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DEPARTMENT OF FOREST INDUSTRY ENGINEERING

PRODUCTION OF BIODEGRADABLE FILM, BIOETHANOL, AND PULP WITH INTEGRATED FOREST PRODUCT BIOREFINERY USING WHEAT STRAW AND CORN STALKS

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

ÖMER ÖZYÜREK

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DÜZCE

APPROVAL PAGE

This postgraduate study which was conducted by Ömer ÖZYÜREK under the title of "*Production of Biodegradable Film, Bioethanol, and Pulp with Integrated Forest Product Biorefinery using Wheat Straw and Corn Stalks"* was approved as a doctoral dissertation of Forest Industry Engineering by the committee convened upon the provision No 2016/439 in 16.05.2016 of the Board of Trustees of the Graduate School of Natural and Applied Sciences, Düzce University, Düzce.

This is to certify that we have read this thesis and that in our opinions it is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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DECLARATION

I hereby declare that this dissertation is my own work and effort from draft to the manuscript without any unethical attitude, and all data included have been obtained following academic and ethical values, and where other sources of information have been used, they have been acknowledged, and all the sources of information I have used are listed in the references, and I have no behavior against any action of breach of register or copyright from draft to the manuscript.

31/05/2016
Ömer ÖZYÜREK

This dissertation is dedicated to my dear family for their support, encouragement, and constant love has sustained me throughout my life.

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LIST OF SYMBOLS AND ABBREVIATIONS

°C	Celsius
4-O-MeGA	4-O-methyl glucuronic anhydride
AcG	Acetyl Groups
AcOH	Acetic Acid
ANOVA	Analysis of Variance
ASL	Acid soluble lignin
C	Carbon
cm	Centimeter
CS	Corn Stalks
DP	Degree of Polymerization
ETOH	Ethanol
FA	Formic Acid
FAO	Food and Agriculture Organization
g	Gram
h	Hour
H_2SO_4	Sulfuric Acid
HCl	Hydrochloric Acid
HMF	5-Hydroxymethyl Furfural
HPAEC	High Performance Anion Exchange Chromatography
HPLC	High Performance Liquid Chromatography
HW	Hot Water
K_2HPO_4	Potasium Phosphate
kDa	Kilodalton
kg	Kilogram
KL	Klason lignin
L	Liter
LAP	Laboratory Analytical Procedures
LiBr	Lithium Bromide
M	Molar
m^2	Square Meter

min	Minute
MJ	Megajoule
ml	Milliliter
mM	Millimolar
mol	Mole
MPa	Megapascal
MT	Metric Ton
MW	Molecular Weight
$NaBH_4$	Sodium Borohydrate
NaOH	Sodium Hydroxide
nm	Nanometer
NREL	National Renewable Resources Laboratory
OD	Oven-dry
PAD	Pulsed Amperometric Detection
RID	Refractive Index Detector
rpm	Revolutions Per Minute
SA	Sulfuric Acid
Tg	Teragram
TUBITAK	The Scientific and Technological Research Council of Turkey
UA	Uronic Anhydride
US	United States
UV	Ultraviolet
v/v	Volume/Volume
w/v	Weight/Volume
w/w	Weight/Weight
WS	Wheat Straw
$CaCl_2.2H_2O$	Calcium Chloride Dihydrate
$MgSO_4.7H_2O$	Magnesium Sulphate Hepta Hydrate
$(NH_4)_2SO_4$	Ammonium Suphate

ABSTRACT

PRODUCTION OF BIODEGRADABLE FILM, BIOETHANOL, AND PULP WITH INTEGRATED FOREST PRODUCT BIOREFINERY USING WHEAT STRAW AND CORN STALKS

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Depending on the production method, traditional paper mills often burn the black liquor for energy. The black liquor undesirably includes some soluble hemicelluloses, which has lower heating value compared to lignin. Therefore, the extraction of hemicelluloses before the pulping process is an alternative for pulp and paper mills and the extracted hemicelluloses could be converted to higher economic value by producing biofuels, biopolymers, paper additives, other chemicals etc. However, the amount of pre-extracted hemicelluloses before pulping will be regulated to maintain pulp yield and paper quality while comparing with untreated pulp.

Wheat straw and corn stalks have a significant amount of carbohydrate contents (70-75% w/w), and thus both were examined in this study in terms of the integrated biorefinery concept. The aim of this study was to pre-extract hemicelluloses from wheat straw and corn stalks by treating them with hot water, alkali (NaOH), and modified alkali ($NaBH_4$+NaOH) at varying temperatures and chemical concentrations. The pre-extracted solid materials were utilized to produce papermaking pulps. In addition, the liquid portions (pre-extracted liquids) consisting mostly of xylan were utilized to produce bioethanol and biodegradable films. Furthermore, to overcome the restrains in hot water pre-extraction, due to "sticky lignin" formation in hot water pre-extraction, formic acid (FA) reinforced autohydrolysis was examined for wheat straw at different chemical concentrations.

The obtained results were statistically analyzed by ANOVA to identify significant differences and the differences between groups were determined by the Duncan test. For wheat straw, the hot water (135 and 150 °C), alkali (16.7% NaOH at 50 °C), and modified alkali (16.7% NaOH+0.1% $NaBH_4$ at 50 °C) pre-extractions removed 16.5%, 41.5%, 33.6% and 33.9% of the xylan from the straw structure, respectively. Ethanol produced from the removed xylan for the hot water (135 and150 °C), alkali (16.7% NaOH at 50 °C), and modified alkali (16.7% NaOH+0.1% $NaBH_4$ at 50 °C) pre-extractions were yielded up to 7.79%, 16.9% 6.81%, and 4.22% (g/100 g soluble material) ethanol, respectively. In addition, good-quality biodegradable films were produced when some gluten and nanocellulose was added to the extracted xylan. The hot water pre-extracted pulp yields and the paper produced from this pulp's physical and mechanical properties were comparable to those of the corresponding conventional soda (control) pulp.

For corn stalks, the results showed that the hot water (150 °C) and alkali (26.7% NaOH at 50 °C) pre-extractions removed 44.8% and 35.6% of the xylan from the stalk structure. When bioethanol was produced from the dissolved liquid, the hot water (150 °C) and alkali (26.7% NaOH at 50 °C) pre-extractions gave 14.7% and 7.66% (g/100g soluble material) ethanol yield. Similar to wheat straw, when gluten and nanocellulose were added to the xylan, good-quality biodegradable films were produced. Additionally, the pulps produced from the hot water pre-extracted solid fractions were comparable in yield and pulp properties to the control soda pulp. It should be mentioned that modified alkali pre-extraction preserved some glucan and dissolved more lignin from the structure of wheat straw and corn stalks.

A cost efficient pretreatment technology is required for competitive sugar production at industrial implementation. Therefore, autohydrolysis would be a cost-effective process because only water/steam is needed in this application. Unfortunately, in this pretreatment technique, "sticky lignin" precipitates are formed in the hydrolysate that leads to severe plugging in the hydrolysis reactor and downstream equipment in a continuous process. In this concept, wheat straw was pretreated at temperature of 150 °C for 100 min at a liquor-to-straw ratio (L/S) of 6 L/kg (oven dry) straw. Different FA concentrations, ranging from 0 to 15 g/L, were applied during autohyrolysis. The results showed excellent sugar and lignin mass balances for pretreatment were obtained, and precipitated lignin in the hydrolysate decreased from 0.94 g/100g straw at autohydrolysis conditions (no FA) to 0.31 g/100g straw at 15 g/L of FA. The average molecular weight of precipitated lignin decreased from 1970 g/mole for autohydrolysis to 710 g/mole at 15 g/L of FA. The monomeric sugar yield during pretreatment improved dramatically with increasing FA concentration relative to autohydrolysis. Without FA the sugar yield in the hydrolysate was only 3.83 g/100g. However at a FA concentration of 15 g/L, the dissolved sugar yield increased to 23.5 g/100g. The results showed that FA reinforced autohydrolysis not only minimize the formation of "sticky lignin" but also significantly increased dissolved sugar yield.

Keywords: Biodegradable films, Bioethanol, Biorefinery, Corn stalks, Pretreatment, Sticky lignin, Wheat straw.

ÖZET

BUĞDAY VE MISIR SAPLARINDAN ENTEGRE BİYORAFİNERİ UYGULAMASI İLE BİYOBOZUNUR FİLM, BİYOETANOL VE KÂĞIT HAMURU ÜRETİMİ

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Üretim yöntemine bağlı olarak geleneksel kâğıt fabrikaları üretim sonrasında açığa çıkan ve lignine göre daha düşük ısı değerine sahip çözünmüş hemiselülozu da içeren siyah çözeltiyi genellikle enerji elde etmek amacıyla yakmaktadırlar. Son dönemlerde yapılan çalışmalarda ise pişirme öncesi hammaddeden bir kısım hemiselüloz ekstrakte edilerek kâğıt ve kâğıt hamuru üretiminin yanı sıra çözünen bu hemiselülozdan ekonomik değeri yüksek biyoyakıt, biyobozunur film ve diğer kimyasal ürünleri de üretecek şekilde araştırmaların yoğunlaştığı görülmektedir.

Bu çalışmada ülkemizde oldukça fazla miktarda atık olarak ortaya çıkan ve ekonomik değeri düşük/olmayan buğday ve mısır sapları yüksek karbonhidrat içeriğine (%70-75) sahip olmaları sebebiyle entegre orman ürünleri biyorafinerisi yöntemi ile değerlendirilerek ekonomik değer kazandırılması ve ülke ekonomisine katkı sağlanması amaçlanmaktadır. Bu amaçla hammaddeler sıcak su, alkali (NaOH) ve modifiye alkali (NaBH$_4$+NaOH) ön ekstraksiyonuyla değişik sıcaklık ve konsantrasyonlarda muamele edilerek sıvı kısımdaki çözünen ekstrakte hemiselülozdan biyoetanol ve biyobozunur film, katı kısımdan ise kâğıt hamuru ve kâğıt üretimi gerçekleştirilmiştir. Ayrıca buğday sapları düşük sıcaklık ve sürede değişik konsantrasyonlarda formik asitle (FA) ön ekstraksiyona tabi tutularak yapışkan lignin oluşumu engellenmeye çalışılmış ve çözünen şeker miktarının da arttırılması amaçlanmıştır.

Çalışma sonucunda elde edilen veriler ANOVA ile grup içi istatistiki değerlendirme yapılmış olup gruplar arası değerlendirmeler Duncan testi ile gerçekleştirilmiştir. Buğday sapında sıcak su (135 ve 150 °C), alkali (%16,7 NaOH, 50 °C) ve modifiye alkali (%16,7 NaOH+%0,1 NaBH$_4$, 50 °C) ön ekstraksiyonu sonucunda sırasıyla %16,5, %41,5, %33,6 ve %33,9 ksilanın yapıdan uzaklaştığı görülmüştür. Sıcak su (135 ve 150 °C), alkali (%16,7 NaOH, 50 °C) ve modifiye alkali (%16,7 NaOH+%0,1 NaBH$_4$, 50 °C) ön ekstraksiyonu sonucunda çözünmüş materyalin her 100 gramına karşılık sırasıyla 7,79 g, 16,9 g, 6,81 g ve 4,22 g etanol üretilmiştir. Ekstrakte edilen ksilana bir miktar gluten ve nanosellüloz ilave edilerek iyi kalitede biyofilm üretimi gerçekleştirilmiştir. Ayrıca, sıcak su ekstraksiyonu sonrası soda yöntemiyle elde edilen kâğıt hamurunun verimi kontrol numunesiyle hemen hemen aynı olup üretilen kâğıdın mekanik ve fiziksel özellikleri de birbirine oldukça yakın tespit edilmiştir.

Mısır sapında sıcak su (150 °C) ve alkali (%26,7 NaOH, 50 °C) ön ekstraksiyonu sonucunda %44,8 ve %35,6 ksilanın yapıdan uzaklaştığı görülmüştür. Sıcak su (150 °C) ve alkali (%26,7 NaOH, 50 °C) ön ekstraksiyonu sonucunda çözünmüş materyalin her 100 gramına karşılık 14,7 g ve 7,66 g etanol üretilmiştir. Ekstrakte edilen ksilana bir miktar gluten ve nanosellüloz ilave edilerek iyi kalitede biyofilm üretimi gerçekleştirilmiştir. Buğday sapında olduğu gibi sıcak su ön ekstraksiyonu sonrası elde edilen kâğıt hamurunun verimi kontrol numunesiyle hemen hemen aynı olup üretilen kâğıdın mekanik ve fiziksel özellikleri de birbirine yakın değerdedir. Ayrıca modifiye alkali yöntemi sonrasında buğday ve mısır saplarının yapısındaki glukanın korunduğu ve yapıdan daha fazla ligninin çözüldüğü görülmüştür.

