed to humins formation.

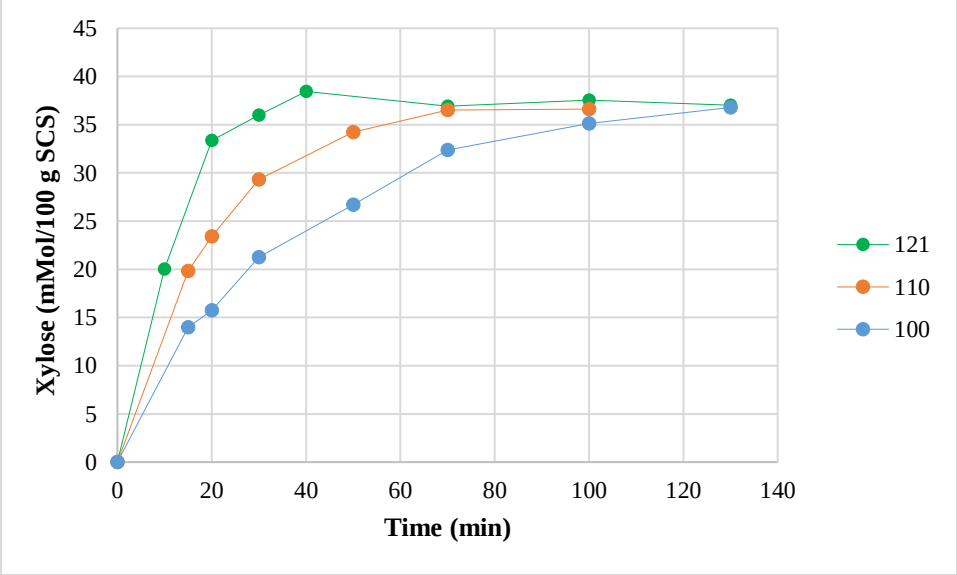

Figure 7.3: Xylose release from ethyl xylosides (EX) hydrolysis

Heat treatment of $EVAP_E$ liquor also releases EtOH as is shown in Figure 7.4. This is expected based on reaction (12). After rotary evaporation of MSEW the resultant liquor ($EVAP_E$) still has liquor ~0.1 g L^{-1} (Table 7.4) EtOH. This corresponds to 0.7 mMol EtOH per 100 g SCS. This amount is subtracted from the EtOH concentration in the hydrolyzed liquor, and the corrected EtOH concentrations are plotted in Figure 7.4. Based on the stoichiometry of reaction (12) and the results in Figure 7.4 it can be calculated that the maximum EtOH concentration after complete

EX hydrolysis would be 38 mMol. However, the maximum EtOH concentration in the heat-treated liquors reaches a value of about 78 mMol which is almost twice as high (see Figure 7.4). The EtOH concentration at complete EX hydrolysis point (121˚C, 70 min) is still higher than expected value (70 mMol vs 38 mMol). Other possible sources of EtOH are ethylated lignin and/or ethyl pyranosides/furanosides of sugars other than xylan. We did not measure ethyl pyranosides of other sugars (arabinose, galactose, glucose, mannose) in MSEW liquors but their presence is likely, albeit at much lower concentrations in comparison to EX. Thus, the most likely source for the additional EtOH is ethylated-lignin. Bauer et al. (2012) shows that acidic organosolv treatment of Miscanthus with EtOH (95% EtOH, 0.2 M HCL, 8 h reflux) results in modification of the lignin structure. Under these conditions, EtOH behaves as a nucleophile and ethoxylates the α-hydroxyl group of the lignin propane side units containing a ß-O-4 linkage. The α-ethoxylation is the only major lignin modification seen in this ethanolsolv pretreatment process. Similar findings have been reported by Lancefield et al. (2017) for organosolv treatment (10 mL g^{-1}, 0.2 M HCL, Alcohol/water: 95:5 ROH/H2O, 6 h reflux) of different lignocellulosic biomass (beech, Walnutshell and Douglas Fir). Interestingly, nearly 96% of the propane side units containing a ß-O-4 linkage are α-ethylated for all these species at 110°C, while a lower degree of ethoxylation is observed under reflux conditions at the azeotropic boiling point of 78.15°C (65% beech, 71% walnutshell, 84% Douglas fir) indicating that lignin ethoxylation occurs at relatively low temperatures. Hydrolysis of these α-ethoxylated linkages are reported to occur under relatively mild acidic conditions (0.1M HCL, 2:1 dioxane/water, 100°C, 4 h) to produce native-like lignin (Lancefield, et al., 2017). Thus, heat treatment of SAW liquor not only leads to xylosides hydrolysis but also to lignin de-ethoxylation which is a very important finding for commercial implementation of organosolv processes since full EtOH recovery is needed for a viable biochemical conversion technology. The advantage of the SAW technology is that there is no need to add any additional mineral/organic acids since the inherently present lignosulfonic acid provides the required acidity for the hydrolysis reactions.

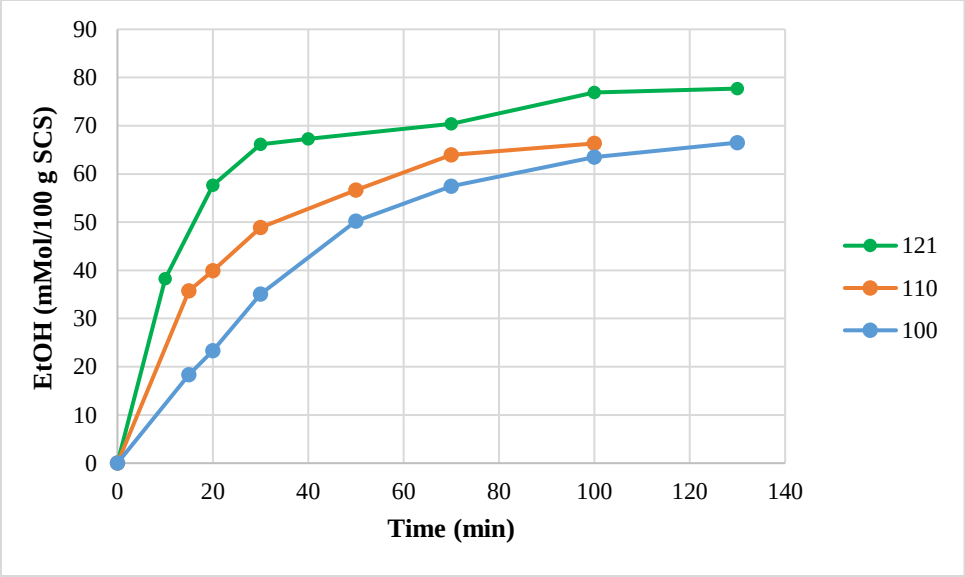

Figure 7.4: Ethanol release from ethyl xylosides (EX) hydrolysis

To confirm lignin ethoxylation, the lignin from fresh spent liquor was precipitated and then subjected to NMR analysis (more details in Chapter 8). The precipitated lignin samples were prepared by removing alcohol and SO_2 from spent liquor through rotary evaporation and then adding DI water (see section 8.2). The degree of ethoxylation was quantified by analyzing the precipitated lignin samples using headspace-isotope dilution (HS-ID) GC-MS method based on hydroiodic acid cleavage of alcoxy groups (Sumerskii, et al., 2017). The NMR data (see section 8.5) confirms that soluble lignin produced during SEW fractionation undergoes alcoholysis reaction as described in earlier studies (Bauer, et al., 2012; Lancefield, et al., 2017). The alcoxy (methoxy, ethoxy and iso propoxy) groups chemically bound to lignin produced in the three SAW processes were quantified and are shown in Figure 7.5. The lignin precipitate is corrected for sugars retained in the samples (~5%), and the molecular weight of lignin is taken as 204.24. Molar weight (MW) of 204.24 g mol^{-1} is used for SCS lignin based on typical structures of G, S and H phenylpropane units and their weight distribution as described in section 4.3. As expected all three lignin samples contain a significant amount of methoxy groups as it is composed of mostly guaiacyl and syringyl units. The methoxyl content is higher in SMW lignin because of methoxylation of lignin in the SMW fractionation process.

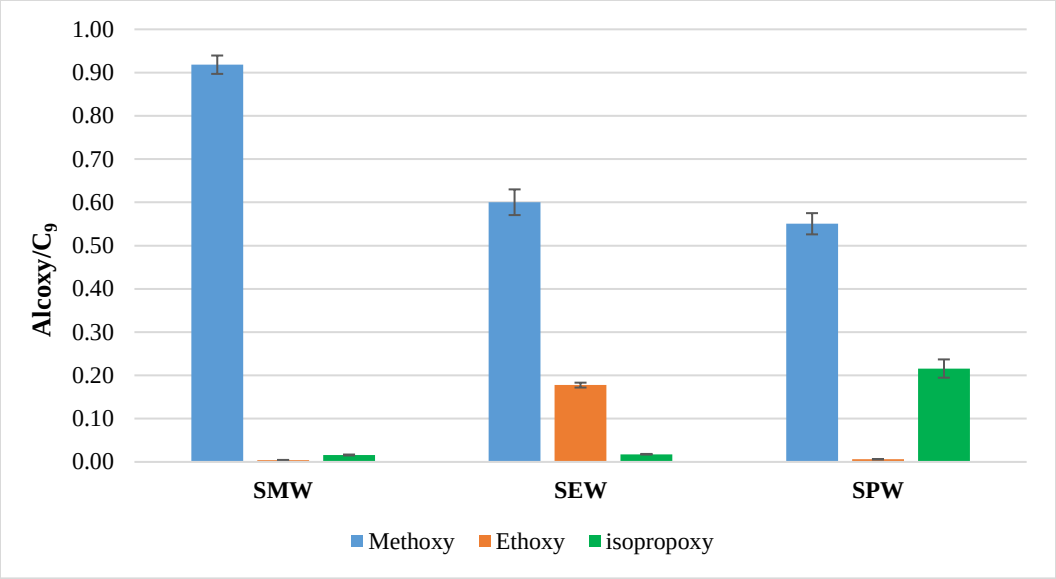

Figure 7.5: Content of methoxy, ethoxy and isopropoxy functional groups in SAW lignins

Based on Figure 7.5 it can be calculated that nearly 0.18 mol ethoxy groups are present per mol of SEW lignin. Hence, there are total 16.4 mMol ethoxy groups (per 100 g SCS) chemically bound to dissolved SEW lignin (based on 18.6% lignin, Table 7.3). Complete hydrolysis of these linkages produces 16.4 mMol EtOH/100 g SCS, so the maximum amount of EtOH produced by EX and SEW lignin hydrolysis is 54.4 mMol of EtOH/100 g SCS (38 from EX + 16.4 from lignin). Since this is still smaller than the maximum of 70 mMol EtOH/100 g SCS at complete EX hydrolysis (see Figure 7.1), it was investigated whether the difference of 15.6 (70-54.4) mMol EtOH/100g SCS could be produced from hydrolysis of ethyl pyranosides/furanosides of other sugars in the spent liquor.