Endüstriyel uygulamada rekabetçi bir şeker üretimi için maliyeti düşük ön muamele yöntemi seçimi önemlidir. Sadece su/buhar kullanılan otohidroliz metodu uygun olmasına rağmen bu yöntem sonucunda oluşan yapışkan lignin sürekli üretim yapan sistemlerdeki hidroliz reaktörlerinde ciddi tıkanmalara sebep olmaktadır. Bu kapsamda, buğday sapları düşük sıcaklık ve sürede çözelti/sap oranı 6 L/kg (tam kuru) olacak şekilde 0-15 g/L arasında değişen konsantrasyonlarda formik asitle (FA) muamele edilmiştir. Mükemmel şeker ve lignin kütle denkliğiyle beraber hidrolizattaki çökelti lignin miktarı da FA konsantrasyonunun 0 g/L (kontrol)'den 15 g/L'ye artmasıyla 0,94'ten 0,31'e (g/100g buğday sapı) düştüğü görülmüştür. Aynı şekilde çökelti ligninin ortalama molekül ağırlığı da 1970 g/mol'den (0 g/L FA) 710 g/mol'e (15 g/L FA) düşmüştür. Monomerik şeker verimi FA konsantrasyonunun artmasıyla çok belirgin şekilde artmıştır. Bu değer kontrol (0 g/L) için 3,83 g/100g iken 15 g/L FA için 23,5 g/100g olarak tespit edilmiştir. Bu da FA destekli otohidroliz yöntemiyle sadece yapışkan lignin oluşumu minimize edilmekle kalmayıp aynı zamanda şeker veriminin de ciddi oranda arttırıldığını göstermiştir.

Anahtar sözcükler: Biyobozunur film, Biyoetanol, Biyorafineri, Buğday sapı, Mısır sapı, Ön muamele, Yapışkan lignin.

GENİŞ ÖZET

BUĞDAY VE MISIR SAPLARINDAN ENTEGRE BİYORAFİNERİ UYGULAMASI İLE BİYOBOZUNUR FİLM, BİYOETANOL VE KÂĞIT HAMURU ÜRETİMİ

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1. GİRİŞ

Dünya nüfusundaki hızlı artış (2030 yılına kadar 8 milyarı aşması beklenmektedir), yaşam standartları ve insan ihtiyaçlarının her geçen gün artması sebebiyle bu ihtiyaçların karşılanabilmesi için üretimin de artırılması gerekmektedir. Üretimin artması ise dünyadaki fosil yakıt kaynaklarına olan baskıyı daha da arttırmaktadır. Fosil yakıtların hızla tüketilmesi küresel ısınmanın yanında hava kirliliğine de sebep olmaktadır. Enerji fiyatlarının giderek artması ve çevre duyarlılığının önem kazanması sebebiyle özellikle ülkemiz gibi petrol gereksiniminin neredeyse tamamını ithalat yolu ile karşılayan ülkeler için yenilenebilir bir enerji kaynağı olarak lignoselülozik maddelerden yararlanmak önem arz etmektedir. Bu çalışmada bu maddelerden biri olan ve tarlalarda çürümeye bırakılan veya yakılmak suretiyle yok edilirken çevreye zarar veren ekonomik değeri düşük/yok olan buğday ve mısır saplarının entegre orman ürünleri biyorafinerisi yöntemi ile değerlendirilerek ekonomik değer kazandırılması ve ülke ekonomisine katkı sağlanması amaçlanmaktadır.

Bu çalışmada buğday ve mısır sapları sıcak su, alkali (NaOH) ve modifiye alkali ($NaBH_4$+NaOH) ön ekstraksiyonu ile değişik sıcaklık ve konsantrasyonlarda muameleye tabi tutularak elde edilen ekstrakte hemiselülozdan biyoetanol ve biyobozunur film, katı kısımdan ise kâğıt hamuru ve kâğıt üretimi araştırılmıştır. Ayrıca, endüstriyel uygulamada rekabetçi bir şeker üretimi için maliyeti düşük ön muamele yöntemi seçimi önemlidir. Sadece su/buhar kullanılan çevre dostu otohidroliz metodu uygun olmasına rağmen bu yöntem sonucunda oluşan yapışkan lignin sürekli üretim yapan sistemlerde hidroliz reaktörlerinde ciddi tıkanmalara sebep olmaktadır. Bu kapsamda, buğday sapları düşük sıcaklık ve sürede değişik konsantrasyonlarda formik asitle muamele edilerek yapışkan lignin oluşumu engellenmeye çalışılmış aynı zamanda çözünen şeker miktarının arttırılması da amaçlanmıştır.

2. MATERYAL VE YÖNTEM

Bu çalışmada kullanılan buğday ve mısır sapı örnekleri Düzce vilayetinden lokal olarak temin edilmiştir. Hammaddeler bir kısım hemisellülozun ekstrakte edilmesi amacıyla sıcak su, alkali (NaOH) ve modifiye alkali ($NaBH_4$+NaOH) yöntemleriyle değişik sıcaklık ve konsantrasyonlarda ön ekstraksiyona tabi tutulmuşlardır. Alkali ekstraksiyonu herbir hammadde için %16,7, %26,7 ve %33,3 (w/v) NaOH (çözelti/sap oranı 4 L/kg) konsantrasyonlarında 50, 70 ve 90 °C sıcaklık ve 4 saat süreyle gerçekleştirilmiştir. Modifiye alkali ekstraksiyonu buğday sapı için %16,7 NaOH ve %0,1, %0,5, %2 ve %4 (w/v) $NaBH_4$, mısır sapı için %26,7 NaOH ve %0,1, %0,5, %2 ve %4 $NaBH_4$ (çözelti/sap oranı 4 L/kg (tam kuru)) konsantrasyonlarında ve 4 saat süreyle gerçekleştirilmiştir. Sıcak su ekstraksiyonu ise her iki hammadde için 90, 120, 135 ve 150 °C sıcaklık, 4 saat süre ve çözelti/sap oranı 10 L/kg (tam kuru) olacak şekilde gerçekleştirilmiştir. Ayrıca, buğday sapları 150 °C sıcaklıkta 100 dakika süreyle çözelti/sap oranı 6 L/kg (tam kuru) olacak şekilde 0-15 g/L arasında değişen konsantrasyonlarda formik asitle (FA) muamele edilmiştir. Ayrıca yüksek asidite etkisini belirlemek için 15 g/L FA+1 g/L sülfürik asit ilave edilerek ön ekstraksiyon işlemi gerçekleştirilmiştir. Ön muamele işlemleri iki tekrar olarak yapılmış olup her iki hammadde dâhilinde toplam 96 adet ön ekstraksiyon işlemi gerçekleştirilmiştir. İşlem sonrası numuneler 200 mesh'lik elekle filtrasyona tabi tutularak sıvı ve katı kısma ayrılmıştır. Sıvı kısımdan elde edilen ekstrakte hemiselülozdan biyoetanol ve biyobozunur film, katı kısımdan ise kâğıt hamuru ve kâğıt üretimi gerçekleştirilmiştir. Üretilen biyobozunur film ve kâğıtlar ilgili fiziksel ve mekanik testlere tabi tutulmuşlardır. FA destekli ön ekstraksiyon işlemi sonrasında reaksiyonun sonlanması için reaksiyon kapları buz banyosunda konulmuştur. Daha sonra sıvı ve katı kısımlar birbirinden ayrılmıştır. Sıvı ve katı kısımların kimyasal içerikleri (tüm şeker, lignin, kül vs.) belirlenerek sıvı ve katı numuneler arasında kütle balansı yapılmıştır.

3. BULGULAR VE TARTIŞMA

Sıcak su ekstraksiyonu sonrası elde edilen veriler incelendiğinde, buğday sapında sıcaklık arttıkça (90-120 °C) şeker (glikoz ve ksiloz) ve lignin çözünürlüğü sınırlı oranda artmakla beraber, 135 ve 150 °C derecelerde ise çok belirgin şekilde şeker ve lignin çözünürlüğünün arttığı gözlemlenmiştir. Hatta 150 °C derecede 135 °C dereceye göre şeker ve lignin çözünürlüğünün iki katından fazla arttığı görülmüştür. Mısır sapında sıcak su ön muamelesinde ise 135 °C dereceye kadar tüm bileşenlerde doğrusal bir çözünme görülürken 150 °C derecede tüm bileşenlerde iki katından fazla bir çözünürlük gözlemlenmiştir. Alkali ekstraksiyonu sonrası elde edilen veriler incelendiğinde, sıcaklık ve NaOH konsantrasyonu arttıkça lignin başta olmak üzere buğday ve mısır saplarında tüm bileşenlerin çözünürlüğünün arttığı görülmüştür. Buna karşın buğday sapında ağırlık, gliloz ve ksiloz kaybı mısır sapına göre daha fazla iken, lignin kaybının ise mısır sapında belirgin bir şekilde daha fazla olduğu gözlemlenmiştir. Modifiye alkali ekstraksiyonunda ise mısır saplarında tüm çözünürlüklerin buğday saplarına göre daha fazla olduğu görülmüştür. Buğday sapında sıcak su (135 °C) ve alkali (%16,7 NaOH, 50 °C) ön ekstraksiyonu sonucunda çözünmüş materyalin her 100 gramına karşılık sırasıyla 7,79 g ve 6,81 g etanol üretilebildiği görülmektedir. Buna karşın 150 °C dereceki sıcak su muamelesinde daha fazla çözünen materyal olması sebebiyle bu değer 16,9 g olarak tespit edilmiştir. Mısır sapında sıcak su (150 °C) ön ekstraksiyonu sonucunda çözünmüş materyalin her 100 gramına karşılık sırasıyla 14,7 g etanol üretilebildiği görülmektedir. Çözünen ksilana bir miktar (0,1 g) gluten ve (0,025 g) nanosellüloz ilave edilerek her iki hammadde için iyi kalitede biyofilm üretimi gerçekleştirilmiştir. Buna karşın mısır sapı ksilanından elde edilen biyofilmlerin direnç değerleri daha yüksek olması mısır sapı ksilanının yüksek DP değeri nedeniyle açıklanabilir. Dahası buğday ve mısır sapları için sıcak su ekstraksiyonu sonrası elde edilen kâğıt hamurunun verimi kontrol numunesiyle hemen hemen aynı olup üretilen kâğıdın mekanik ve fiziksel özellikleri de birbirine yakın elde edilmiştir.

Formik asit (FA) destekli sıcak su ekstraksiyonu sonrası elde edilen veriler incelendiğinde, buğday sapında FA konsantrasyonu arttıkça verim kontrole (0 g/L FA) nazaran %91,7’den %76,3’e kadar düşmekte ve buna bağlı olarak çözünen şeker (arabinan, galaktan, glukan, ksilan ve mannan) miktarlarının da arttığı gözlemlenmiştir. En fazla çözünen şeker miktarı (%23,5 g/100g buğday sapı) buğday sapları 15 g/L

FA+1g/L SA ile muamele edildiğinde bulunmuştur. Çözünen şekerler içerisinde beklendiği gibi ksilan (%18,2 g/100g buğday sapı) en önde gelmektedir. Oluşan yan ürünlere baktığımızda ise en yüksek furfural (%1,68 g/100g buğday sapı) ve asetik asit (%2,12 g/100g buğday sapı) buğday sapları 15 g/L FA+1g/L SA ile muamele edildiğinde bulunmuştur. Yine pH değerinin de (4,75-2,09) FA konsantrasyonunun artmasıyla düştüğü görülmektedir. Elde edilen veriler bağlamında sıvı ve katı numuneler arasında kütle balansı yapıldığında mükemmel şeker ve lignin kütle denkliğiyle beraber yapışkan lignin konsantrasyonu da FA konsantrasyonunun 0 g/L (kontrol)'den 15 g/L'ye artmasıyla %0,94'ten %0,31'e (g/100g buğday sapı) düştüğü görülmüştür. Monomerik şeker verimi de FA konsantrasyonunun artmasıyla ciddi oranda artmıştır. (%3,83'ten %23,5'e g/100g buğday sapı)

4. SONUÇ VE ÖNERİLER

Çalışma kapsamında buğday sapı ve mısır sapı numuneleri sıcak su, alkali ve modifiye alkali yöntemiyle hemiselülöz ön ekstarksiyonuna tabi tutulmuş ve ekstrakte hemiselülozdan biyoetanol ve biyobozunur film, katı kısımdan ise kâğıt hamuru ve kâğıt üretimi gerçekleştirilmiştir. Elde edilen veriler genel olarak incelendiğinde, kontrol soda pişirmesiyle elde edilen hamurdan üretilen kâğıtlarla sıcak su ön ekstraksiyonu sonrası soda pişirmesiyle elde edilen hamurdan üretilen kâğıtların birbirine çok yakın mekanik ve fiziksel özelliklere sahip olduğu tespit edilmiştir. Saf ksilanın tek başına film tabakası oluşturamadığı ancak ksilitol, nanoselüloz ve gluten ilave edildiğinde hem düzgün bir film tabakası oluştuğu hem de mekanik özelliklerinin önemli derecede arttığı tespit edilmiştir. Ayrıca buğday sapları sıcak su hidrolizi sonucunda oluşan yapışkan ligninin oluşumunu engellemek veya konsantrasyonunu azaltmak amacıyla değişik konsantrasyonlarda formik asitle muamele edilmiştir. Sonuç olarak formik asit konsantrasyonunun artmasıyla sadece yapışkan lignin oluşumunun engellenmekle kalmayıp aynı zamanda şeker veriminin de ciddi oranda artırıldığı tespit edilmiştir. Endüstriyel öneme sahip ve kâğıt hamuru üretiminde kullanılan hammadde kaynaklarının farklı/geliştirilmiş ön ekstraksiyon yöntemleri kullanılarak kâğıt hamuru eldesi sonrasında ortaya çıkan siyah çözelti içerisinde kalan hemiselülozların bir kısmının ekstrakte edilerek yüksek endüstriyel değere dönüşümü olanakları araştırılabilir. Aynı şekilde siyah çözelti içerisindeki ligninin de endüstriyel değere dönüşüm olanakları araştırılabilir.

1. INTRODUCTION

In the introduction, the need for bioenergy was first detailed and then the raw materials utilized in this study were explained. Then, the information on the integrated forest products biorefinery (IFBR) concept was given. The products produced with IFBR were detailed in the last part of the introduction.

The rapid increase in global population which is expected to be over eight billion by 2030, and improved living standards are gradually depleting the world fossil fuel resources. In addition, the combustion of these fossil fuels leads to the release of greenhouse gases, pollute on air quality and cause global warming. Therefore, considerable funds and efforts have been invested in alternative and renewable energy resources such as biomass, wind, geothermal, hydroelectric, and solar energy in order to prevent the negative effect of these fossil fuels. Lignocellulosic biomass such as wood, various agricultural residues, grasses and energy crops is the fourth largest source of energy in the world after coal, petroleum, and natural gas, and provides about 14% of the world's energy consumption. Renewable biomass is an important energy resource to generate electricity and fuels for vehicles and provide heat for industries (Bridgewater et al. 1999). However, woods have been commonly used to produce panels, boards, paper etc. They are also utilized as a source of renewable energy, which affects world forests negatively (Anonymous 2014). Deforestation in large scale results in soil erosion, damages in water supplies, destroys the habitats, and increases the air pollution. The increase of the wood demand may cause the shortage of wood raw materials and gradual deforestation. Therefore, nonwoody lignocellulosic materials are taking into consideration as feedstock for pulping and papermaking (Moryia et al. 2007). Agricultural residues and energy crops are one of the low cost nonwoody biomass which is abundant in many countries. The high growth rate and adaptability to various soil types makes them a good alternative to wood (Rocero et al. 2003).

Wheat straw is the second largest agricultural residue in the world (Talebnia et al. 2010) after sugarcane straw at 867 million ton/year in 2008/09. Turkey, having a rich agricultural potential and large agricultural tradition, is an important wheat producer in the world and hence there are huge quantities of underutilized and low-cost wheat straw available. Approximately 40 to 53 million tons of straw are produced in Turkey per year (Ergudenler and Isigigur 1994). Wheat straw with low-no economic value, burnt in the field after harvest creates environmental pollution. Similar to other biomass, wheat straw consists of cellulose (33-40% w/w), hemicelluloses (20-25% w/w), lignin (15-20% w/w), and a small amount of extractives and mostly silica-containing ash (Prasad et al. 2007). The variation in composition depends on the wheat species, soil, climate conditions, etc. (Utne and Hegbom 1992). With a significant carbohydrate content of about 70% w/w, wheat straw is a potential cheap and abundant feedstock for production of fermentable sugars for bioethanol.

Corn stalks have a high carbohydrate content (75% w/w) in addition an abundant, renewable, low-cost and widely available resource in Turkey and have an industrial potential to produce bioethanol, biodegradable film, papermaking pulp etc. Similar to wheat straw, corn stalks burnt in the field after harvest creates environmental pollution and they consist of mostly cellulose (35-47% w/w), hemicelluloses (22-25% w/w), lignin (17-22% w/w), and a small amount of extractives and ash. The differences in composition depend on the corn species, growth environment, growing season, growing location, soil conditions, analysis methods, etc. (Amores et al. 2013).

The new concept of an integrated biorefinery proposed by van Heiningen (2006) pre-extracts a part of the hemicellulose prior to pulp production. This application is expected to improve the competitiveness of pulp producers without disrupting the pulp and paper production. In conventional kraft chemical pulp production, the black liquor includes about 80% of the dissolved hemicelluloses. To separate these hemicelluloses from black liquor is technically very difficult before it is burnt to recover chemicals and to generate steam and electricity (Biermann 1996, Sjostrom 1993) and it should be noted that the heat value of hemicelluloses is only half than that of lignin. Therefore, the hemicelluloses in black liquor could be pre-extracted and used to produce higher economic value products of chemicals, biofuels, biopolymers, etc. by the IFBR (van Heiningen 2006, Ragauskas et al. 2006, Mendes et al. 2009). The pre-extraction prior to pulping dissolves a part of the hemicelluloses and it decreases the required chemicals and cooking times. In addition, pre-extraction increases the digester capacity because the pre-extracted materials have a lower mass compared to unextracted materials (Huang et al. 2010).

Hot water extraction, also called autohydrolysis, could be considered as an effective and environmentally friendly technique, because no chemicals are used in this technique. The obstacle of this technique is the presence of “sticky lignin” precipitates in the hydrolysate, which leads to severe plugging in the hydrolysis reactor and downstream equipment in a continuous process. For this reason, formic acid reinforced autohydrolysis was studied to minimize the formation of sticky precipitates and convert the remaining precipitates in non-sticky particles. Massive problems related to the precipitation of resin-like materials have also been reported during sulfuric acid post-hydrolysis of autohydrolysates to convert the dissolved hemicelluloses into xylose (Stanciu and Ciurea 2008, Stanciu 1974). Leschinsky et al. (2009) found that during autohydrolysis, lignin is degraded through cleavage of aryl-ether bonds resulting in a reduced molecular weight. As a result, the degraded lignin is characterized by an increased content of phenolic hydroxyl groups and a reduced content of aliphatic hydroxyl groups. In parallel to the cleavage reactions, condensation reactions of lignin take place which leads to increased molecular weight of lignin and precipitation. The formation of these sticky lignin precipitates explains why hot water pre-extraction is not commercially applied. The presence of formic acid at relatively low concentrations (5-20 g/L) during hot water (160 °C) treatment for mixed hardwood chips significantly reduced the amount of lignin precipitates in the pre-hydrolysate (Yasukawa et al., 2014).

With IFBR, several products of bioethanol, biodegradable films, pulp and paper, several chemicals etc. could be produced using lignocellulosic biomass. Bioethanol is mainly produced from feedstock that contains natural sugars or starch in the structure. Natural sugars or starch can easily be converted to monomeric sugars. On the other hand, bioethanol production from food like corn and wheat grains may cause a major disruption in the food markets (McElroy 2006). Therefore, several agricultural residues are studied regarding the bioethanol production capabilities (Balat et al. 2008).

In addition, synthetic polymers are mainly produced from petrochemicals. Most of these type polymers do not degrade under standard environmental conditions. So, these persistent polymers cause environmental pollution, harming marine life when they are dispersed in the nature. The costs of recycling these materials are very expensive as well as require a high energy. Therefore, in recent years there has been an increase in interest in biodegradable polymers. Biodegradable polymers have a wide variety of uses such as packaging, agriculture, medicine, and other areas (Kolybaba et al. 2003). In addition, these materials bring a significant contribution to the sustainable development due to the large variety of disposal options with minor environmental impact. Consequently, the market of these environmentally friendly materials is in rapid expansion, 10-20% per year (Avérous and Pollet 2012).

World demand for paper and paper products is also expected to rise from 300 million tonnes to over 490 million tons by the year 2020 (Agnihotri et al. 2010). The aim of the pulping process is to remove lignin and preserve the polysaccharides, especially the cellulose for pulp and paper production (Fengel and Wegener 1989). The chemical pulps are commonly manufactured from alkali processes namely soda and kraft pulping. The soda pulping application is restricted for various agricultural residues and annual crops and uses mainly NaOH and pulping additive may be used such as anthraquinone (AQ), to decrease the carbohydrate degradation. It should be mentioned that agricultural residues paper production is produced around 5-10% in all over the world (Anonymous 2016). The use of agricultural residues in pulping have increased more than two times as fast as wood pulping volumes on the worldwide since 1970 (Hedjazi et al. 2009).

The main objective of this dissertation was to determine whether an integrated biorefinery is technically feasible to produce bioethanol and biodegradable films as well as pulp and paper when large quantities and inexpensive wheat straw and corn stalks w.ere used as a feedstock. Hot water, alkali and modified alkali pre- extraction techniques were examined regarding the process yields. In addition, for the first time, the hypothesis was made in this study to modify the alkali pre-extraction technique by $NaBH_4$. The modification was expected to remove more lignin during extraction. It should also preserve more carbohydrates in the structure of wheat straw and corn stalks that were expected to improve the process yield. The aim was to produce pulp having almost similar properties from pre-extracted and un-extracted (control) wheat straw and corn stalks. In addition, the obstacle of sticky precipitates in hot water pre-extraction was examined to be overcome by using formic acid reinforced pre-hydrolysis of wheat straw.

1.1. LITERATURE REVIEW

1.1.1. Lignocellulosic Biomass and Global Demands

Increasing of global population, food demand, oil prices, consumption of lignocellulosic biomass and fossil fuels are very strictly interlinked. The rapid increase in global population, which is expected to be over eight billion by 2030, causes increasing demand of food and paper products (Pimentel 2007, Nellemann et al. 2009). On the other hand, increasing of crude oil prices and environmental concerns calls for the replacement of fossil fuels by biofuels. Many researchers have reported production of biofuels such as bioethanol from lignocellulosics (Tozluoğlu et al. 2015, Çöpür et al. 2012, Talebnia et al. 2010, Prasad et al. 2007, Moryia et al. 2007). Forests have been cleared for cultivation of crops to meet the increasing food requirements. However, the food supply chain needs large scale paper and board production from lignocellulosics. According to recent facts and figures (Anonymous 2013) wrapping and packaging accounted for half of the total paper and pulp production in 2013.

Commonly wood from lignocellulosic biomass has been used for the production of a wide range of biomass products in addition to energy sources (Anonymous 2013). Worldwide air pollution is an important factor encouraging interest in alternative fuels. The global increase in the use of fossil fuels has not only changed the world economics but also affected air quality. Therewithal the cutting of trees on a large scale to meet the ever increasing pulp and paper demand has adversely affected the global environment, water supplies, biodiversity, animal habitats and landform. Food and Agriculture Organization (Anonymous 2013) has demonstrated an enormous decrease in forest area from 1990-2010 as shown in Figure 1.1.