Hydrolysis converts all pyranosides/furanosides as well as sugar oligomers into monomeric sugars. Table 7.6 shows the concentration of non-xylose monosaccharides in $EVAP_E$ and $EVAP_P$ spent liquors before and after hydrolysis. Non-xylose monomeric sugars in fresh $EVAP_E$ are measured and compared with their corresponding quantities in the completely hydrolyzed liquor (121˚C, 70 min). Similar analysis is done for fresh $EVAP_P$ and hydrolyzed liquor (121˚C, 30 min) discussed later in this chapter (section 7.5). The increase in non-xylose sugar content after hydrolysis indicates the contribution from hydrolysis of corresponding pyranosides/furanosides and oligomers in fresh spent liquors. When neglecting the contribution from oligomers (only glucomannan) it can be calculated that the maximum increase in monosugars caused by hydrolysis of pyranosides/furanosides could be 15.2 mMol of EtOH/100 g SCS, which is nearly the same as the earlier identified difference of 15.6 mMol/100 g SCS.

However, for unequivocal verification that the additional ethanol (15.2 mMol) is coming from the non-xylose pyranoside/furanosides quantification is needed of the pyranosides/furanosides of all other sugars than xylose.

Table 7.6: Non-xylose sugars mass and molar balance for fresh and hydrolyzed spent liquors

	$EVAP_E$			**$EVAP_P$**		
	Fresh	Hydrolyzed	Pyr/Fur	Fresh	Hydrolyzed	Pyr/Fur
Arabinose	2.4 (16.0)	3.2 (21.3)	0.8 (5.3)	2.5 (16.6)	2.8 (18.8)	0.3 (2.2)
Galactose	0.5 (2.8)	0.7 (4.1)	0.2 (1.3)	0.6 (3.1)	0.6 (3.6)	0.0 (0.5)
Glucose	3.5 (19.4)	4.7 (26.3)	1.2 (6.9)	4.4 (24.4)	4.7 (25.9)	0.3 (1.5)
Mannose	0.4 (2.0)	0.7 (3.7)	0.3 (1.7)	0.5 (2.6)	0.7 (3.8)	0.2 (1.2)
Total	6.8 (40.2)	9.3 (55.4)	2.5 (15.2)	8.0 (46.7)	8.8 (52.1)	0.8 (5.4)

Quantities are reported as g/100 g SCS. The numbers in brackets shows concentration as mMol/100 g SCS

7.5 Isopropyl pyranosides hydrolysis

$EVAP_p$ contains 4.7 g PX (per 100 g SCS) as shown earlier in Table 7.4. This represents 3.7 g of the equivalent xylose. Comparing this with isopropyl xylosides in fresh SPW spent liquors and MSPW (Table 7.4) indicates that addition of the wash liquors increases the amount of PX confirming that PX is washed out of the cooked SCS. Table 4.7 also shows that rotovap treatment of MSPW liquor at 80°C to produce $EVAP_P$ is unable to hydrolyze PX at this temperature as was also found for EX. Subsequently the $EVAP_P$ liquors which are essentially free of SO_2 and IPA are subjected to heat treatment at temperature >80°C. The effect of heat treatment temperature on PX hydrolysis is shown in Figure 7.6. The PX hydrolysis rate increases with increasing temperature and complete hydrolysis is achieved in about 30 minutes at 121°C. It takes about 100 minutes at 110°C for complete hydrolysis but $EVAP_P$ still has about 0.3 g or 6.4% PX remaining after 2 h heat treatment at 100°C.

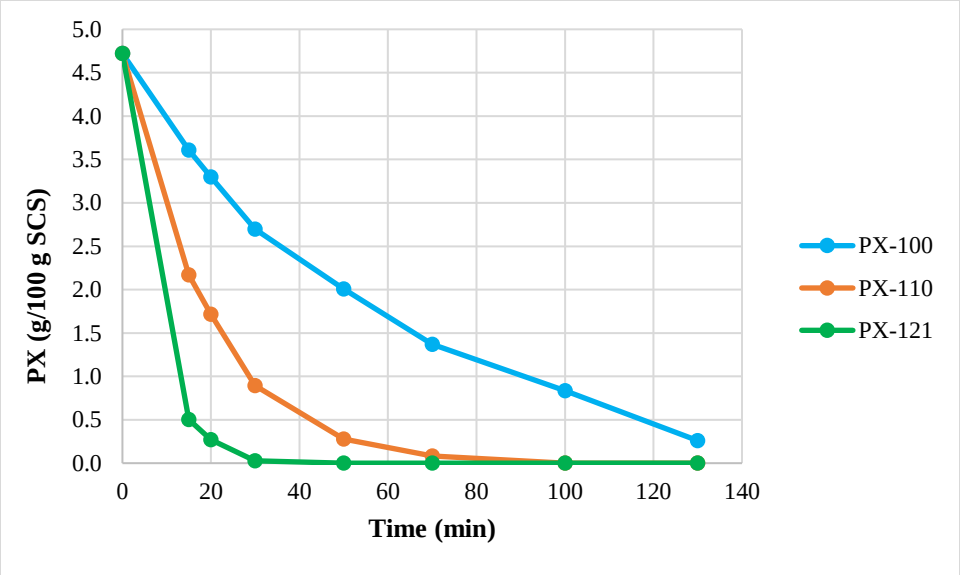

Figure 7.6: Isopropyl xylosides (PX) hydrolysis at different temperatures

The initial PX content of $EVAP_P$ of 4.7 g/100 g SCS is equivalent to 25 mMol/100 g SCS. As for EX, a natural logarithm plot of the molar concentration of PX in the heat-treated liquors vs reaction time at different temperatures is shown in Figure 7.7. The straight lines again indicate a first order hydrolysis reaction for PX as is found for EX. The first order reaction rate constants calculated from the slopes of straight lines (Figure 7.7) are listed in Table 7.5. It shows that the PX hydrolysis rate constants are higher than those of EX at 100, 110 and 121°C, and the PX hydrolysis is 10 times faster at 121°C than at 100°C. This is reflected by the high activation energy of PX hydrolysis (130.7 kJ mol^{-1}) which is about two times higher than that of EX. This high activation energy might be related to steric hindrance offered by the isopropyl groups. Therefore, it is more efficient to perform heat treatment on $EVAP_P$ at high temperatures for complete IPA recovery and xylose release.

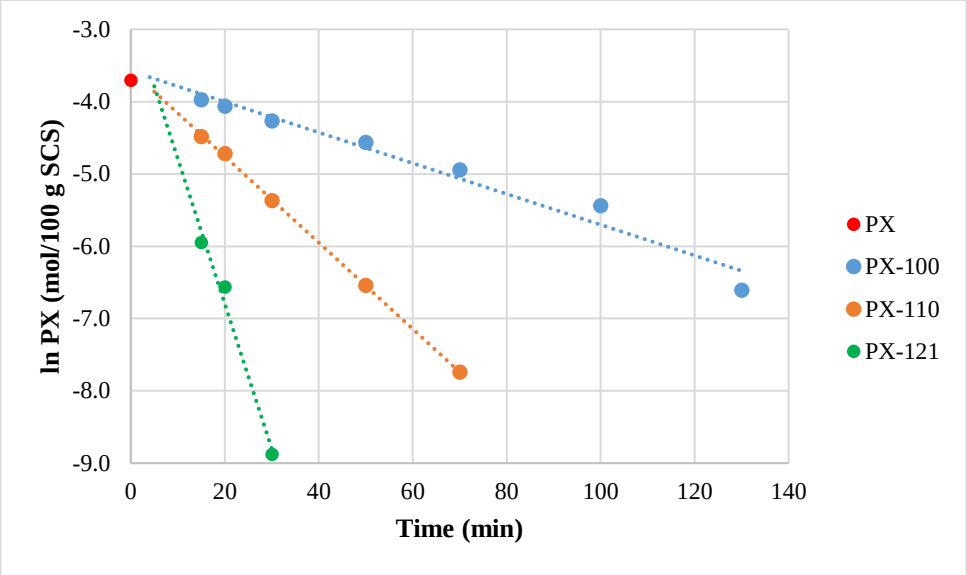

Figure 7.7: Kinetics of isopropyl xylosides (PX) hydrolysis

The maximum amount of xylose released during heat treatment of $EVAP_P$ at different temperatures is 22 mMol as shown in Figure 7.8. This is slightly smaller than the initial amount of 25 mMole PX/100 g SCS. However, the maximum molar amount of IPA formed is significantly larger than 25 mMole/100 g SCS at 110 and 121˚C as can be seen in Figure 7.9. Based on the stoichiometry of the PX hydrolysis reaction:

$$C_8H_{16}O_5 + H_2O \leftrightarrow C_3H_7OH + C_5H_{10}O_5 \quad (13)$$

Complete hydrolysis of PX should produce 25 mMol of xylose and IPA per 100 g SCS. The maximum 22 mMol of xylose obtained suggests that a small amount of xylose is dehydrated/condensed to furfural and humins respectively. This is supported by the finding that about 4 mMol furfural was present in heat-treated liquor after 130 minutes at 121°C.

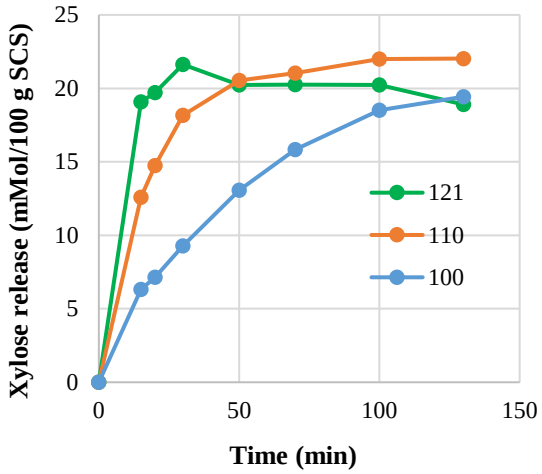

Figure 7.8: Xylose release from isopropyl xylosides (PX) hydrolysis

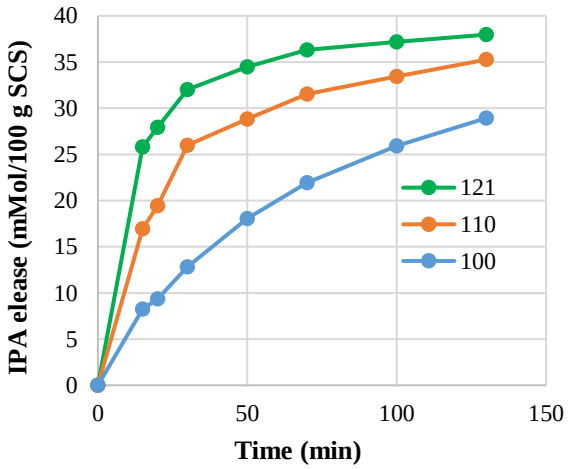

Figure 7.9: Isopropanol (IPA) release from isopropyl xylosides (PX) hydrolysis

As with EX hydrolysis, the concentrations of IPA higher than 25 mMol suggests that the solvent is also covalently bound to lignin and other sugars than xylose. $EVAP_P$ liquor treated at 121°C for 30 minutes contains 32 mMol IPA, i.e. 7 mMol (or 28%) more than expected from PX hydrolysis stoichiometry. Figure 7.5 shows that SPW lignin contains 0.22 mol isopropoxy units per mol of lignin. This isopropoxy/C_9 ratio is similar to ethoxy/C_9 suggesting that lignin alcoholysis is determined by the reactivity of lignin and not the solvent. This data implies that complete de-isopropoxylation of dissolved lignin from SPW fractionation will produce 20 mMol IPA. Thus, complete hydrolysis of PX and lignin produces 45 mMol IPA which is more than the maximum (32 mMol) found in $EVAP_P$ heat treated liquor (121˚C, 30 min). This can be related to comparatively slower de-alcoxylation of isoproxylated than ethoxylated lignin in the presence of lignosulfonic acids. The argument is supported by Lancefield et al. (2017) who reports better de-etherification of ethanol than butanol lignins in acidic media (0.1 M HCL in 2:1 dioxane/water at 100˚C).