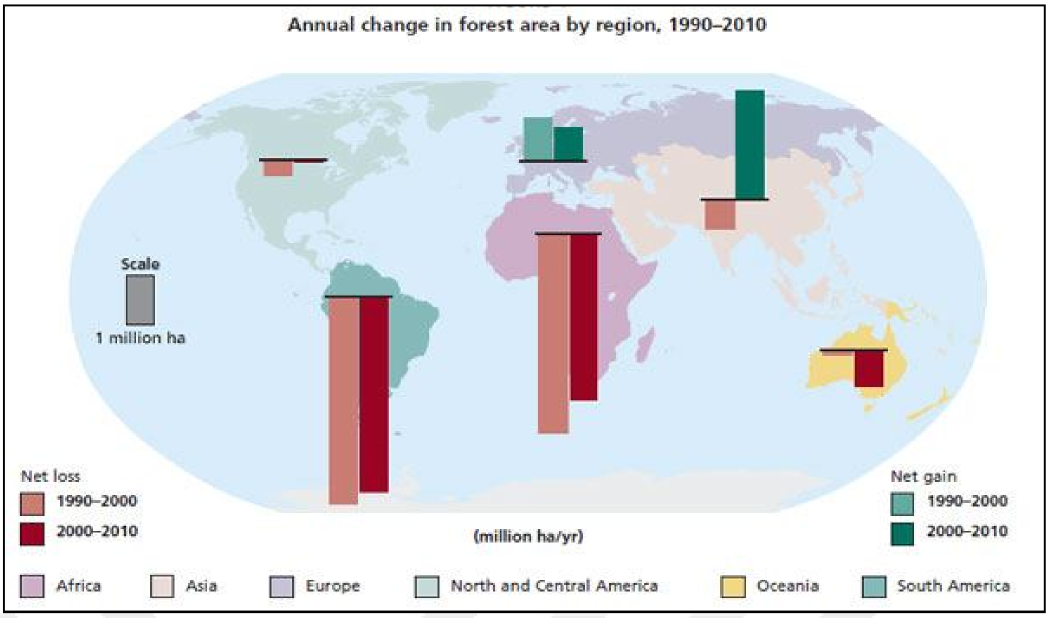

Figure 1.1. Dramatic loss in forest area by region (Adapted from FAO 2013).

1.1.2. Agricultural Residues

The enormous portion of forest land has already turned into cultivated agriculture land is limited and even decreasing due to the soil degradation (desertification, salinization) (Pimentel 2007, Nellemann et al. 2009). Efforts to meet the global food, fibers and fuel demands for sustainable development, agricultural residues may be a good alternative due to the following reasons;

- Agricultural residues are available in abundance.
- Biomaterials production instead of woody biomass.
- The use of agricultural residues as a raw material for fibers or fuel doesn't affect food supply on the contrary when wheat, corn, etc. is used for bioethanol production.
- Agricultural residues protect the environment because of they are usually burnt in the fields.
- They preserve forests and develop biological diversity.

Agricultural residues include a wide range of lignocellulosic biomass such as wheat straw, corn stalks, rice straw, sugarcane bagasse, sugarcane straw etc. These agricultural materials are widely-available worldwide and their accessibility depends on production region and the production of the individual crop. Figure 1.2. shows the major crops production data (2005-2013) of wheat, maize (corn), sugarcane, barley, rye, cotton lint, flax fibre and tow, rice and paddy are the most abundantly cultivated crops worldwide (Anonymous 2013). Owing to abundantly available of these above mentioned agriculture crops may provide significant amount of residues that can contribute for the production of pulps, chemicals, and biofuels without affecting the food supply chain.

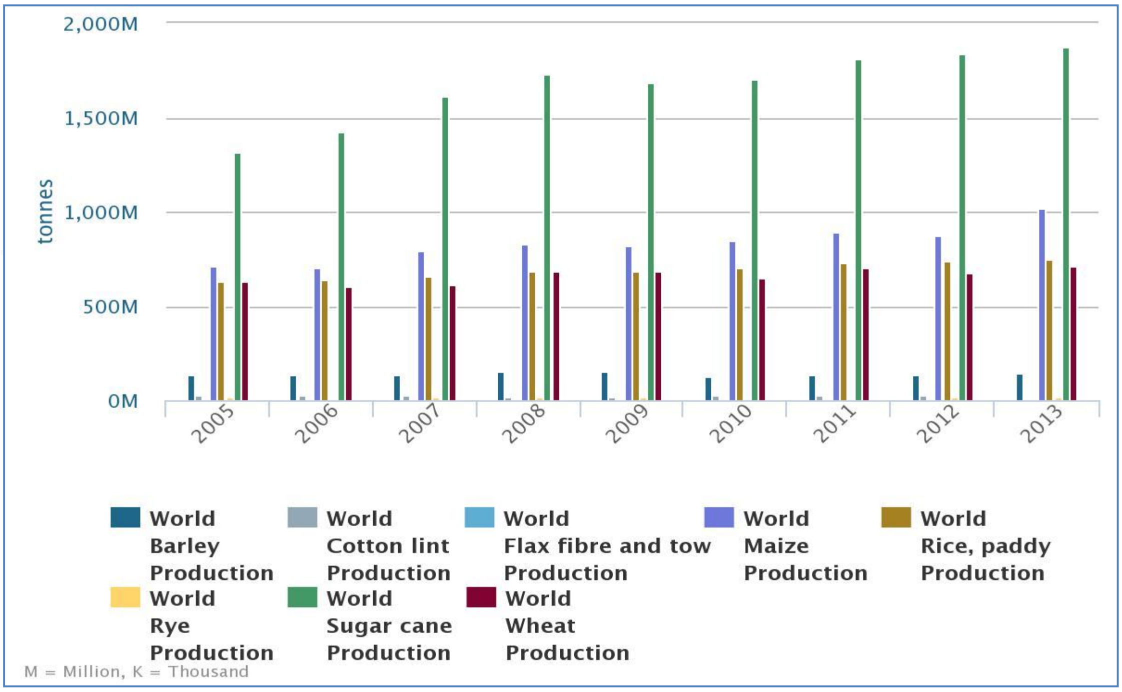

Figure 1.2. World production of major crops (Adapted from FAO 2013).

1.1.2.1. Feedstocks used in this study:

Wheat straw (*Triticum aestivum* L.): Wheat straw is the second largest agricultural residue in the world (Talebnia et al. 2010) after sugarcane straw at 867 million ton/y in 2008/09. It is also the largest crop cultivated in Europe (Kim and Dale 2004). Turkey, having a rich agricultural potential and large agricultural tradition, is an important wheat producer in the world and hence there are huge quantities of underutilized and low-cost wheat straw available. Approximately 40 to 53 million tons of straw are produced in Turkey per year (Ergudenler and Isigigur 1994). Wheat straw with low-no economic value, burnt or left in the field after harvest creates environmental pollution. Burning wheat straw has been done for a long time and burning causes large amount of air pollutants (Li et al. 2008) and health problems. Similar to other biomass, wheat straw consists of cellulose (33-40% w/w), hemicelluloses (20-25% w/w), and lignin (15-20% w/w) and a small amount of extractives and mostly silica-containing ash (Prasad et al. 2007). The variation in composition depends on the wheat species, soil, climate conditions, etc. (Utne and Hegbom 1992).

Corn stalks (*Zea mays indurata* Sturt.): Corn (maize) is another most abundantly cultivated crop throughout the world. The world production of corn has been about 520 $x10^9$ kg/annum. The main production regions are North America (42%), Asia (26%), Europe (12%) and South America (9%) (Kim and Dale 2004). According to the Turkish Statistical Institute's report (Anonymous 2014), Turkey's corn production was 6 million ton in 2014 increased by 1.0%. Corn stalks have a high carbohydrate content (75% w/w) in addition an abundant, renewable, low-cost and widely available resource in Turkey and have an industrial potential to produce bioethanol, biodegradable biofilm, papermaking pulp, etc.

1.1.3. Chemical Compositions

Lignocellulosic materials including agricultural residues (e.g. straws, stover, stalks, cobs, bagasses, shells), industrial residues (e.g. sawdust and paper mill discards), urban solid wastes (e.g. garbage) consists of primarily three different types of polymers, namely cellulose, hemicellulose, and lignin, with minor amounts of extractives and ash, which are related which each other (Fengel and Wegener 1989). Structural characteristics of agricultural residues play a critical role in the delignification process and end use of products obtained. Therefore, it is essential to study thoroughly the isolation and structural characterization of the hemicelluloses, cellulose, lignin, extractives, and ash.

1.1.3.1. Cellulose

Cellulose is the most abundant biopolymer in the world and the main constituent of plant cell walls. Many industrial products (fibers, paper, biofilm, additives etc.) are made from cellulose. It is isolated from wood through a well-known process called pulping. As shown in Figure 1.3, cellulose is a linear monopolymer of anhydro D-glucose subunits, linked by β-1,4 glycosidic bonds 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 chemical formula of cellulose is $(C_6H_{10}O_5)_n$. Cellulose molecular weight (MW) ranging from 50 to 2500 kDa and degree of polymerization (DP) of 305-15.300 depending on the cellulose source. For instance, wood cellulose normally has around 10.000 units whereas cotton has about 15.000 (Fengel and Wegener 1989).

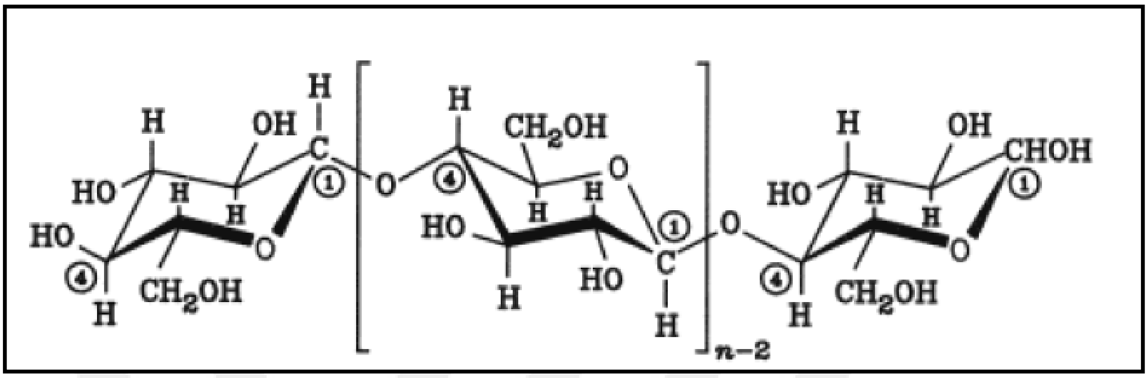

Figure 1.3. The structure of cellulose molecule.

The cellulose in lignocellulosic biomass consists of primarily two different parts, namely crystalline structure, and amorphous structure. It consists of inter and intramolecular hydrogen bonds that cause it to have amorphous and crystalline regions in the plant cell wall. The cellulose chains (rods) are bundled together and form hence called cellulose fibrils. These fibrils are highly ordered in the crystalline region, while they are less ordered in the amorphous regions (Figure 1.4). This is important property as the crystalline regions cause cellulose to be relatively inactive and insoluble in the most of solvents. These cellulose fibrils are mostly independent and weakly bound through hydrogen bonding (Laureano-Perez et al. 2005). The cellulose microfibrils are primarily embedded in a matrix of hemicellulose, pectin and variety of proteins (Sun 2002), which complicates hydrolysis of cellulose to glucose even further.

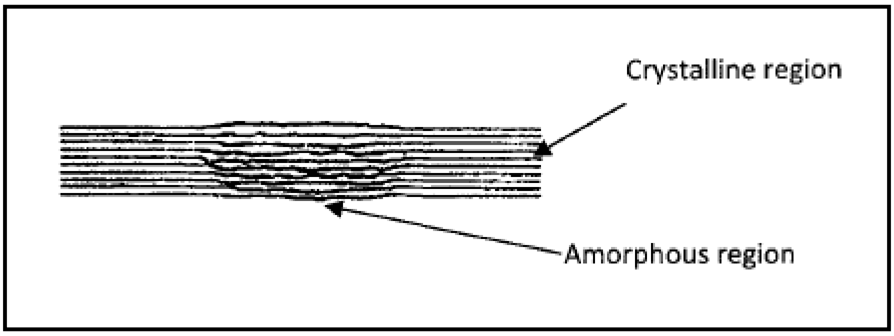

Figure 1.4. Cellulose fibrils of crystalline and amorphous regions.

1.1.3.2. Hemicellulose

After cellulose, hemicelluloses are the second common polysaccharide in the biomass, represent about 20-35%. Hemicelluloses differ from cellulose in three distinct ways (1) by a composition of different sugar units, (2) by much shorter molecular chains, and (3) by a branching of the chain molecules. However, unlike cellulose, hemicelluloses have complex carbohydrate structures that consist of different branched heterogeneous polymers like pentoses (usually D-xylose and L-arabinose), hexoses (usually D-mannose, D-glucose, and D-galactose), acetic acid, and uronic acid. The major component of hemicellulose from hardwood is xylan, whereas softwood hemicellulose contains mostly glucomannans. Agricultural residues, such as corn stalks and wheat straw, contain large amounts of xylan, some arabinan, and only very small amount of mannan. The structure and composition of hemicellulose varies by species and in some cases by cell location and type (Fengel and Wegener 1989). Unlike cellulose, hemicelluloses are non-crystalline, with a MW ranging from 10-26 kDa and a highly low DP of 80-200. The low crystallinity is responsible for their low thermal and chemical stability as well as easy dissolution in pulping and pretreatment processes.

Xylan is the most abundant hemicelluloses. Xylan is a complex polysaccharide composed of a backbone of β-1,4-linked D-xylopyranose units that, depending on the biomass source. Xylan side chains may be containing arabinosyl, glucuronosyl, methylglucuronosyl, acetyl, feruloyl and p-coumaroyl residues. The ratio of xylose to other units and composition of branches are dependent on the source of the xylan (Aspinall 1980). The backbone comprise of O-acetyl, α-L-arabinofuranosyl, glucuronic or 4-O-methylglucuronic acid substituents. Nevertheless, linear xylans have been isolated from some lignocellulosic materials such as guar seed husk, esparto grass, and tobacco stalks (Eda et al. 1976). So xylans could be classified as linear homoxylan, arabinoxylan, glucuronoarabinoxylan, and glucuronoxylan.

The composition of xylans varies depending on the sources such as grasses, cereals, softwood, and hardwood. For instance; birch wood xylan comprises 89.3% xylose, 1% arabinose, 1.4% glucose, and 8.3% anhydrouronic acid (Kormelink and Voragen 1993), while rice bran xylan comprises 46% xylose, 44.9% arabinose, 6.1% galactose, 1.9% glucose, and 1.1% anhydrouronic acid (Shibuya and Iwasaki 1985). However, wheat straw arabinoxylan comprises mostly xylose and arabinose and little mannose, galactose, and glucose (Gruppen et al. 1992). Corn fiber xylan is a complex heteroxylans that comprises 48-54% xylose, 33-35% arabinose, 5-11% galactose, and 3-6% glucuronic acid (Doner and Hicks 1997). Figure 1.5 shows schematic structure of corn fiber heteroxylan (Saulnier et al. 1995). The corn fiber cell wall model is given Figure 1.6 (Saha 2003).

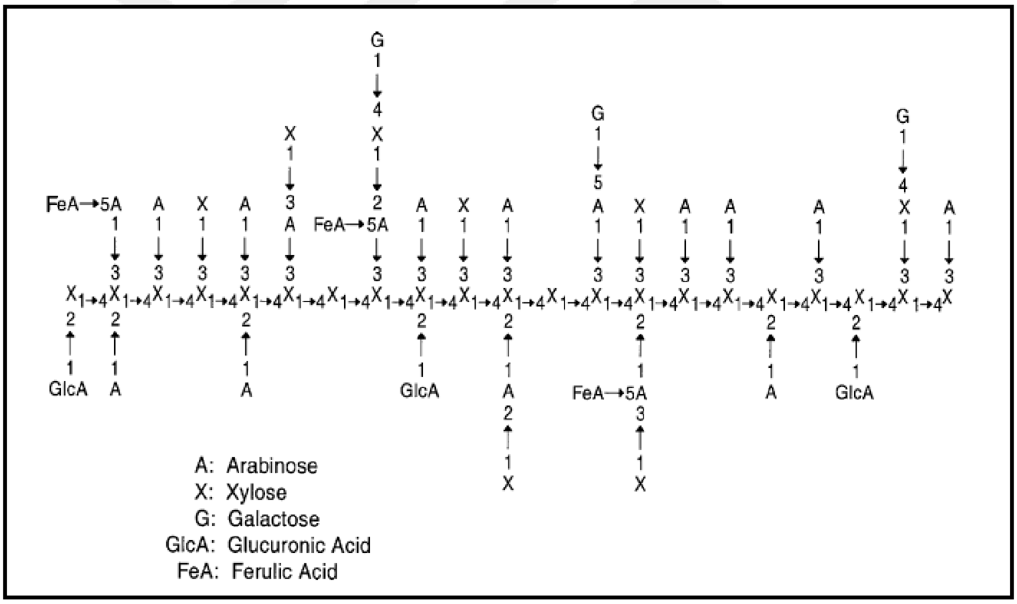

Figure 1.5. Schematic structure of corn fiber heteroxylan (Adapted from Saulnier et al. 1995).

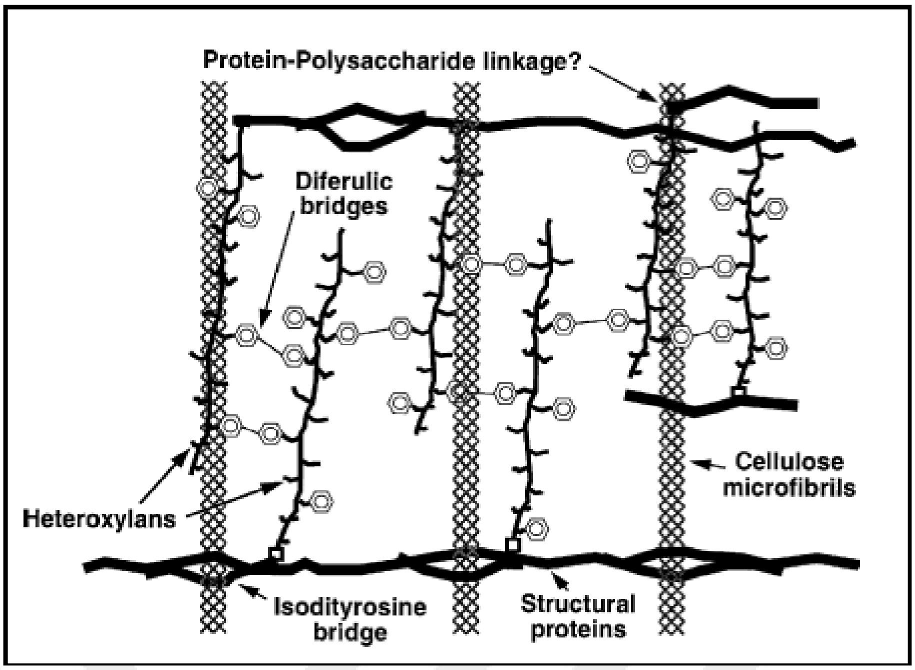

Figure 1.6. Model for corn fiber cell walls (Adapted from Saulnier and Thibault 1999).

1.1.3.3. Lignin

Lignin is an amorphous aromatic polymer that consists of repeating units of phenyl propane (Sjöström 1993). In contrast to cellulose and hemicellulose, lignin a complex three-dimensional cross-linked polymer. It is built by three monomers have an aromatic ring with different substituents: coniferyl alcohol, sinapyl alcohol, and p-coumaryl alcohol (Brown 2003). It is amorphous in nature and plays a critical role in giving structural rigidity to hold cell wall fibers together. There is some evidence for bonding between lignin and carbohydrates, especially hemicelluloses, but the nature of this bonding is not well understood. The carbohydrate polymers are tightly bound to the lignin by hydrogen bond and some covalent bonds (Fan et al. 1982). Lignin cannot be separated from biomass without degrading it, and the molecular weight of native lignin still remains unclear.

Lignin also plays an important role in the transport of water and nutrients and decreases the penetration of water throughout the cell walls. Lignin is proven as resistant to microbial degradation, playing a vital role in a lignocellulose's natural defense (Basaglia et. al. 1992, Chandler et al. 1980). The lignin in plant cell walls must be separated from carbohydrates during biomass conversion to open the protective lignin structure. The main target of successful pretreatment is to weaken the protective linkage between lignin and carbohydrates (lignin carbohydrate complexes, LCC). Chemical bonds between lignin and hemicellulose components are reported and these bonds can be ester, ether or glycosidic type linkages. Ether type linkages between LCC are more common and stable whereas the ester linkages are easily cleaved by alkali (Sjostrom 1993). Lignin is so complex that its structure is not yet completely understood. Also it has no structural regularity. The structure of a small section of a lignin polymer is shown below in Figure 1.7.

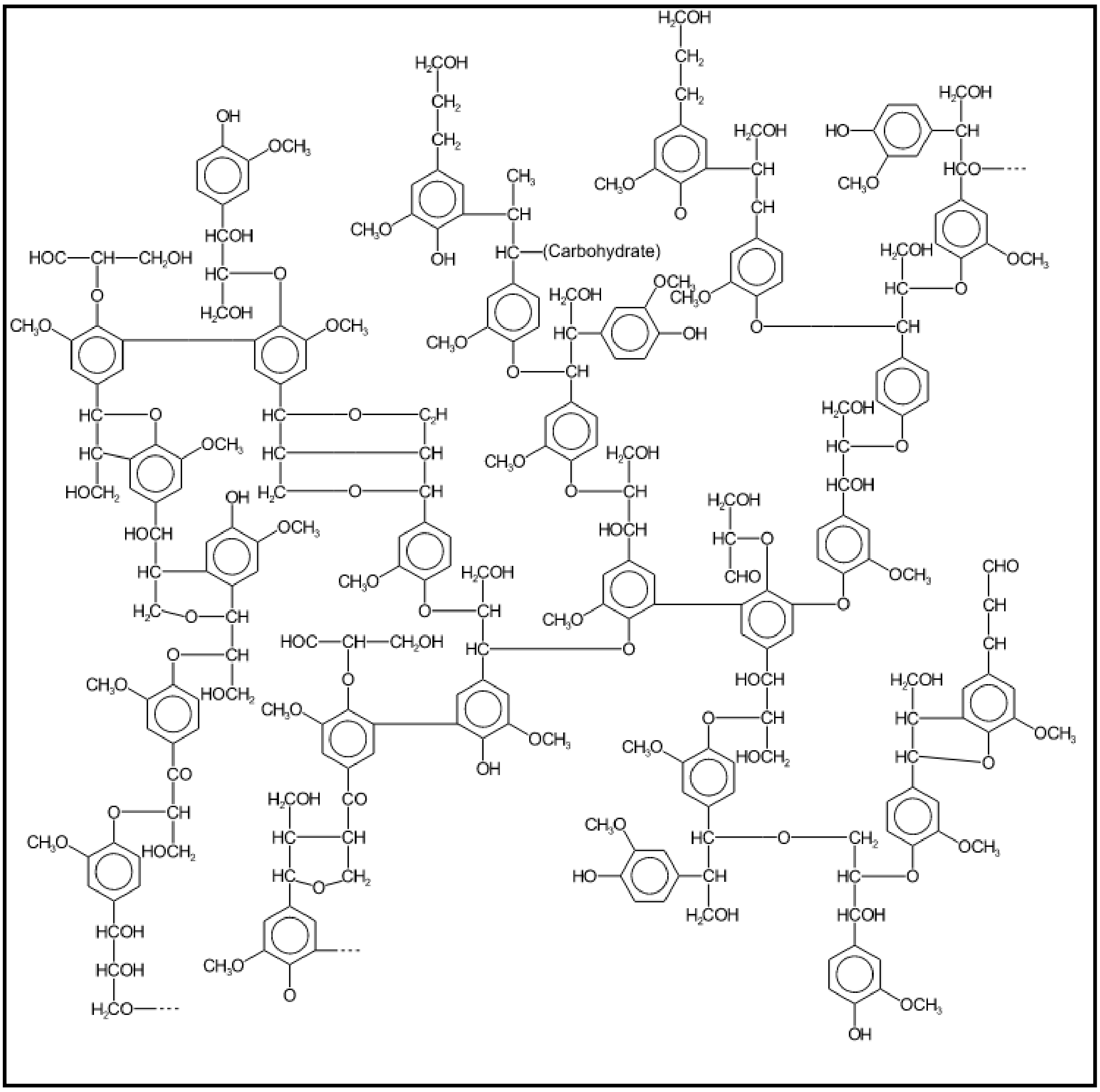

Figure 1.7. The structure of a lignin polymer.