We have not analyzed isopropyl pyranosides/furanosides of non-xylose sugars in MSPW liquors for two reasons; (1) No standard compounds are available for quantification, (2) the concentrations of non-xylose isopropyl pyranosides/furanosides are likely much lower than the corresponding ethyl compounds because of the smaller extent of this reaction with longer chain alcohols (Drouet, et al., 1994). However, an analysis of hydrolyzed and fresh $EVAP_P$ liquors from Table 7.6 suggests that hydrolysis of pyranosides/furanosides of non-xylose sugars can produce no more than 5.4 mMol IPA. This amount might have contributed to extra IPA found in the hydrolyzed $EVAP_P$ liquors.

7.6 Conclusions

Spent liquors from SEW and SPW fractionation of SCS containing significant amounts of ethyl xylosides (EX) and isopropyl xylosides (PX) respectively are subjected to heat treatment to recover alcohol and chemically bound xylose. The kinetics of alcohol xylosides hydrolysis shows that EX and PX are completely hydrolyzed to xylose and their respective alcohols. The hydrolysis does not require addition of any mineral acids since lignosulfonic acids, present in the spent liquors, provide the required acidity for the alcohol xylosides hydrolysis reaction. The rate of hydrolysis is higher for PX than EX at temperatures of 100 °C or higher, while the hydrolysis does not or only slightly occur at 80°C. At 121°C the hydrolysis is complete after 30 or 70 minutes respectively. Longer times at this temperature promotes xylose dehydration reactions. Higher concentrations of alcohols (EtOH or IPA) are obtained than the stoichiometric amounts expected from hydrolysis of the respective alcohol xylosides. It is argued that the additional alcohol is mostly coming from hydrolysis of ethoxylated (or isopropoxylated) lignin and of alcohol pyranosides of other sugars than xylose (i.e. arabinose, galactose, glucose and mannose) which were not quantified in the current study. SEW and SPW lignins contains 16.4 and 20 mMol ethoxy or isopropoxy groups (per 100 g SCS) respectively and their molar ratio suggests that lignin alcoholysis is determined by the reactivity of lignin and not the solvent. These alcoxy groups are most likely linked to alpha position of ß-O-4 linkages in lignin based on earlier literature. The present study reveals that alcohol and monomeric sugars are completely recoverable from the SAW process by simple heat treatment of the solvent-depleted spent liquor at 100 to 121 °C.

Chapter 8

LIGNIN MODIFICATIONS: EFFECT OF SOLVENTS

8.1 Introduction

Lignin is the 2nd most abundant renewable material on earth with cellulose being the first (Hu, et al., 2011; Min, et al., 2013; Kuhad & Singh, 2007). In contrast to most of other natural polymers, which have single inter-monomeric linkage, lignin contains many different carbon-to-carbon and ether linkages. It is almost impossible to isolate lignin in pure form because of strong physical and chemical linkages between lignin and the cell wall polysaccharides (Holtmam, et al., 2003). That's why it is hardly being used for making value added products despite its high availability. Lignin can be separated from lignocellulosic biomass by mechanical or chemical means. Milled wood lignin (MWL) is considered native-like lignin that is extracted from finely ball milled biomass using aqueous dioxane (Bjorkman, 1954; 1956). Some modification in MWL, including cellulolytic enzymatic lignin (CEL) (Chang, et al., 1975) and enzymatic mild acidolysis lignin (EMAL) (Wu & Agrypopoulos, 2003) are included to decrease the amount of carbohydrates contamination. These separation technologies produce lignin at low yields and are energy intensive because biomass milling process extends hours to weeks (Guerra, et al., 2006). Kraft and acid sulfite are two well-known chemical pulping processes that produce cellulosic pulp and a spent liquor stream rich in lignin. In the Kraft process, lignin undergoes major modifications and the lignin containing spent liquor is burnt in a chemical recovery cycle to meet energy requirements and recover the inorganic pulping chemicals (Kouisni, et al., 2011). Kraft lignin can be recovered from the spent pulping liquor by acid precipitation (Kouisni, et al., 2011) However, Kraft lignin is not as reactive as sulfite lignin, called lignosulfonates, has many applications such as surfactants, dispersants, binder, emulsion agent etc. (Yang, et al., 2007). The number of mills producing lignosulfonates has reduced over time because of the difficulty to compete with Kraft pulp. In contrast to Kraft and acid sulfite, organosolv pulping processes are suggested as a route to produce pure and sulfur-free lignin as a byproduct. Such lignin without major chemical modifications is referred to as native lignin. It is reported that SO_2-ethanol-water (SEW) produces pure lignin that can be easily separated from spent liquor stream without further acidification by simple evaporation of ethanol (Retsina & Pylkkanen, 2013). However, the lignin obtained from SEW fractionation contains sulfonate groups just as sulfite lignin. The degree of sulfonation of spruce SEW lignin has been reported (Iakovlev & van Heininhen, 2012b) but no mention was made of possible modifications such as ethoxylation of the lignin. In view of recent findings that lignin

was modified during organosolv fractionation (Bauer, et al., 2012; Lancefield, et al., 2017), SAW lignin obtained from SCS fractionation was further analyzed, and the effect of the solvent used (methanol, ethanol and isopropanol) on lignin modification was studied.

8.2 Lignin separation and analysis

SCS was fractionated for 1 hour at 155˚C. The cooking liquor composition and other pulping conditions have been described earlier (see Chapter 3 and Chapter 5). Fresh spent liquors (SMW, SEW and SPW) were subjected to rotary evaporation to remove SO_2 and alcohol. After 50-60% mass reduction of the spent liquors by rotary evaporation, the remaining suspension was transferred into 50 mL Corning vials and DI water (30-35 mL) added to further precipitate the lignin. The precipitated lignin was separated by centrifugation (4000 rpm, 10 minutes). The obtained samples were freeze dried (-80˚C) and then put into a vacuum oven dryer (vacuum ~10^{-4} Torr) for at least 48 hours. The final lignin samples obtained after processing of SMW, SEW and SPW spent liquors are shown in Figure 8.1, 8.2 and 8.3 respectively. The obtained SPW lignin after above mentioned processing was lighter in color in comparison to SMW and SEW lignins. One of the reasons may be less lignin condensation reactions in SPW fractionation or removal of certain chromophores in the washing step. The precipitated lignin samples shown in Figures 8.1-8.3 were analyzed for sulfur, molecular weight distribution and degree of alcoholysis/alkylation.

Figure 8.1: Precipitated lignin from SMW fractionation

Figure 8.2: Precipitated lignin from SEW fractionation

Figure 8.3: Precipitated lignin from SPW fractionation

The amount of lignin remaining in the pulp was calculated from the kappa number and pulp yield (see equation 1, Chapter 4). The amount of lignin in the spent liquors was calculated from the difference between the original lignin content in SCS and that in the pulp. Original SCS and pulps were ground in a Wiley mill before total sulfur analysis.

The amount of sulfur in these solids and in the precipitated lignin samples was determined after digesting the solids following the EPA-3051 method and then analyzing for sulfur using ICP-AES.

Molecular weight distributions of the dissolved lignin samples were obtained by dissolving 5 mg of lyophilized samples in a 2 mL of DMSO+0.5% LiBr (w/w) solution. After filtration of the samples through 0.45 µm PTFE filters, size exclusion chromatography (SEC) was performed with SEC 1260 Infinity (Polymer Standard Services, Germany). The equipment consisted of an isocratic pump (G1310B), a micro degasser (G1379B) and a standard autosampler (G1329B). The detection system included a UV detector (G1314B) in series with a refractive index detector (G1362A). The mobile phase was DMSO+0.5% LiBr set to a constant flow rate of 0.5 mL min^{-1} for a total run time of 65 minutes. The injection volume was 100 µL. The separation system consisted of PSS GRAM Precolumn, PSS GRAM 100 Å and PSS GRAM 10 000 Å analytical columns thermostated at 60˚C and connected in series. The pullulan standards with nominal masses of 708 kDa, 337 kDa, 194 kDa, 47.1 kDa, 21.1 kDa, 9.6 kDa, 6.1kDa, 1.08 kDa and 342 Da were used for standard calibration.

2D-NMR was performed on the precipitated lignin samples dissolved in DMSO: Pyridine = 4:1. All spectra were acquired on a Bruker Avance II 400 MHz with a BBFO broadband probe head with z-gradients. HSQC spectra were acquired in the edited mode to get information about the different carbon bonds visualized in plots with different colors: CH + CH_3 blue and CH_2 in red. The relaxation delay in the HSQC was set to 0.5 s. Methoxy, ethoxy and isopropoxy functional groups in SMW, SEW and SPW precipitated lignin samples were quantified using a recent method developed based on headspace-isotope dilution (HS-ID) GC-MS (Sumerskii, et al., 2017). This method employs an isotopically labeled internal standard 4-(methoxy-d3)-benzoic acid and 4-(ethoxy-d5)-benzoic acid, which, together with lignin, undergoes standard hydroiodic acid cleavage of methoxyl and ethoxyl groups, followed by headspace GC-MS analysis of the respective deuterated and non-deuterated iodomethanes and iodoethanes.

8.3 Lignin sulfonation

The degree of sulfonation of SCS lignin for the three SO_2-organosolv pulping processes is shown is Table 8.1. The results from other recent studies conducted on SEW fractionation of spruce, softwood and hardwood biomass and SCS (Iakovlev & van Heiningen, 2012b; Sklavounos, et al., 2013a; Yamamoto, et al., 2014b) are also included in Table 8.1. The degree of sulfonation is expressed as the S/C_9 ratio for the pulp lignin and the precipitated lignin. Molar weight (MW) of 204.24 g mol^{-1} is used for SCS lignin based on typical structures of G, S and H phenylpropane units

(see section 4.3) and their weight distribution (G:S:H of 68:28:4) (del Río, et al., 2015; Quesada-Medina, et al., 2010). All other studies cited in Table 8.1 employ a lignin MW of 190 for different biomass species for S/C_9 determination, however using same MW for SCS makes insignificant difference (0.01). The yield of precipitated lignin was 30-45% (based on total dissolved lignin) for 50-60% spent liquor mass reduction by rotary evaporation. The precipitated lignins also contained 4.5-8.0% sugars that were corrected for sulfur and latter alcoxy groups analysis. It can be seen in Table 8.1 that the S/C_9 ratio of residual lignin for the three SAW fractionation systems is the same (about 0.17) within experimental error. You et al. (2017a) reports same findings for SEW fractionation of SCS. This shows that the solvent has no effect on the degree of lignin sulfonation, in agreement with Iakovlev and van Heiningen (2012b) who found the same S/C_9 ratio for AS and ethanol-AS pulping systems and concluded that the presence of ethanol does not affect lignin sulfonation. The percentage sulfur on oven dried feedstock (odf) basis in the three residual lignins is also same. The higher sulfur content in SEW pulps (0.03 vs 0.06 %odf) reported by You et al. (2017a) can be explained by sulfur present in the original SCS that does not participate in sulfonation process. Despite the same S/C_9 and percentage sulfur (%odf), SMW pulp has higher lignin content (2.11%). The difference in percentage residual lignin can be explained by solubility of lignin in methanol, ethanol and isopropanol cooking liquors (see section 4.3). The comparison of δ-values of the cooking liquors with δ-value of SCS lignin (28.47 $MPa^{1/2}$) shows that the SPW liquor is the best SCS lignin solvent among three solvents, while SMW cooking liquor is the poorest one. Iakovlev and van Heiningen (2012b) suggest that less degree of sulfonation at which lignosulfonates dissolution become possible, is required in a cooking liquor of significantly higher solubility (water vs ethanol-water). Therefore, a high degree of sulfonation is required to dissolve lignin in methanol in comparison to ethanol and isopropanol. The S/C_9 comparison of residual lignin from SEW fractionation of SCS with other biomass species indicates the ratio is slightly higher for SCS lignin. These small differences may be related to variations in the lignin structures of SCS and wood biomass. One major difference between wood and annual plant lignin is that the latter contains all three lignin units (G, S and H) and significant amount of hydroxycinnamic acids (Lam, et al., 1992). Also, straw lignin is more susceptible towards sulfonation and condensation due to presence of more phenolic groups in comparison to wood species.