The composition of lignin differs from species to species. Hardwood lignin consists of mainly guaiacyl and syringyl with nearly equally amounts, whereas, guaiacyl is major unit in softwood. Grass lignins contain p-coumaryl alcohol derived units along with sinapyl and coniferyl. The lignin content varies greatly between species. Generally softwood contains 26-32% and hardwood 20-28% lignin (Sjöström, 1993), while the lignin content of agricultural residues is comparable to that of hardwood. Also significant structural differences exist between softwood and hardwood lignin. Hardwood lignin is less branched, cross-linked and condensed and has a lower molecular weight and lower share of C-C bonds than softwood lignin (Sjöström, 1993). These differences are important from a delignification point of view. Scheme of secondary plant cell wall structure is shown below in Figure 1.8 (Adapted from Bidlack et al. 1992).

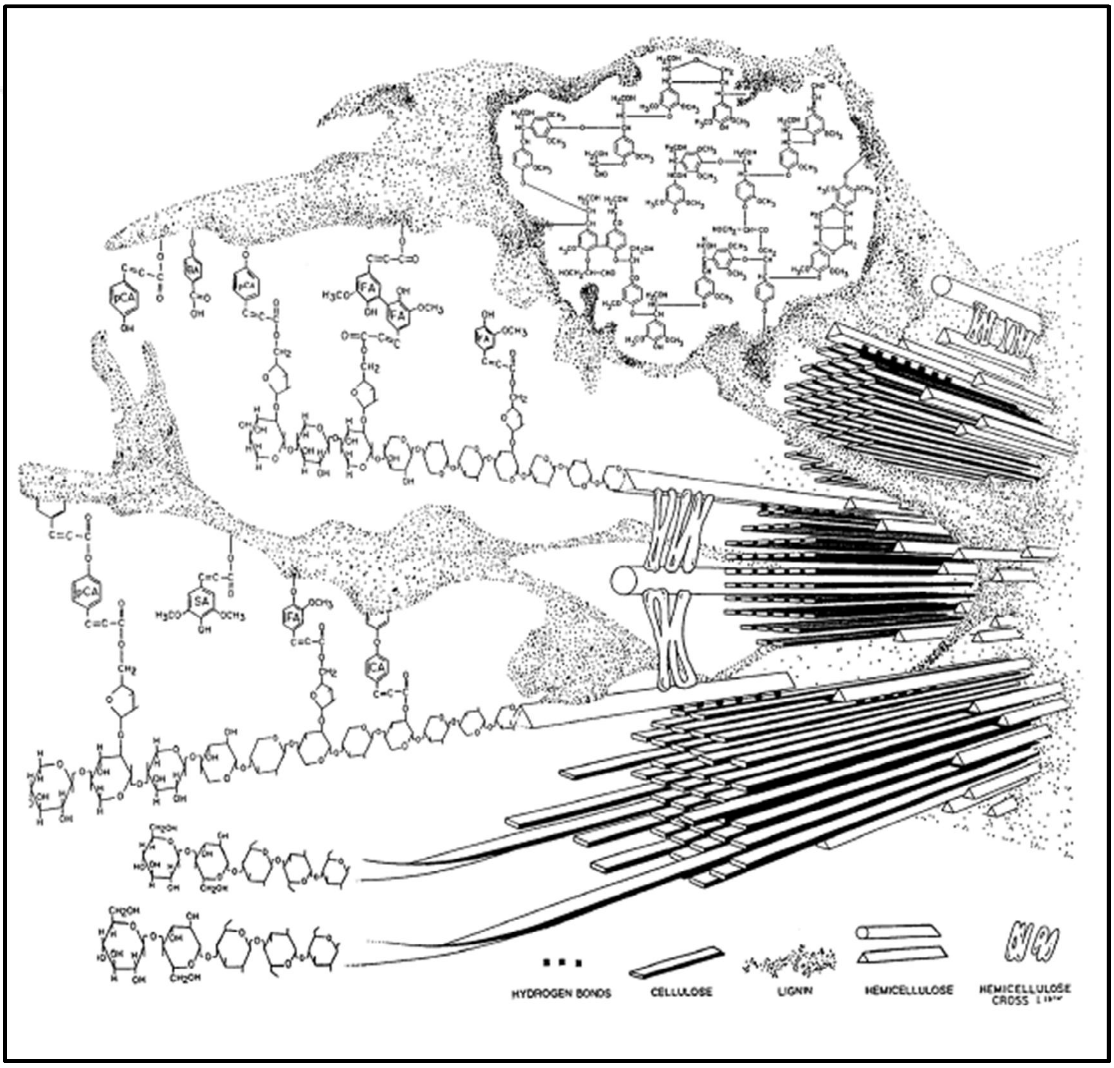

Figure 1.8. Scheme of secondary plant cell wall structure (Adapted from Bidlack et al. 1992).

1.1.3.4. Extractives and Ash

Extractives are a large variety of non-cell wall components, although generally representing a minor fraction, and can be easily removed from biomass using neutral organic solvents or water. Extractives mainly include resin acids, acids fats, fatty acids, terpenes, waxes, tannins, and wide variety of phenolic compounds. Phenolic compounds have a tendency to form cross-links with lignin and cause impaired delignification in acid sulfite process. As increases storage time of biomass decreases and changes the composition of extractives and thus their adverse effects in pulping process can be minimized (Sjöström, 1993).

Ashes are mainly inorganic components such as alkali earth carbonate and oxalates, either in structural or extractable form. Structural ash is an inorganic material that is bound into the physical structure of the biomass, whereas extractable ash is inorganic material that can be removed by washing or extracting the material. 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 and Wegener 1989).

1.1.3.5. Hemicellulose Extractions

Hemicelluloses 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 and Wegener 1989).

The extraction of hemicelluloses for lignocellulosic biomass has been extensively studied during the last decades. The new concept of an integrated biorefinery proposed by van Heiningen (2006) pre-extracts a part of the hemicellulose prior to pulp production. This application is expected to improve the competitiveness of pulp producers without disrupting the pulp and paper production. In conventional kraft chemical pulp production, the black liquor includes about 80% of the dissolved hemicelluloses. To separate the hemicellulose part from black liquor is even difficult (Biermann 1996, Sjostrom 1993). The heat value of hemicelluloses is only half than that of lignin. Therefore, the hemicelluloses in black liquor could be pre-extracted and used to produce chemicals and biofuels. The new concept, IFBR, aims removing some hemicelluloses prior to pulping (van Heiningen 2006). The extracted hemicellulose may be utilized to produce materials having economic value.

Previous studies have examined hemicellulose pre-extractions along with pulp production, and several techniques have been applied in the hemicellulose pre-extraction process. Mendes et al. (2009) examined an acid-catalyzed pre-hydrolysis of eucalyptus, with the pre-extracted hemicelluloses being further fermented to produce bioethanol. Furthermore, the pulp produced had lower bleaching requirements and brightness reversion. Al-Dajani and Tschirner (2008) pre-extracted poplar chips with NaOH and obtained a comparable yield and higher pulp quality when the alkaline technique was compared with acidic pre-extraction. The alkaline technique extracted more hemicelluloses from the structure and resulted in less degradation to furfural substances compared to the acidic one. Alkaline techniques are carried out under much milder treatment conditions (temperature, pressure, and chemical dosage). In addition, neither adjustment of the pH nor washing of the chips is required before alkaline pulping. Copur and Tozluoglu (2008) and Çöpür et al. (2012) modified the alkaline pre-extraction technique by adding a small amount of sodium borohydrate ($NaBH_4$), and results showed that, thanks to this modification, the pulping selectivity was improved by preventing peeling reactions during cooking (lowering hemicelluloses degradation), and the pulp yield was increased by providing a higher rate of delignification.

Techniques for pre-extracting hemicelluloses before pulping were evaluated for the woody biomass of eucalyptus by Mendes et al. (2009), for aspen by Al-Dajani and Tschirner (2008), and for loblolly pine by Yoon et al. (2011). However, studies with wheat straw and corn stalks/stover in this context are more limited (Jahan and Rahman, 2012, Resalati et al. 2012, Cheng et al. 2014). Studies of agricultural residues have mainly focused on hemicellulose pre-extraction but have not examined the subsequent pulping process and pulp properties. Wheat straw (Feng et al. 2014), corn stalks (Egüés et al. 2012), and sugarcane bagasse (Batalha et al. 2015) have only been studied for hemicellulose extraction.

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1.1.4. Bioethanol Production

Increasing crude oil prices and environmental concerns calls for the replacement of biofuels instead of fossil fuels. In addition, the dependency on crude oil has two main drawbacks: burning fossil fuels can contribute to global warming, and importing oil causes a foreign dependency. Therefore, increasing of global population, energy demand, concerns about greenhouse gas emissions from fossil fuels and energy security have led to great interest in the production of fuels from alternative renewable biomass. Bioethanol has been used an economically feasible fuel for transportation for several decades. Bioethanol an alternative fuel is renewable bio-based resource and it ensures to diminish the emissions (Hansen et al. 2005). It is rapidly becoming a common alternative to gasoline in some parts of the world, such as in Brazil. Currently, ethanol is mostly produced from any feedstocks that contain sugars or starch that can be readily converted to sugar, such as corn, wheat, and sugarcane in the world. However, it is recognized that food ethanol cannot meet the requirement without causing a major disruption of the food markets (McElroy 2006). Also, there has been considerable debate about “Food vs. fuel”. The years 2007–2008 noticed an enormous increase in food prices, making a global crisis and leading to political, economical, and social uneasiness in both developing and developed countries in the world. Universal causes for the throughout the world rises in food prices continue to be a subject of debate. Recent developments can allay in the cellulosic ethanol production and commercialization some of these concerns. According to Kim and Dale (2004), there is enough biomass from wasted agricultural residues to potentially substitute 32% of the global fuel consumption with bioethanol. For instance; the largest ethanol producers in the world are the US, Brazil, and China. Production of bioethanol from sugarcane in Brazil in 2004 accounted for nearly 18% of the country’s automotive fuel needs (Brown et al. 1998).

1.1.5. Bioethanol Production from Hemicellulosic Hydrolyzates

Hemicellulosic sugars are converted to bioethanol. The conversion should be efficient and cost-effective. In 2002, over 2 billion gallons of bioethanol was produced mainly by fermenting corn starch. The demand for bioethanol is expected to rise very rapidly.

1.1.5.1. Fermentation

Lignocellulosic materials are frequently hydrolyzed by acid hydrolysis; then obtained hydrolysate is used for bioethanol fermentation. Fermentation is the phase of the bioconversion process that converts sugars to ethanol and carbon dioxide or other valuable products such as numerous organic acids. This fermentation reaction is carried out by microorganism such as yeast or bacteria that feed on the sugars. Since such lignocellulosic hydrolysate contains not only glucose, but also various monosugars, such as xylose, mannose, galactose, arabinose, and oligosugars, the microorganisms should be required to ferment these sugars efficiently for the successful industrial production of bioethanol (Katahira et al. 2006). According to the reactions, the theoretical maximum yield is 0.51 kg bioethanol and 0.49 kg carbon dioxide per kg of xylose and glucose (Vessia 2005):

$$3C_5H_{10}O_5 \rightarrow 5C_2H_5OH + 5CO_2$$
$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

Several different waste and underused agricultural residues could avail as inexpensive feedstocks for bioethanol production. Hemicelluloses produce a mixture of sugars by different pretreatment methods. The sugar mixture of hemicelluloses can involve 5C and 6C sugars such as xylose, arabinose, glucose, galactose, mannose, fucose and rhamnose depending on the hemicellulose source. The most commonly used yeasts, *Saccharomyces cerevisiae* and *Zymomonas mobilis,* easily and efficiently ferment the 6C sugars of glucose, however, they cannot ferment 5C sugars of xylose and arabinose. On the other hand, the yeasts such as *Pichia stipitis*, *Pachysolen tannophilus*, and *Candida shehate* can ferment xylose to ethanol (Bothast et al. 1997, Schneider et al. 1981, Wang et al. 1980). Commercial use of these yeasts for bioethanol production from xylose is limited mostly by their low ethanol tolerance, slow rates of fermentation, difficulty in controlling the rate of oxygen supply at the optimal level, plus sensitivity to inhibitors generated during pretreatment and hydrolysis of lignocellulosic substrates (Du Perez 1994, Hahn-Hagerdal et al. 1994). Another pentose sugar, arabinose, is often present certain amount in hemicellulosic hydrolyzate depending on the source. However, only a few yeast strains can hardly ferment arabinose to ethanol (Dien et al. 1996, Saha and Bothast 1996). Consequently, no naturally occurring yeast may ferment all these sugars to ethanol. However, among the 5C-fermenting yeasts, *P. stipitis* has shown promise for industrial applications, so it ferment quickly xylose to ethanol with high yield and pruduces no xylitol. Moreover, *P. stipitis* has no absolute vitamin requirement for xylose fermentation and is can ferment a wider range of sugars including cellobiose (Nigam 2001).