Table 8.1: Sulfonation of lignin in SAW fractionation of SCS

Fractionation		Residual lignin	Sulfur in pulp		Sulfur in precipitated lignin	
Feedstock	**Process**	**% odf**	**% odf**	**S/C$_9$**	**% odf**	**S/C$_9$**
SCS	SMW	2.11±0.49	0.05±0.02	0.14±0.03	0.10±0.00	0.10±0.00
SCS	SEW	1.06±0.09	0.03±0.01	0.18±0.01	0.10±0.02	0.09±0.00
SCS	SPW	0.95±0.07	0.03±0.00	0.17±0.02	0.09±0.00	0.08±0.00
SCS[a]	SEW		0.06	0.15	0.22	0.13
Spruce[b]	SEW	3.33	0.07	0.12	n.m	n.m
Spruce[c]	SEW	3.06±0.34	0.05	0.09	n.m	n.m
SW[d]	SEW	8.7	0.16	0.11	n.m	n.m
HW[e]	SEW	2.0	0.04	0.11	n.m	n.m
SW[f]	SEW	9.7	0.13	0.08	n.m	n.m
Spruce[g]	SEW	5.0	0.08	0.10	n.m	n.m

n.m – not measured
a- (You, et al., 2017a) (L/F = 4 L kg^{-1}, 155˚C, 68 min, 12% SO_2)
b- (Iakovlev & van Heiningen, 2012b) (L/F = 4.8 L kg^{-1}, 135˚C, 80 min, 12% SO_2)
c- (Iakovlev & van Heiningen, 2012b) (L/F = 6 L kg^{-1}, 135˚C, 80 min, 12% SO_2)
d,e- (Yamamoto, et al., 2014b) (L/F = 6 L kg^{-1}, 150˚C, 30 min, 12% SO_2)
f,g- (Sklavounos, et al., 2013a) (L/F = 3 L kg^{-1}, 150˚C, 30 min, 12% SO_2)

Table 8.1 also shows the amount of sulfur in the precipitated lignin. About same amount of sulfur (0.10% odf) is found in methanol, ethanol and isopropanol precipitated lignins and their S/C$_9$ values are also nearly identical (~0.09). Again, this confirms that degree of lignin sulfonation is independent of the solvent used. Iakovlev and van Heiningen (2012) report that highly sulfonated lignin is preferentially dissolved in SEW fractionation but the S/C$_9$ values of the current SCS precipitated lignin is two times smaller than its corresponding residual lignin (0.09 vs 0.18). It means that dissolved lignin is composed of highly sulfonated and less sulfonated lignin and the sulfur lean lignin get precipitated upon solvent removal. Therefore, the lignin is not evenly sulfonated in SAW fractionation. This agrees with earlier studies on SEW fractionation of different biomass species including SCS (Iakovlev & van Heiningen, 2012b; Sixta, et al., 2013; You, et al., 2017a). However, nearly same S/C$_9$ for pulp and precipitated lignin reported by You et al. (2017a) seems doubtful based on this analysis. The consumption of sulfur by lignin (residual + precipitated) in three solvent systems is same indicating that the choice of solvent in SAW fractionation does not affect the SO_2 recovery process. The cooking liquor in SCS fractionation has a high initial concentration of SO_2, (~125 g L^{-1}) but sulfonation degree is low for each system. It indicates that only small amount of SO_2 is consumed in the

fractionation process and the rest can be recovered by distillation as suggested by Iakovlev and van Heiningen (2012b) and You et al. (2017a).

The high residual lignin in pulps of biomass other than SCS is also seen in Table 8.1, indicating that SCS lignin is more easily removed than wood species. The amount of sulfur in the pulp based on original feedstock is high for SW biomass (Table 8.1). However, the corresponding amounts in pulps from other species including SCS are one order of magnitude smaller (% odf). Yamamoto et al. (2014b) attributes this to high inorganics (especially sulfur) in the original feedstock. The SW and HW biomass used in Yamamomo et al. (2014b) had 0.03 and 0.01 (%odf) sulfur respectively. SCS used in the current study has high sulfur content (0.06%) but the amount of sulfur in SEW pulp is 0.04% similar to spruce and HW pulps. Therefore, the initial amount of sulfur in feedstock may change the sulfur content of pulp but it does not significantly affect the lignin sulfonation.

The effect of fractionation temperature on degree of lignin sulfonation is shown in Table 8.2. The S/C_9 values for SMW, SEW and SPW pulp lignin are the same irrespective the temperature and fractionation system (within experimental error). Comparison with the results obtained in other SEW studies (Table 8.1) show that the type of feedstock and severity of fractionation have only minor influence on the S/C_9 ratio (values of about 0.1-0.2). However, these values are smaller than those obtained in acid sulfite process (S/C_9 of 0.34 for pulp) which confirms that the absence of bisulfite anions in SMW, SEW and SPW fractionation process leads to a much lower lignin sulfonation degree (Iakovlev & van Heiningen, 2012b).

Table 8.2: Temperature effect on lignin sulfonation

	Temperature	**Lignin**	**Sulfur in pulp**	
	°C	**%odf**	**%odf**	**S/C_9**
SMW	145	2.82	0.07	0.16
	155	2.67	0.07	0.17
SEW	145	1.58	0.05	0.19
	155	1.16	0.03	0.19
SPW	145	1.25	0.04	0.20
	155	1.03	0.03	0.19

8.4 Molecular weight distribution of SAW lignin

The physiochemical properties of lignin are very important in polymer product development. The molecular weight of lignin is a key parameter that affects its physiochemical properties. The data from Figure 8.4 and Table 8.3 suggest that SAW lignin possess characteristics of many other organosolv lignins and therefore may find applications

in the synthesis of materials that require good tensile strength, toughness, brittleness, hardness and abrasive resistance. Figure 8.4 shows the molecular weight distribution for precipitated lignin obtained from SMW, SEW and SPW fractionation of SCS. The curves are calculated based on first order calibration curve (log M vs elution volume) combined with concentration data obtained from an RI detector. All three lignin samples show a very similar and broad molecular weight distribution with a clear low MW lignin fraction.

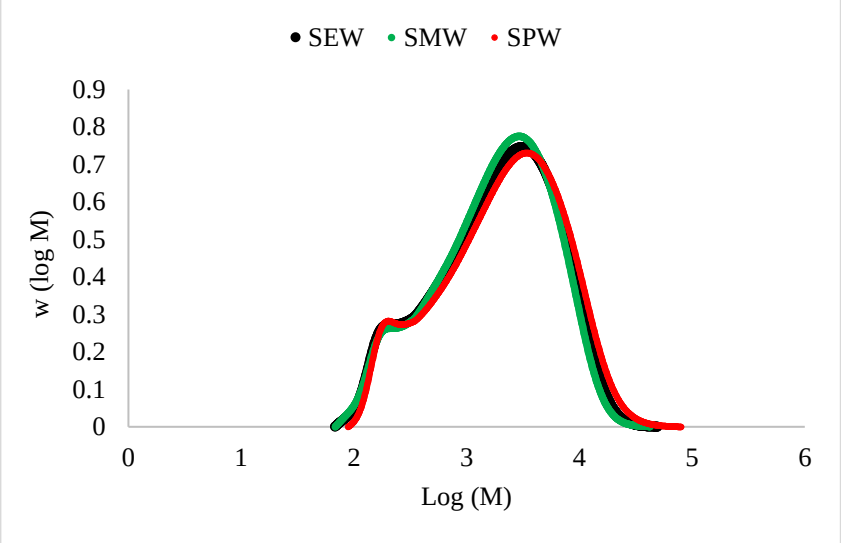

Figure 8.4: Molecular weight distribution of SMW, SEW and SPW lignin

The different average molecular weight numbers are shown in Table 8.3. The molecular weights in Table 8.3 are obtained from calibration curves based on pullulan standards. Therefore, these values should be considered as relative instead of absolute weights. The average molecular weights (M_w, M_n, M_z and M_p) increase in the following order SMW<SEW<SPW and same trend is seen for the polydispersity index (D). It is interesting to notice that alcohols used in the SAW fractionation of SCS can be arranged in same order based on their molar mass (g mol^{-1}) i.e. MeOH (32.04)< EtOH(46.06)<IPA (60.1). Therefore, alcoholysis of lignin might be partially (if not completely) responsible for molecular weight increase from methanol to isopropanol lignin. Likewise, with carbohydrates covalently attached to lignin (Sun, et al., 2012), extra alcohol groups (methoxy, ethoxy or isopropxy) may increase the hydrodynamic volume and hence MW of lignin. Table 8.4 shows the extent of alcoxy groups in precipitated lignin samples (corrected for sugars). The values ≤0.1 shows the contamination and experimental errors in the analysis. Each lignin sample has about 2.8 mM methoxy groups per gram of precipitated lignin. SMW lignin shows 4.5 mM methoxy groups indicating that 1.7 mM (4.5-2.8 = 1.7) methanol from SMW liquor is covalently bound to SCS lignin. The degree of lignin

alcoxylation for SPW is lower than SMW but higher than SEW {SEW (0.9)<SPW (1.1)<SMW (1.7)} therefore higher M_w for SPW lignin is plausible. Sulfonation also changes the MW (Fredheim, et al., 2003) but same degree of sulfonation is seen for the three lignins (see Table 8.1). Therefore, difference in MW of SMW, SEW and SPW lignin because of sulfonation is not convincing.

Table 8.3: Average molecular weights and polydispersity of SAW lignin

g mol^{-1}	**SMW**	**SEW**	**SPW**
M_n	798±20	868±57	912±0
M_w	2807±351	3368±122	3831±16
M_z	5994±903	7559±402	9447±69
D (M_w/M_n)	3.5±0.4	3.9±0.1	4.2±0.0
M_p	2091±195	2368±58	2561±21

Table 8.4: Alcoxy groups in precipitated lignin (mM/g lignin)

Lignin/Alcoxy group	**Methoxy**	**Ethoxy**	**Isopropoxy**
SMW	4.5+0.1	0.0+0.0	0.1+0.0
SEW	2.9+0.1	0.9+0.0	0.1+0.0
SPW	2.7+0.1	0.0+0.0	1.1+0.1

Determined using HS-ID GC-MS method (Sumerskii, et al., 2017)

The weight average molecular weight (M_w) of SMW, SEW and SPW lignin is within the range (500-4000) of organosolv lignins in contrast to lignosulfonates which have one order of magnitude higher M_w (20,000-50,000) (Espinoza-Acosta, et al., 2016). The high M_w of lignosulfonates is attributed to their high sulfur content (5%) as described by Espinoza-Acosta et al. (2016) while this is significantly lower for SAW lignins (0.1%). Polydispersity index values of SAW lignins are also similar to organosolv lignin (1.3-4.0) (Delmas, et al., 2011; Espinoza-Acosta, et al., 2016). A comparatively high M_w (5715) and plausible polydispersity (2.96) are reported for organosolv lignin obtained from Miscanthus when treated with ethanol (50%) and H_2SO_4 (0.5% w/w) at 150˚C for 60 minutes (Obama, et al., 2012). In contrast, She et al. (2012) reports low values of M_w (1248-1462) for lignins obtained from organosolv treatment of rice straw with methanol, ethanol, n-propanol, and butanol at low temperature treatment (75˚C, 3 h, L/F = 25). No clear correlation between MW of wheat straw lignin and the employed solvent is seen in that study. It is anticipated that no lignin alcoxylation occurs at low temperature treatment. Tolbert et al. (2014) relates all these differences to physiochemical properties of the lignin that depend on biomass source, pretreatment conditions and separation method.

8.5 2D HSQC NMR analysis of precipitated lignin

The three precipitated SAW lignin samples were analyzed by NMR to identify the structural modifications in SCS lignin when subjected to SAW fractionation using methanol, ethanol and isopropanol. The HSQC spectra for SMW, SEW and SPW lignin were acquired in edited mode to get information about different carbon types. The final HSQC spectra for SMW, SEW and SPW lignin are shown in Figure 8.5, 8.6 and 8.7 respectively. In the HSQC plots CH +CH_3 carbons are shown in blue while CH_2 is in red. Lignin and carbohydrates correlation assignments based on the earlier literature (Rencoret, et al., 2011; She, et al., 2012; Bauer, et al., 2012; Santos, et al., 2015; Lancefield, et al., 2017) are shown in Table 8.5 and Table 8.6 respectively. The HSQC 2D NMR spectra show three regions corresponding to aliphatic (δ_C/δ_H 10-50/0.5-3.0 ppm), side chain (oxygenated aliphatic) and sugar anomerics (δ_C/δ_H 50-110/3.0-5.8 ppm) and aromatic/olefinic ^{13}C-^{1}H (δ_C/δ_H 90-150/5.5-8.0 ppm) correlations for SMW, SEW and SPW lignins. The aliphatic region shows high intensities of a wide variety of saturated aliphatic moieties. Strong signals observed in the aliphatic region (δ_H 0.8 and 1.2-1.3) are assigned to extractives, mainly of lipid nature in accordance with earlier literature (Balakshin, et al., 2003; Martin-Sampedro, et al., 2011). For SEW and SPW lignins, signals at 1.0-1.2 ppm correspond to alcoholic groups (i.e. ethoxy or iso-propoxy) introduced by lignin reaction with employed alcohol in the SCS fractionation process confirmed by HMBC and HSQC-TOCSY experiments.

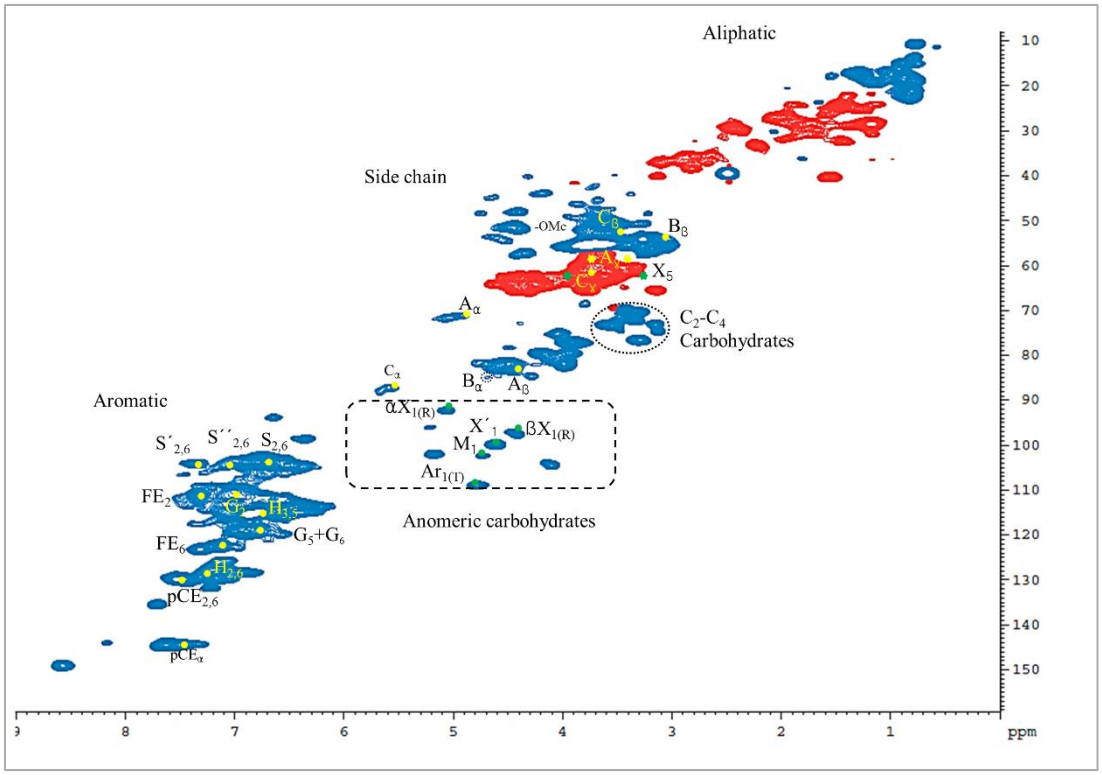

Figure 8.5: 2D-HSQC spectrum of SMW lignin from SCS fractionation

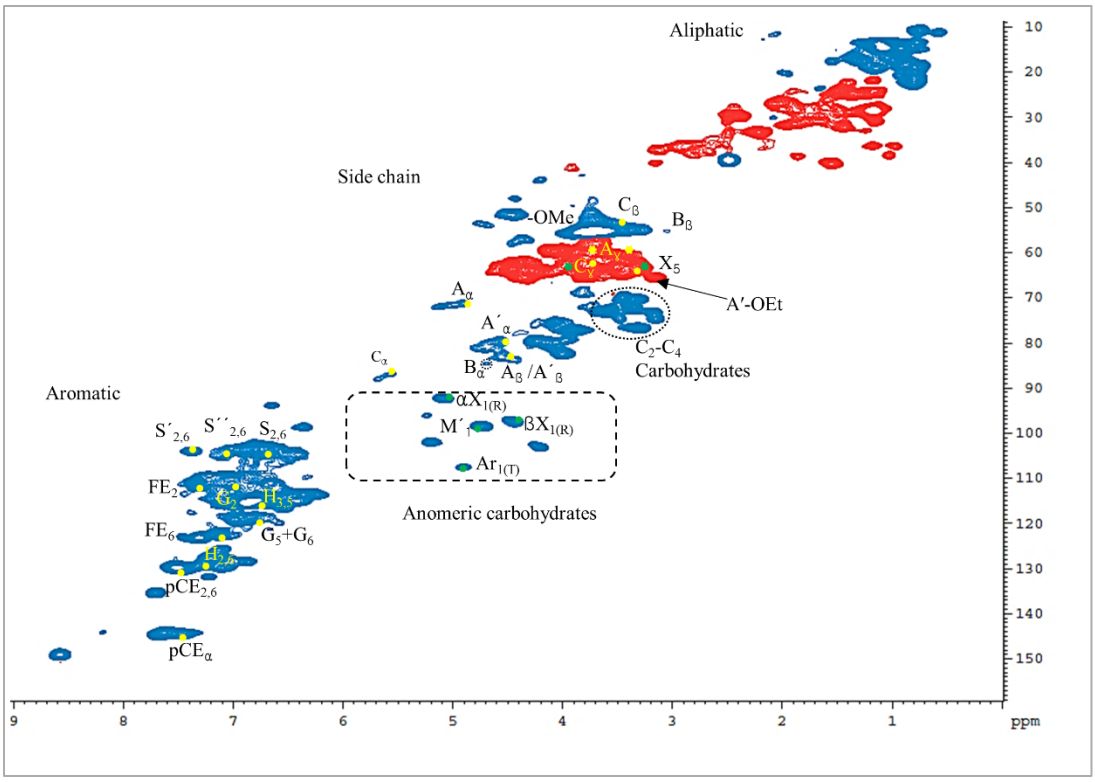

Figure 8.6: 2D-HSQC spectrum of SEW lignin from SCS fractionation

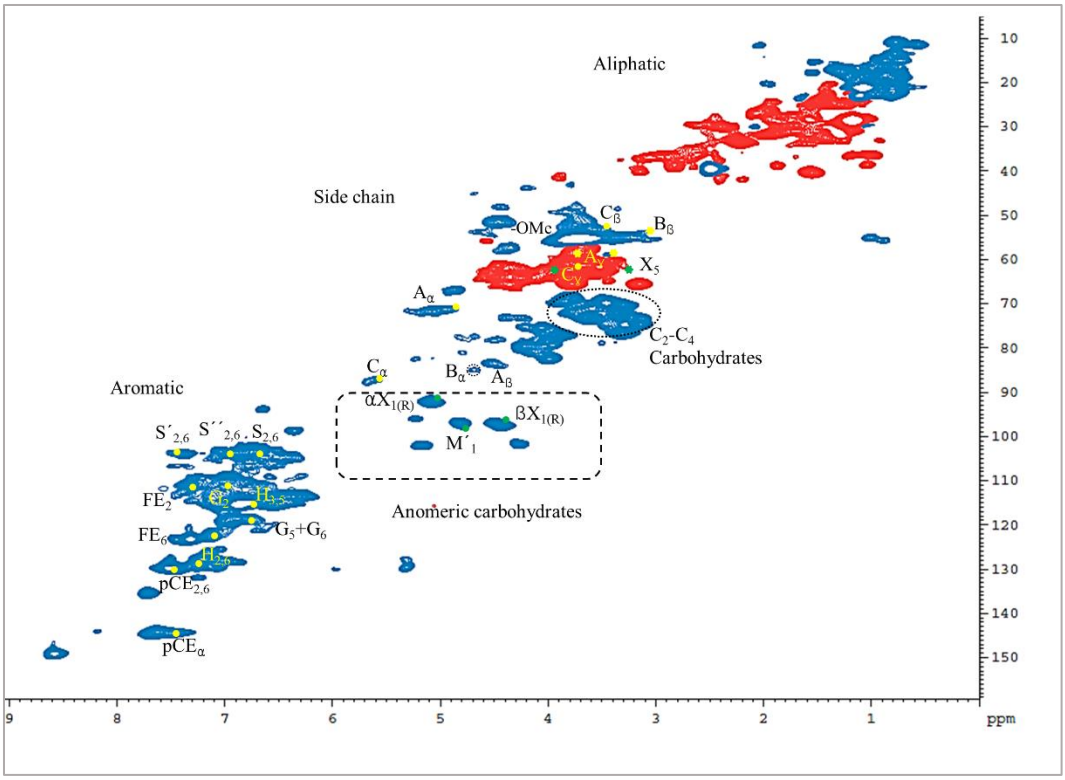

Figure 8.7: 2D-HSQC spectrum of SPW lignin from SCS fractionation

Table 8.5: Assignments of the lignin ^{13}C-^{1}H correlation signals in the HSQC spectra of SAW lignin from SCS