Also some ethanologenic bacteria, such as *Escherichia coli*, *Klebsiella oxytoca* ve *Zymomonas mobilis* currently use the most promise for industrial exploitation (Dien et al. 2003). *Z. mobilis* also efficiently produces bioethanol from the hexose sugars glucose and fructose, but not from pentose sugars, although a xylose-fermenting *Z. mobilis* was generated by introducing a xylose-metabolizing pathway from *E. coli* (Hahn-Hagerdal et al. 2006). Despite its advantages as an ethanologen, *Z. mobilis* is not well suited to biomass conversion because it ferments only glucose, fructose and sucrose. However, over the last decade, researchers at the National Renewable Resources Laboratory (NRRL, US Department of Energy) have successfully engineered strains capable of fermenting xylose and arabinose (Dien et al. 2003). *E. coli* and *K. oxytoca* naturally metabolize arabinose, such that the ethanologenic strains ferment all lignocelluloses-derived sugars (Hahn-Hagerdal et al. 2006).

Fermentation process can be carried out three different ways namely batch, fed batch, and continuous process. The select of the most suitable process depends on the microorganisms and the source of lignocellulosic hydrolysate as well as the economic aspects of the process (Chandel et al. 2007). Fed-batch process is commonly used in industrial applications since they combine the advantages of both batch and continuous processes (Saarela et al. 2003). Compared to batch process, the main advantage of fed-batch process is the ability to increase maximum possible cell concentration, prolong culture life, and allow product accumulation to a higher concentration (Frison and Memmert 2002).

1.1.6. Biodegradable Film Production from Hemicellulosic Hydrolyzates and Their Applications

In recent years, biodegradable materials have increased great interest due to sustainable development policies be inclined to transform with the decreasing the fossil fuel reserve and environmental reasons. These materials have a wide variety of uses such as packaging, agriculture, medicine, and other areas. In addition, these materials bring a significant contribution to the sustainable development due to the large variety of removal possibilities with zero environmental impact. Consequently, these environmentally friendly materials market has a rapid expansion, 10–20 % per year. Thus, biodegradable polymers are the topics of much research (Avérous and Pollet 2012).

According to the ASTM D-5488-94d, "biodegradable" means "capable of undergoing decomposition into carbon dioxide, methane, water, inorganic compounds, and biomass methane, water, inorganic compounds or biomass in which the predominant mechanism is the enzymatic action of micro-organisms, that can be measured by standardized tests, in a specified period of time, reflecting available disposal conditions". On the other hand, according to the ASTM D 6400-04, "biodegradable plastic" means "a degradable plastic in which the degradation results from the action of naturally occurring microorganisms such as bacteria, fungi, and algae".

Biodegradable polymers are natural polymers derived from four broad feedstock areas. Some are from animal sources like collagen, gelatin; from marine sources like chitin and chitosan. However, the remaining two feedstock areas are the most attention from scientists, and are the sources thought to be the most promising for future development and expansion. These are from microbial sources like polylactic acid (PLA), polyhydroxyalkanoates (PHAs) and from agricultural feedstock like lipids, proteins and polysaccharides (Tharanathan 2003).

Biodegradability and renewability are the main advantages of natural biopolymers on synthetic polymers. Because of these materials are abundant, low-cost, and annually renewable, especially in countries where landfill is the main agricultural sector and environmentally friendly, they are considered attractive alternatives for the petrochemical based non-biodegradable plastics (Rhim and Perry 2007).

Food packaging, an important discipline in the area of food technology, is concerned with the preservation and protection of all types of foods and their raw materials (Tharanathan, 2003). There are various materials used in food packaging. The most commonly used ones are petrochemical based polymers like polyolefins, polyesters, polyamides, and polyethylene. They are mostly desired due to their availability in vast amount at low prices, favorable functionality characteristics like good tensile and tear strength, good barrier properties to oxygen and aroma compounds and heat sealability. However, they are completely non-biodegradable, demonstrating very low water vapor transmission rates, and leading to ecological problems. Therefore, producers shift their production to the biodegradable packaging materials (Tharanathan, 2003).

Xylans from hemicelluloses are especially interesting as sustainable raw materials since they are easily available prior the pulping, in pulp residues, and side-streams of various stages of pulp production. Hemicelluloses from agricultural residues mainly consist of xylan. Xylans are considered to be a promising raw material for the manufacturing of films for packaging purposes (Hansen and Plackett 2008, Mikkonen and Tenkanen 2012). Xylan films are considered a potential future additional for widely used non-degradable oxygen barrier materials, including aluminum foil and synthetic polymers. When cast from aqueous solutions, some xylans can form dense, hydrogen-bonded structures with low oxygen permeability (OP). The film formation and barrier properties depend on xylans source and chemical structure besides on the plasticization of the films by water or added plasticizers such as glycerol and xylitol (Mikkonen and Tenkanen 2012).

In previous studies show that xylan film production was seen to be possible, however pure extracted xylan from different lignocellulosic biomass such as hardwood, cotton stalks, aspen, and birch wood did not form continuous and self-supporting films. On the other hand the addition of different additives as plasticizers such as sorbitol, glycerol, or xylitol improves its film-forming properties (Gabrielii and Gatenholm 1998, Gabrielii et al. 2000, Göksu et al. 2007, Gonçalves 2011). To increase the water vapor permeability of xylan films, glycerol was added as an extra plasticizer to xylan film solutions. Moreover, some researchers have been added gluten to increase the mechanical properties (Karamanlıoğlu 2008, Göksu et al. 2007, Kayserilioğlu et al. 2003). Xylan was in addition combined with wheat gluten films to produce biodegradable composite films. The results show that up to 40% (w/w) addition of xylan, the films were obtained without altering the properties of gluten films. When different types of xylan like birchwood, corncob and grass xylan was used at different concentrations, the gluten films demonstrated 1 to 8 MPa tensile strength, 20 to 650% strain at break and 5 to140

MPa elastic modulus. Moreover, it was observed that only slightly affected water vapor transfer rate of films by the addition of xylan (Kayserilioğlu et al. 2003).

Agricultural residues including fibers are used as reinforcing additives in the biopolymer industry. Natural cellulose fibers such as flax and hemp fibers are cost-efficient, biodegradable, and also have strong mechanical properties (Bismarck et al.2002). Due to these properties cellulose fibers the most common natural additives in biopolymer industry. Hornsby et al. (1997) reported that the addition of 25% w/w of cellulose fibers in a polypropylene matrix results in a significant increase in tensile strength. Nanocellulose is a versatile material with a wide array of possible commercial applications.

1.1.7. Soda Pulping

Soda pulping was one of the first chemical pulping methods and was invented in 1851 by Hugh Burgees and Charles Watt in England. Because of the finding little interest in England for this process, Burgess brought this method to the USA in 1854 and the first commercial production of soda pulp was in 1866. Soda pulping is the most common method of chemical pulping for various agricultural residues and annual crops including wheat straw and corn stalks all over the world. However, the soda pulping method usually gives lower yield and tear strength than other chemical pulping processes (sulfite method and kraft method), but has still limited use for easy pulped materials like straw and some hardwoods. The soda pulping process was experimentally produced from wood by cooking it with caustic soda at elevated temperature and pressure (Biermann 1996). The first corn stalks pulping plant was installed to produce packaging paper and paperboard pulp in Austria by the beginning of 1800. Then followed by Weber in 1929 (McGovern et al. 1987).

1.2 OBJECTIVE OF THE THESIS

Turkey is a vast agricultural region and has high agricultural waste potential. Turkey is a significant wheat and corn producer in the world. Therefore, large quantities of underutilized and inexpensive wheat straw and corn stalks residues exist after harvest. Approximately 40-53 million tons of straw (Ergudenler and Isigigur 1994) and 6 million tons of corn (TUIK 2014) were produced in Turkey per year. In addition, it should be mentioned that wheat straw is the second largest agricultural residue in the world after sugarcane straw (867 million ton/y in 2008/09).

The residues are mostly burnt or left in the field after harvest and may create environmental pollution. These residues are abundant and overall have little economic value. On the other hand, they with high carbohydrate content (70-75% w/w) have a significant potential for industrial applications.

These biomaterials in addition includes hemicelluloses and lignin in the structure and the heat value of hemicelluloses is low than that of lignin (van Heiningen 2006). Therefore, in pulping industry, hemicelluloses can be pre-extracted from the biomaterials structure prior to pulping operations. The extracted hemcielluloses could be utilized to produce chemicals, biofuels, etc. Regarding this, the developed new concept, Integrated Forest Products Biorefinery (IFBR), pre-extracts some (not all) hemicelluloses prior to pulping (van Heiningen 2006). The extract could be used to produce economically profitable materials and expected to add extra income to pulp or paper mills.

The main objective of this dissertation was to determine whether an integrated biorefinery is technically feasible when large quantities and inexpensive wheat straw and corn stalks were used as a feedstock.

The side objectives of this research

- The pre-extraction methods of hot water, alkali (NaOH), and modified alkali ($NaBH_4$+NaOH) were applied to assess these techniques. Modified alkali method ($NaBH_4$+NaOH) was hypothesized to remove more lignin during pre-extraction and preserve more carbohydrates in the structure to improve the process yield.

- Pre-extracted hemicelluloses were utilized to produce bioethanol and biodegradable films.
- It was also aimed to produce pulp and paper with almost equal properties of pre-extracted and control pulp.
- In addition, formic acid reinforced hot water pre-extraction was studied to convert sticky precipitates occurred during hot water pre-extraction to non-sticky particles or eliminate their formation in the extracted liquors. This application may expect to increase the sugar recovery in the extracted liquors.

2. INTEGRATED PRODUCTION OF BIOFILM, BIOETHANOL, AND PAPERMAKING PULP FROM WHEAT STRAW

2.1. ABSTRACT

Depending on the production method, traditional paper mills often utilize the black liquor by burning it for energy. Hemicelluloses extracted from the raw material prior to pulping could be utilized to produce biochemical fuels. The aim of this study was to pre-extract hemicelluloses from wheat straw by treating the biomass with hot water and alkali (NaOH or the combination $NaOH+NaBH_4$) at varying temperatures and chemical concentrations, and also to integrate resulting solid material to produce pulp and to produce bioethanol and biodegradable films from extracted liquor consisting mostly of xylan. Optimum hot water (135 °C) and alkali pre-extractions (16.7% NaOH at 50 °C) removed 16.5% and 33.6% of the xylan from the straw structure, respectively. The liquid portion of the hot water (135 °C) and alkali (16.7% NaOH at 50 °C) pre-extracted oven-dry (OD) straw yielded up to 7.79% and 6.81% (g/100 g soluble material) ethanol. Good-quality biodegradable films were produced when some gluten and nanocellulose was added to the extracted xylan. The hot water pre-extracted unscreened pulp yields were almost same; so its physical and mechanical pulp properties were comparable to those of the corresponding conventional soda pulp.

2.2. INTRODUCTION

Deforestation is a major global problem that raises the demand for alternatives to wood for the production of pulp and paper, as well as for chemicals, fuel, and other purposes. On the other hand, fibrous wheat straw is abundant and is likely to increase in quantity as the needs of human cereal consumption are met. Wheat is the world’s most widely grown crop, and its annual production is estimated to be over 650 Tg (Atwell 2001) with wheat residues of 850 Tg produced annually (Talebnia et al. 2010). Recycling these organic raw materials would not only diminish environmental pollution, but would also utilize these wastes as a source of energy (Rajoka 2005).

The physical structure of straw makes it applicable for obtaining pulp fibers, and several researchers have investigated using straw for nanocomposite production (Helbert et al. 1996) and in the fermentation (Yasin et al. 2010) and medical (Kumar et al. 2011) industries, among others.