Labels	δ_C/δ_H ppm	Assignment
$C_ß$	53.5/3.46	$C_ß$-$H_ß$ in phenylcoumaran substructures (C)
$B_ß$	55.0/3.05	$C_ß$-$H_ß$ in resinol substructures (B)
-OMe	55.9/3.73	C-H in methoxyls
$A_ɣ/A'_ɣ$	59.3/3.46 and 3.70	$C_ɣ$-$H_ɣ$ in ß-O-4 substructures (A/A´)
$C_ɣ$	62.5/3.72	$C_ɣ$-$H_ɣ$ in phenylcoumaran substructures (C)
A-OEt	63.6/3.32	C-H in α-OEt ß-O-4 substructures A´
$B_ɣ$	71.0/3.83 and 4.19	$C_ɣ$-$H_ɣ$ in resinol substructures (B)
$A_α$	71.1/4.88	$C_α$-$H_α$ in ß-O-4 substructures (A)
$A'_α$	79.9/4.61 and 81.2/4.76	$C_α$-$H_α$ in ß-O-4 substructures (A´)
$A'_{ß(G)}$	82.1/4.39 and 83.1/4.30	$C_ß$-$H_ß$ in ß-O-4 linked to G units (A´)
$A_{ß(G)}$	83.5/4.28	$C_ß$-$H_ß$ in ß-O-4 linked to G units (A)
$A'_{ß(S)}$	84.4/4.20 and 84.6/4.11	$C_ß$-$H_ß$ in ß-O-4 linked to S units (A´)
$B_α$	84.2/4.7	$C_α$-$H_α$ in resinol substructures (B)
$A_{ß(S)}$	85.8/4.11	$C_ß$-$H_ß$ in ß-O-4 linked to S units (A)
$C_α$	86.8/5.53	$C_α$-$H_α$ in phenylcoumaran substructures (C)
$S_{2,6}$	103.8/6.7	$C_{2,6}$-$H_{2,6}$ in etherified syringyl units (S)
$S'_{2,6}$	104.2/7.37	$C_{2,6}$-$H_{2,6}$ in oxidized ($C_α$ = O) phenolic syringyl units (S´)
$S''_{2,6}$	104.5/7.03	$C_{2,6}$-$H_{2,6}$ in oxidized ($C_α$OOH) phenolic syringyl units (S´´)
G_2	110.8/6.96	C_2-H_2 in guaiacyl units (G)
$H_{3,5}$	115.2/6.74	$C_{3,5}$-$H_{3,5}$ in p-hydroxyphenyl units (H)
FE_2	110.8/7.30	C_2-H_2 in ferulic ester (FE)
G_5	115.5/6.84	C_5-H_5 in guaiacyl units (G)
G_6	119.0/6.77	C_6-H_6 in guaiacyl units (G)
FE_6	122.4/7.09	C_6-H_6 in ferulic ester (FE)
$H_{2,6}$	129.7/7.02	$C_{2,6}$-$H_{2,6}$ in p-hydroxyphenyl units (H)
$pCE_{2,6}$	129.82/7.48	$C_{2,6}$-$H_{2,6}$ in p-coumaroylated substructures (A´´)
$pCE_α$	144.1/7.47	$C_α$-$H_α$ in p-coumaroylated substructures (A´´)

Assignments are based on (Rencoret, et al., 2011; She, et al., 2012; Bauer, et al., 2012; Santos, et al., 2015; Lancefield, et al., 2017)

In the side chain region, methoxylated lignin (-OMe) was prominent and detected at δ_C/δ_H 55.9/3.73 ppm. Different interunit linkages present in SAW lignin and carbohydrates were also observed in this region. The carbohydrates were distinct in two differentiated regions of the spectra: side chain region and anomeric carbohydrates region. All spectra showed prominent signals for ß-O-4 ether units (substructure A, see Figure 8.8). The $C_α$-$H_α$, $C_ß$-$H_ß$ and $C_ɣ$-$H_ɣ$ correlations in in ß-O-4 substructures were seen at δ_C/δ_H 71.1/4.88 ppm, δ_C/δ_H 83.4-85.9/4.10-4.29 ppm and δ_C/δ_H 59.3/3.46-3.70 ppm respectively. However, these $C_ß$-$H_ß$, correlations showed overlapping signals for structures linked to G or S lignin units. Likewise, $C_ɣ$-$H_ɣ$ in ß-O-4 substructure overlapped with other signals. Some additional α-ethoxylated ß-O-4 substructures (A´) were also seen in SEW lignin samples by comparing its ^{13}C-^{1}H

correlations with recent literature (Bauer, et al., 2012; Lancefield, et al., 2017). The C_{α}-H_{α} correlations for this substructure were detected at δ_C/δ_H 79.9/4.61 and δ_C/δ_H 81.2/4.76 ppm. Bauer et al. (2012) showed that $C_{ß}$-$H_{ß}$ correlations for α-ethoxylated ß-O-4 substructures appeared at δ_C/δ_H 82.1/4.39 ppm and δ_C/δ_H 83.1/4.30 ppm when linked to G lignin units while the corresponding chemical shifts for S units were 84.4/4.20 and 84.6/4.11 ppm. No change in chemical shift for C_{γ}-H_{γ} correlations was reported when ß-O-4 substructures were ethoxylated at the α-position (Bauer, et al., 2012). No signals were detected for $C_{ß}$-$H_{ß}$ correlations for α-ethoxylated ß-O-4 substructures when linked to S units however, low intensity cross peaks could be found for G units. The signals for CH_2 in α-OEt ß-O-4 substructures A´(δ_C/δ_H 63.6/3.32) could overlap with X_5 signals (δ_C/δ_H 63.2/3.26 and 3.95 ppm) in ß-O-4-xylopyranoside (see Figure 8.6). Therefore, a clear distinction was not possible. However, current analysis and comparison with earlier findings show that SCS lignin has undergone some degree of ethoxylation during SEW fractionation as three different types of ethoxy groups were identified in SEW lignin. Ether type ethoxy groups were found at 63.8/3.30-3.50 ppm as well as at 14.8/1.10 ppm. These linkages are most likely α-OEt ß-O-4. Other ether type ethoxy groups were seen at chemical shift of 62.4/3.60-3.75 and 14.8/1.10 ppm. Ester-type ethoxy groups appeared at 59.5-60.2/4.0-4.15 and 13.7/1.10-1.20 ppm. SPW lignin also showed signals for two different type of linkages with isopropoxy groups. These linkages were ether (69.3/3.80 ppm and 23.2/1.10 ppm) and ester (67.4/4.90 ppm and 21.4/1.10 ppm) type. Therefore, alcoholysis reactions are certain when biomass is treated with alcohols (i.e. methanol, ethanol, isopropanol) in acidic media. The hydroiodic acid cleavage quantification of methoxy, ethoxy and isopropoxy groups in SMW, SEW and SPW lignin samples also supports this argument (see Table 8.4). However, it was hard to confirm alcoxy linkages with HMBC as well as HSQC-TOCSY as sugars obscured the signals from lignin side chains.

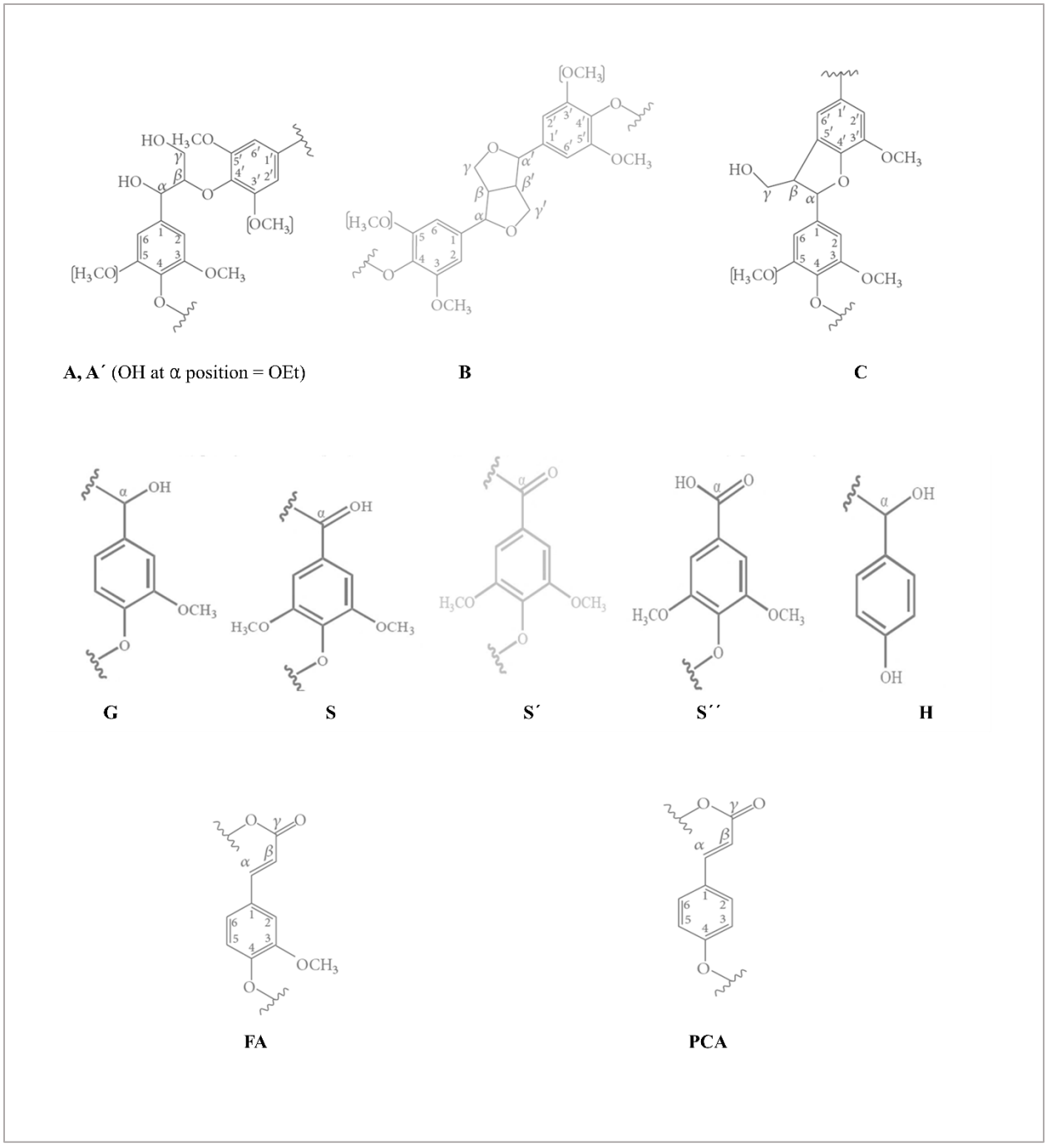

Figure 8.8: Typical structures of identified aromatic units in SAW lignin [structures adapted from (She, et al., 2012; Santos, et al., 2015)]

Besides the major ß-O-4 substructures, other linkages such as ß-ß (B, resinol) and ß-5´(C, phenylcoumaran) were also found in the side chain region of all lignin samples (model compounds shown in Figure 8.8). The C_{α}-H_{α} and $C_{ß}$-$H_{ß}$ correlations for resinol (B) were observed at δ_C/δ_H 84.2/4.7 ppm and 55.0/3.05 ppm respectively however

no signals were detected for C_{γ}-H_{γ} at δ_C/δ_H 71.0/3.83 and 4.19. Similarly signals at 86.8/5.53, 53.5/3.46 and 62.5/3.72 ppm corresponded to C_{α}-H_{α}, C_{β}-H_{β} and C_{γ}-H_{γ} correlations in phenycoumaran (C) substructures. B substructures were relatively low in comparison to C substructures as shown by low intensity of their corresponding signals for C_{α}-H_{α}. The C_{β}-H_{β} and C_{γ}-H_{γ} correlations for these structures were difficult to compare because of the overlapping signals with other structures. It proves that ß-ß and ß-5´linkages remain stable in SAW fractionation of SCS and solvent has no apparent effect on these linkages.

All the lignin samples also contained sugars that are shown as cross peak signals in the δ_C/δ_H 67-75/3.0-3.8 ppm region. The C-H correlations for anomeric carbohydrate were reflected by δ_C/δ_H signals in the 90-110/4.0-5.5 region as shown in Table 8.6. No signals were detected for anomeric (1→4)-ß-D-galactopyranoside and (1→4)-ß-D-glucopyranoside in this region. The SMW lignin was different because it showed cross peaks for (1→4)-ß-D-mannopyranoside (M_1 at δ_C/δ_H 101.2/4.69) that was absent in SEW and SPW lignin samples. The SEW lignin was different from the SPW in respect of α-L-Arabinofuranoside (T) ($A_{r1(T)}$ at δ_C/δ_H 108.3/4.91) cross peaks appearing in SMW and SEW samples only. No ^{13}C-^{1}H cross peaks were identified for α-L-Arabinofuranoside (A_{r1} at δ_C/δ_H 107.3/5.05 ppm) for any of the lignin samples. The presence of carbohydrates especially xylan in SAW lignins shows that a certain proportion of carbohydrates was removed as lignin carbohydrate complex (LCC) in SAW fractionation of SCS.

Table 8.6 Assignments of the carbohydrates ^{13}C-^{1}H correlation signals in the HSQC spectra of SAW lignin from SCS

Labels	δ_C/δ_H ppm	Assignments
$\alpha X_{1(R)}$	92.5/5.02	α-D-xylopyranoside (R) [α-D-Glucopyranoside (R)]
$\beta X_{1(R)}$	97.7/4.39	ß-D-xylopyranoside (R) [ß-D-Glucoopyranoside (R)]
U_1	97.7/5.32	4-O-Methyl-α-D-GlcUA
M'_1	99.1/4.78	2-O-Acetyl-ß-D-mannopyranoside
X'_1	99.8/4.58	2-O-Acetyl-ß-D-xylopyranoside
M_1	101.2/4.69	(1→4)- ß-D-mannopyranoside
Gl_1	103.3/4.41	(1→4)- ß-D-glucopyranoside
Ga_1	105.6/4.40	(1→4)- ß-D-galactopyranoside
Ar_1	107.3/5.05	α-L-Arabinofuranoside
$Ar_{1(T)}$	108.3/4.91	α-L-Arabinofuranoside (T)

(R)- Reducing end; (T)- Terminal (non-reducing) end
Assignment are based on Rencoret et al., 2011

In the HSQC spectra of SWM, SEW and SPW lignin, the main signals at δ_C/δ_H 100-150/6.0-8.0 corresponded to aromatic ring based lignin units. In all three lignin samples tricin could be detected. The chemical shifts of 94.0/6.1 and 98/4.2 ppm in Figure 8.5-8.7 corresponded to these structures. The S lignin units showed a dominant signal for $C_{2,6}$–$H_{2,6}$ correlations at 103.8/6.7 ppm. The overlapping signals for oxidized $C_{2,6}$–$H_{2,6}$ syringyl units (S´ and S´´) were observed at 104.5/7.32 and 104.5/7.03 ppm. The G units gave different correlations for C_2-H_2 (δ_C/δ_H 110.8/6.96 ppm), C_5-H_5 (δ_C/δ_H 115.5/6.84 ppm) and C_6-H_6 (δ_C/δ_H 119/6.77 ppm). Cross peaks for p-hydroxyphenyl units (H) were also detected in the HSQC spectra at 115.2/6.74 ppm and 129.7/7.02 ppm corresponding to $C_{3,5}$-$H_{3,5}$ and $C_{2,6}$-$H_{2,6}$ correlations respectively. The $C_{2,6}$-$H_{2,6}$ signals of H units were partially overlapped with esterified p-coumarates (pCE) signals that had $C_{2,6}$-$H_{2,6}$ correlations at δ_C/δ_H 129.8/7.48 ppm. Besides the p-coumarates, ferulates ester (FE) were also found in SAW lignin. Two different peaks at 110.8/7.30 ppm and 122.4/7.09 belonged to 2-position and 6-position in ferulic ester (FE) respectively. The presence of ferulates (FE) and p-coumarates (pCE) in SCS suggests the association of lignin with polysaccharides through these linkages (Kim & Ralph, 2010). These linkages typically acylate arabinoxylans in grasses with higher degree of acylation with ferulates (Lam, et al., 2001; Kim & Ralph, 2010). She et al. (2012) suggests that ferulates are also involved in lignification, cross linking with lignin monomers and oligomers. The presence of FE and pCE in SAW precipitated lignin samples again confirms that a certain portion of lignin is removed as LCC in the SAW fractionation process.

8.6 Conclusions

SCS lignin is easily separated from SAW spent liquor because its low degree of sulfonation renders the lignin insoluble in a more aqueous environment after alcohol evaporation and wash water addition. Despite the high initial concentration of SO_2, (~125 g L^{-1}) in the cooking liquors, sulfonation degree is low for SMW, SEW and SPW fractionation systems. The degree of sulfonation (S/C_9) of residual lignin is also low (0.1-0.2) in comparison to the AS process (S/C_9 = 0.34) and this agrees with earlier studies on SEW fractionation of different species. The use of methanol, ethanol or isopropanol in SAW pulping leads to the same degree of lignin sulfonation. However less lignin dissolves in SMW because of the poorer solubility of sulfonated lignin in this liquor. The fractionation temperature does not affect the lignin sulfonation. Moreover, the amount of sulfur in feedstock material hardly change the degree of sulfonation. The MWD of SAW lignin shows that it has characteristics like many other organosolv lignins with a M_w of ~3000 g mol^{-1} and polydispersity index of ~4.0. The SEW and SPW lignins have slightly higher MW's than SMW lignin, possibly because of lignin alcoholysis in acidic media in accordance with the recent literature. The HSQC spectra of precipitated SAW lignins show that it contain different ether-type and ester-type covalently bound alcoxy groups. The HSQC-NMR spectra and quantification of alcoxy groups by hydroiodic acid cleavage confirm lignin alcoholysis reactions in SAW fractionation of SCS. However, we do not have absolute remarks regarding alcoxy cross linkages with lignin side chains as these signals are obscured by sugars in HMBC or HSQC-TOCSY spectra. Some sugars, ferulates esters and esterified p-coumarates are present in precipitated SAW lignin samples, a typical characteristic of grass and straw lignins. It indicates that some lignin is removed as LCC in SAW fractionation of SCS retaining the linkages between lignin and carbohydrates present in native lignin.

Chapter 9

CONCLUSIONS AND RECOMMENDATIONS

The SO_2-Alcohol-Water (SAW) process was evaluated in terms of solvent effects on sugarcane straw (SCS) fractionation. The fractionation experiments were conducted using methanol, ethanol or isopropanol in a cooking liquor of fixed composition (SO_2:Alcohol:Water = 12:44:44 w/w%) and liquor to feedstock ratio (4 L kg^{-1}). SCS was successfully fractionated into cellulosic pulp, dissolved hemicelluloses and lignin using either of the solvents. Delignification kinetics were developed by correcting Klason lignin for acid insoluble ash and permanganate non-oxidizable material. SCS delignification kinetics can be described by three phases; initial, bulk and residual. About 50-60% lignin was removed in the initial phase. The bulk delignification was pseudo 1st order in lignin, while the residual phase showed very slow delignification like wood species. In terms of delignification, isopropanol showed the most promising results because of its higher lignin dissolution characteristics. The delignification rate constants for ethanol at each temperature (135, 145 and 155˚C) were close to that of isopropanol, while methanol resulted in slower delignification. The delignification activation energy data indicated that temperature had a more pronounced effect with ethanol and isopropanol (E_a = 60-65 kJ mol^{-1}) than with methanol (E_a = 35 kJ mol^{-1}). Overall the bulk delignification rate constants for present SEW fractionation system were lower in comparison to wood species (spruce and beech) and this was attributed to decreased effective acidity because of the high inorganics content of SCS (6.1%).

Xylan was the most abundant hemicellulose found in SCS (21.2%) and its removal proceeded in two phases; initial and bulk phase. About 50% xylan was removed in the initial phase most probably in the form as lignin carbohydrate complex (LCC). The bulk xylan removal phase followed pseudo 1st order kinetics for each solvent system. The xylan removal rate was the lowest with isopropanol at low temperature (135˚) in comparison to methanol and ethanol. However, at higher temperatures (145, 155˚) the rate difference became negligible leading to comparatively high activation energy for the isopropanol system. This could be explained by high increase in proton activity with temperature and less polar solvents i.e. isopropanol. Also, the delignification vs xylan removal selectivity decreased with increasing temperature for each solvent system. Therefore, the process temperature affects the product properties.

Although cellulose was almost completely retained in the solid phase, it underwent hydrolysis as indicated by its decrease in degree of polymerization. Ethanol and isopropanol pulps showed a sharp decrease in intrinsic viscosity

at kappa number around 25, while the same trend was observed at much higher lignin content for methanol pulps (kappa ~45). The slow hydrolysis in the first phase was attributed to lignin and carbohydrates providing a protective barrier reducing cellulose accessibility. It was concluded that cellulose hydrolysis was more influenced by hemicellulose removal than delignification. Cellulose hydrolysis followed zero order kinetics, and the rate constants for cellulose hydrolysis at different temperatures (i.e. their activation energies) showed a trend very similar to the xylan removal kinetic parameters. The activation energy for cellulose hydrolysis was highest with isopropanol (130 kJ mol^{-1}) in comparison to methanol and ethanol (80 kJ mol^{-1}) and it was explained by increased hydronium ion activity with temperature and isopropanol. The selectivity of delignification vs cellulose hydrolysis was highest for isopropanol at low temperature. Therefore, isopropanol was recommended as the most suitable solvent to produce high viscosity and low lignin content pulp especially at lower temperatures. High viscosity pulp could also be produced using ethanol but with comparatively less selectivity. The use of methanol for paper grade pulp production was not suggested as lower viscosity (<900 mL g^{-1}) was observed even at high kappa number values (~45).

The spent liquors produced from SMW, SEW and SPW fractionation of SCS were composed of dissolved lignin, monomeric and oligomeric sugars and alkyl pyranosides. Carbohydrates oxidation, dehydration and degradation products were insignificant in the spent liquor streams. The product distribution was highly dependent on the severity of fractionation designated as CSF. The highest concentration of total carbohydrates in the spent liquors (27.5% w/w SCS or 60 g L^{-1}) was obtained at CSF of 2.4±0.1 (145°C, 100-120 min and/or 155°C, 60 min). It was recommended to stop the fractionation at CSF of 2.4±0.1 because at higher severity carbohydrates degradation reactions resulted in decreased dissolved sugars yield. The maximum level of monomeric sugars in SMW, SEW and SPW spent liquors were 20.8±0.7, 31.1±1.7 and 37.0±0.5 g L^{-1} respectively. This represented 36%, 54% and 64% of the total sugars in the respective spent liquors. The spent liquors were analyzed for alkyl xylosides being the most prominent alkyl pyranosides in SMW, SEW and SPW spent liquors. The maximum concentrations of alkyl xylosides and monomeric xylose were also found at same CSF (2.4±0.1) indicating that they are in equilibrium at the fractionation operating conditions. The concentration of monomeric xylose in SPW and SEW spent liquor were 3.0 and 1.9 times more than respective alkyl xylosides. On the other hand, SMW spent liquor contained 1.4 times more methyl xylosides than monomeric xylose. In view of these results it was suggested that SMW fractionation process could generate a high yield byproduct i.e. methyl xylosides. However, the pulp obtained would not be suitable for

paper production because of its low viscosity and high lignin content (η~850 with kappa 45 or η ~500 with kappa 36) instead it might be further hydrolyzed for biofuel production.

SEW and SPW spent liquors were further subjected to heat treatment after alcohol and SO_2 evaporation to recover chemically bound xylose and alcohol from alkyl xylosides. The kinetics of alkyl xylosides hydrolysis showed that the ethyl xylosides (EX, sum of alpha and beta) and isopropyl xylosides (PX, sum of alpha and beta) were completely hydrolyzed to xylose and respective alcohols at each temperature (100, 110, 121˚C). At 121˚C, PX and EX were completely hydrolyzed in about 30 and 70 minutes respectively while hydrolysis was slower at 100 and 110 ˚C. The hydrolysis of alkyl xylosides was performed without addition of any mineral acid as the acidity provided by lignosulfonic acids formed during fractionation was sufficient for alkyl xylosides hydrolysis. The rates of alkyl xylosides hydrolysis and activation energy were higher for PX in comparison to EX. The alkyl xylosides hydrolysis rate constants for PX were 1.3 times and 3.8 times higher than corresponding EX rate constants at 100˚C and 121˚C respectively. In both cases, alkyl xylosides hydrolysis followed 1st order kinetics with activation energies of 66 and 130.7 kJ mol^{-1} for EX and PX respectively. The hydrolysis was much faster at high temperature treatment (121˚C) but it also promoted xylose dehydration to furfural. Therefore, low temperature treatment (100, 110˚C) of spent liquor was recommended for alkyl xylosides hydrolysis. More alcohol (ethanol or isopropanol) was obtained than the stoichiometric from hydrolysis of the respective alkyl xylosides. It was suggested that hydrolysis of alkyl pyranosides of non-xylose sugars (mostly glucose) and some lignin de-etherification were responsible for the excess alcohol release.

Lignin analysis revealed that only a small amount of sulfur was consumed in SAW fractionation of SCS for each solvent system indicating the potential of nearly complete recovery of SO_2. The same degree of sulfonation was found for residual lignin (in pulp) and precipitated (from spent liquor) lignin for the methanol, ethanol and isopropanol systems. Therefore, lignin sulfonation is not affected by the nature of the solvent, but the high residual content of methanol pulps suggests that lignin dissolution is highly affected by solvent solubility properties. The molecular weight distribution (MWD) curves of SCS lignin precipitated after SMW, SEW or SPW fractionation showed, like many other organosolv lignins, a M_w of ~3000 g mol^{-1} and polydiversity index of ~4.0. However, SEW and SPW lignins showed a slightly higher MW's than SMW lignin that was attributed to lignin alcoholysis in acidic media in accordance with the recent literature. An effort was made to identify extra alcoxy groups in SAW lignin through 2D

NMR analysis. Lignin alcoholysis was confirmed by identifying different types (ether and ester) of alcoxy linkages in HSQC spectra of precipitated lignin samples. The hydroiodic acid cleavage quantification of methoxy, ethoxy and isopropoxy groups also supported the lignin alcoholysis argument. However, no absolute conclusions were possible regarding alcoxy group linked to lignin side chains because of interfering signals from sugars in HMBC or HSQC-TOCSY spectra. 2D HSQC spectra also indicated the presence of remaining sugars, ferulates esters and esterified p-coumarates in SMW, SEW and SPW lignin samples. It confirmed that some lignin was removed as LCC in SAW fractionation of SCS.

High mass losses (65-160 %SCS) were observed while handling fractionated SCS in open containers. These losses mostly resulted from escaping SO_2 and highly volatile solvent used in the fractionation process. Therefore, it is suggested to perform all washings and handlings in closed vessels to prevent evaporative losses. Also, SAW fractionation appears to be well suited for biofuel productions (ABE or IBE) where solid residues (containing residual solvent) and spent liquor would be enzymatically hydrolyzed to produce biofuel. Another important recommendation is to heat treat the evaporated spent liquor stream to hydrolyze the solvent pyranosides as well as the solvent etherified/esterified lignin, In that case, the solid residue stream (rich in cellulose) would be utilized to produce biofuel/solvent and the spent liquor stream, rich in xylose, would be directed towards synthesis of xylose based products such as xylitol. The lignin produced in SAW fractionation process had properties similar to the lignin produced from other organosolv process and could find applications in the production of adhesives and high strength materials. However, further research would be required in this area.

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APPENDIX A

SMW PULPS AND SPENT LIQUORS

Table A.1: Properties of pulps and spent liquors from SMW fractionation of SCS

Process parameters		**Spent liquor**	**Pulp properties**			
Temperature	**Duration**	**pH**	**Pulp yield**	**Pulp kappa**	**Pulp viscosity**	**Cellulose DP**
˚C	minutes		% SCS		mL g^{-1}	
135	30	1.20	58.9	69.9	919	6817
	50	1.21	52.4	64.2	917	5934
	70	1.14	48.6	54.1	882	5303
	90	103	44.5	43.3	843	4466
145	20	1.04	57.7	88.1	879	6240
	40	1.03	48.6	62.4	919	5213
	60	1.01	45.1	45.8	827	4385
	80	1.00	43.1	39.4	719	3636
	100	1.00	41.5	37.7	608	2961
	120	0.98	39.8	35.5	503	2282
155	20	1.06	53.6	78.1	994	6521
	30	1.05	48.1	66.9	919	5390
	40	1.06	46.3	56.7	827	4430
	60	1.00	41.1	44.7	719	3553
	80	0.97	40.8	40.7	608	2741
	100	0.93	36.8	40.3	503	2234

SO_2/MeOH/H_2O = 12:44:44 (w/w%), L/F = 4 L kg^{-1}. Cooking duration includes 10 min of heat-up.

APPENDIX B

SEW PULPS AND SPENT LIQUORS

Table B.1: Properties of pulps and spent liquors from SEW fractionation of SCS

Process parameters		Spent liquor	Pulp properties			
Temperature	**Duration**	**pH**	**Pulp yield**	**Pulp kappa**	**Pulp viscosity**	**Cellulose DP**
˚C	minutes		% SCS		mL g^{-1}	
135	30	1.20	57.7	68.5	915	6341
	50	1.17	50.3	52.2	939	5580
	70	1.13	46.3	38.9	863	4911
	90	1.13	44.2	29.6	848	4429
145	20	1.23	57.0	70.5	910	6155
	40	1.13	45.8	39.6	919	5049
	60	1.10	42.3	27.8	827	4209
	80	1.07	39.7	22.1	719	3339
	100	1.04	38.7	20.1	608	2832
	120	1.00	36.5	17.4	503	2105
155	20	1.10	49.3	55.6	935	5594
	30	1.04	44.2	40.4	919	5067
	40	0.99	41.6	28.2	827	4196
	60	0.97	38.3	22.6	719	3342
	80	0.92	36.4	20.1	608	2635
	100	0.90	34.9	21.7	503	2044

SO_2/EtOH/H_2O = 12:44:44 (w/w%), L/F = 4 L kg^{-1}. Cooking duration includes 10 min of heat-up.

APPENDIX C

SPW PULPS AND SPENT LIQUORS

Table C.1: Properties of pulps and spent liquors from SPW fractionation of SCS

Process parameters		**Spent liquor**	**Pulp properties**			
Temperature	**Duration**	**pH**	**Pulp yield**	**Pulp kappa**	**Pulp viscosity**	**Cellulose DP**
˚C	minutes		% SCS		mL g^{-1}	
135	30	1.22	57.7	64.8	873	6072
	50	1.16	48.6	50.2	940	5506
	70	1.08	45.1	33.7	922	4979
	90	1.06	44.5	27.6	921	4581
145	20	1.16	56.4	63.5	863	5575
	40	1.01	45.9	35.4	919	4919
	60	0.98	41.4	22.6	827	4254
	80	0.94	40.4	20.2	719	3493
	100	0.93	38.2	17.4	608	2692
	120	0.89	35.6	15.2	503	2070
155	20	1.20	51.2	60.0	953	5521
	30	1.10	45.2	35.1	914	4901
	40	1.04	42.5	28.6	817	4035
	60	0.96	37.9	20.4	620	2838
	80	0.89	38.0	18.2	-	-
	100	0.85	36.0	18.4	-	-

SO_2/IPA/H_2O = 12:44:44 (w/w%), L/F = 4 L kg^{-1}. Cooking duration includes 10 min of heat-up.

BIOGRAPHY OF THE AUTHOR

Asif Masih Sharazi was born in Toba Tek Singh, Punjab, Pakistan on August 20, 1988. He was raised in Toba Tek Singh, Pakistan where he completed his elementary, high school and pre-engineering (community college) education. In 2005, he moved to Lahore, the 2nd biggest city in Pakistan. Four years later he graduated with Bachelor of Chemical Engineering from the University of Engineering and Technology (UET) Lahore, Pakistan in 2009. Upon graduation, Asif enrolled in UET graduate school where he pursued his Master of Science in Chemical Engineering. In summer 2012, he was awarded a Fulbright International Scholarship to continue his studies in the United States. He entered the chemical engineering graduate program at The University of Maine Orono in the fall of 2012. He has published one article in Tappi Journal and another one in Holzforschung as a first author. Currently he has one article as a first author under peer review. He has presented his doctoral research at two conferences to audiences of his peers. He is a member of the Technical Association of Pulp and Paper Industry (TAPPI). Asif loves snow related activities including skating and skiing. He also greatly enjoys playing cricket and tennis. Asif is a candidate for the Doctor of Philosophy in Chemical Engineering from the University of Maine in August 2017.