Turkey, with its rich agricultural potential and vast agricultural regions, is one of the world’s most important wheat producers and subsequently is a source of large quantities of underutilized and inexpensive wheat straw. Approximately 40 to 53 million tons of straw is produced in Turkey per year (Ergundenler and Isigigur 1994). Similar to other biomass, straw contains cell walls made of highly lignified structural carbohydrates and small amounts of extraneous materials. Chemical analysis shows that straw is not only rich in carbohydrates, but also rich in bioactive compounds and vitamins (Slavin 2003). The cell walls consist primarily of cellulose and hemicellulose. In straw, cellulose and xylose are the predominant carbohydrate components (Liu et al. 2005). A few pectic compounds and mannans are also present in the structure. Lignin is covalently bound to the side chains of xylan and is an obstacle to carbohydrate digestion because of the phenolic compounds in the structure (Antongiovanni and Sargentini 1991). One major mineral component of straw is silica, which also tends to impede polysaccharide degradation.

The new concept of an integrated biorefinery proposed by van Heiningen (2006) pre-extracts a part of the hemicellulose prior to pulp production. This application is expected to improve the competitiveness of pulp producers without disrupting the pulp and paper production. In conventional chemical pulp production, the black liquor, which includes a fraction of hemicelluloses, is burnt to recover pulping chemicals and to generate steam and electricity. On the other hand, hemicelluloses pre-extracted with hot water (Yoon et al. 2008) and mild alkali (Al-Dajani and Tschirner 2008) prior to pulping could be converted to soluble sugars and utilized to produce ethanol and other valuable chemicals (Xing and Huber 2011). In addition, literature (Copur et al. 2012, Copur and Tozluoglu 2008) has disclosed that adding small amount of $NaBH_4$ in alkali pulping improves pulping selectivity by preventing peeling reactions and hemicelluloses degradation. Using $NaBH_4$ is expected to preserve more glucan and xylan and to degrade lignin more selectively.

Several researchers have examined this new concept regarding woody materials; however, there is limited information about agricultural residues (Resalati et al. 2012). In the present study the objective was to determine whether an integrated biorefinery is technically feasible for pulp producers using wheat straw as a raw material and to assess the potential benefits of pre-extractions with hot water and alkali (NaOH and $NaBH_4$).

2.3. MATERIALS AND METHODS

2.3.1. Materials

Wheat straw (*Triticum astivum*), locally obtained in the Düzce province of Turkey, was cut to suitable sizes (3 to 8 cm) using a garden chopper. The chopped material was air-dried at room temperature. The moisture content was determined according to the TAPPI T 412 om-11 test method, and the material was sealed in plastic bags and stored at -5 °C.

2.3.2. Methods

The wheat straw was first pre-extracted using hot water, NaOH, and a mixture of $NaOH+NaBH_4$. The extracted liquids were hydrolyzed and fermented to produce ethanol from xylan and arabinan. The solid parts were further cooked to produce soda pulps.

2.3.2.1. Pre-extractions

For this study, the pre-extractions were conducted in a 3 L rotating digester. In the hot water extractions, the equivalent of 100 g of oven-dry (OD) straw and the required amount of water were added to the digester. The liquor-to-straw ratio was 10 L/kg oven-dry (OD). The digester was heated to the maximum temperature in 30 min and then kept at the maximum treatment temperature for 4 h. The maximum treatment temperatures in the hot water pre-extractions were 90, 120, 135, and 150 °C.

In the alkali (NaOH) pre-extractions, the equivalent of 100 g of OD straw and the required amount of alkali solutions were added to the digester. The liquor-to-straw ratio was 4 L/kg OD. The digester was heated to the maximum temperature in 30 min and then kept at the maximum treatment temperature for 4 h. The effects of NaOH concentration (16.7%, 26.7%, and 33.3% w/w) and treatment temperature (50, 70, and 90 °C) were investigated in the alkali pre-extractions. The optimum alkali treatment condition was determined regarding the ratios of carbohydrates to lignin in the solid materials and $NaBH_4$ was added (only for the optimum alkali concentration). Additions of 0.1%, 0.5%, 2%, and 4% $NaBH_4$ were made to the alkali (16.7% NaOH w/w), and pre-extractions for the OD wheat straw were conducted at 50 °C for 4 h. The other conditions were kept constant.

After pre-extraction, the liquor portions were separated from the solids by filtering through a cloth, and the liquid and solid materials were collected for further testing. All pre-extractions were performed in duplicate.

2.3.2.2. Analysis of pre-extracted liquids

The pre-extraction liquors were first centrifuged (5000 rpm) to separate the resultant solids from the liquid. To hydrolyze the oligomers in the liquid portion into monomeric sugars, 50 mL of liquid was incubated in an autoclave with 1000 mL of 4% sulfuric acid at 121 °C for 1 h (Li et al. 2010). The liquids were neutralized with NaOH to pH 7 before analyzing the monomeric sugar contents in the liquids using high performance liquid chromatography (HPLC) (Agilent 1200 series, USA). The above procedure was conducted to determine the total sugar content of the liquids before fermentation.

2.3.2.3. Fermentation and ethanol production

The obtained liquids were fermented in a bioreactor (Sartorius Stedim Biotech Fermenter) to produce ethanol. The pH was maintained at 6.0 throughout the fermentation by the automatic addition of 0.5 M NaOH and 0.5 M HCl. The medium was supplemented with 1.70 g/L $(NH_4)2SO_4$, 0.96 g/L K_2HPO_4, 0.17 g/L $MgSO_4.7H_2O$, 0.23 g/L $CaCl_2.2H_2O$, and 0.02 g yeast extract. For fermentation, *Pichia stipitis* from overnight cultures was added and incubated in a bioreactor. Fermentation was conducted with 100 rpm for 48 h at 30 °C. Samples were taken out periodically (3, 6, 12, 24, and 48 h) and centrifuged at 10,000 × g for 10 min; then, the supernatants were filtered through 0.45-µm filters and stored at -20 °C until HPLC analysis. The fermentation experiments were performed in duplicate.

2.3.2.4. Biodegradable film production

The pre-extracted liquid was first filtered and then centrifuged (5000×g) for 5 min to completely remove small particles. Hemicellulose in liquid was precipitated with 2500 mL of refrigerated acetic acid-ethanol (1:10 v/v). The precipitate was desalted while washing with deionized water (200 mL) and ethanol (600 mL) three times. The hemicellulose left was dried at room temperature.

Mixtures of 0.6 g of precipitated material (xylan) and 0.4 g of xylitol were stirred in deionized water (15 mL), and the solutions were then poured into polystyrene petri dishes (9 cm). The samples were dried in a conditioned room (50% relative humidity and 23 °C) for 3 days. In addition, 0.10 g of gluten and 0.025 g of nanocellulose were used in biodegradable film production to improve the mechanical properties.

The produced films were conditioned according to TAPPI standard method (T 402 sp-08). The thickness of the samples was determined with a digital micrometer (40 EXL, Mahr GmbH, Esslingen, Germany) with a sensitivity of 0.1 µm. The tensile strength of the samples was tested (Zwick/Roell Z250 Strength Testing Device with 100 N/10 kN load cell). Samples for testing were prepared according to TAPPI standard method (T494 om-96). Elastic modulus and tensile strength are reported.

2.3.2.5. Pulping

As a control, conventional soda pulp was prepared from wheat straw to provide a basis for comparison with hot water (135 °C) pre-extracted pulps. On the other hand, the alkali and $NaBH_4$ pre-extracted materials resulted in lower yields and mechanical pulps were produced from them according to method explained by Chang et al. 2012.

The liquor-to-straw ratio was 10 L/kg OD. Regarding the optimum alkali pre-extraction condition, the soda pulps were cooked at 16.7% alkali charge. Furthermore, the samples were cooked to the target H factor of 460 at 160 °C for 65 min. The maximum temperature was achieved in 30 min. The H factor of the hot water pre-extraction was calculated and excluded from the total H factor. Pulps obtained from each cook were defibrillated in a laboratory disintegrator and thoroughly washed on a 200-mesh screen with warm (40 °C) tap water.

To determine paper properties, the control and the hot water pre-extracted pulps were subjected to refining. A Waring blender (Chang et al. 2012, Chang et al. 2010, French and Maddern 1994, Shaw 1984) was used to evaluate the refining response, and pulps were refined for 0, 2, and 4 min. The freeness level of the pulps was determined according to ISO standard method (5267-1). Handsheets were produced in a Rapid Kothen machine in accordance with ISO standard method (5269-2). The paper physical properties were determined according to TAPPI standard method (T 220 sp-01). The optical properties of the pulps were tested according to ISO standard methods (2469 and 2471).

2.3.2.6. Analytical Tests

The straw for the study was sampled and prepared according to TAPPI standard method (T 257 sp-14) for chemical tests. The ash content was determined by TAPPI standard method (T 211 om-12). Alcohol-benzene, hot water, and 1% NaOH solubilities were examined according to TAPPI standard methods of T 204 cm-07, T 207 cm-08, and T 212 om-12, respectively.

The yield determined by gravimetric measurements was calculated from the dry weight of the straw and the weight of the initial sample. The moisture content was obtained by drying the samples to a constant weight at 105 °C. The kappa number and the viscosity of the pulps were determined according to TAPPI standard method of T 236 om-99 and SCAN standard method of cm 15:88, respectively.

The laboratory analytical procedures from the National Renewable Energy Laboratory (NREL) were used to determine the sugar (glucose, xylose, and arabinose) and lignin (acid-insoluble and acid-soluble) contents of the samples (Sluiter et al. 2011). The sugar and ethanol contents were determined using HPLC (Agilent 1200 series, USA) equipped with a Shodex SH1011 sugar column (mobile phase: 5 mM H_2SO_4, flow rate: 0.5 mL/min, column temperature: 60 °C) and a refractive index detector. The acid-insoluble lignin was obtained by weighing the solid samples. The acid-soluble lignin was analyzed at the adsorption of 320 nm against blank deionized water.

The percentage of solids recovered was calculated on an oven-dry basis as follows in Equation 1, The percentage of solids recovered = $(W_2 \div W_1) \times 100$ (1)

where W_1 is the dry weight of the sample before pretreatment (g) and W_2 is the dry weight of the treated sample (g).

The reduction in lignin was calculated with respect to the initial dry weight of lignin in the untreated material (LU) and the dry weight of lignin in the remaining solids after treatment (LP). The percentage of lignin reduction was calculated with the following equation,

The percentage of lignin reduction = $[(LU-LP) \div LU)] \times 100$ (2)

where LP is the dry weight of lignin in the pre-treated sample and LU is the dry weight of lignin in the untreated biomass.

In addition, the solubilization of glucan and xylan in the pre-treated samples was calculated in the same manner by substituting the appropriate percentage for glucan and xylan.

During fermentation, the ethanol yield was calculated as a percentage of the theoretical maximum yield (Kim and Lee 2005) using the following equation,

The percentage of theoretical ethanol yield = $E \div (X \times 0.511) \times 100$ (3)

where E and X represent ethanol (g) produced during fermentation and xylose (g) in the liquid, respectively. The constant 0.511 is the theoretical yield of ethanol produced from xylose and arabinose.

2.3.2.7. Statistical analyses

The data obtained were statistically analyzed via the SPSS packet program. The analysis of variance (ANOVA) was used to identify significant differences and the differences between groups were determined according to the Duncan test.

2.4. RESULTS AND DISCUSSION

2.4.1. Chemical Composition

Table 2.1 summarizes the chemical composition of the wheat straw and the literature values for hard/softwood (Fengel and Wegener 1989) and wheat straw (Ertas et al. 2014, Montané et al. 1996). The polysaccharides content (sum of glucose, xylose, and arabinose) was 60.1%, and lignin and ash accounted for 29% of the dry wheat straw. The wheat straw had higher extractives and ash contents but lower lignin and carbohydrate contents compared to hard/softwood. In addition, all solubility values of the wheat straw were noticeably higher than those of hard/softwood. In the straw structure, the main part of glucan was cellulose and xylan comprised most of the hemicelluloses. The obtained data were within the range found in the literature (Ertas et al. 2014, Mosier et al. 2005, Montané et al. 1996). Slight differences in values could be due to growth environment, soil conditions, or other factors (Utne and Hegborn 1992).

Table 2.1. Chemical composition of wheat straw and hard/softwood.

Chemical composition	Raw material (%)*			
	Wheat straw (Current work)	Wheat straw (Ertas et al. 2014)	Wheat straw (Montané et al. 1996)	Hard/Softwood (Fengel and Wegener 1989)
Extractives	7.62±0.33	1.21	5.40	2-6/2-8
Hot water solubility	15.1±0.11		6.22	2-7/3-6
1% NaOH solubility	46.4±0.17			14-20/9-16
Lignin	21.4±0.31**	22.6	17.1	30-35/25-35
Ash	7.56±0.03	4.40	8.00	0.35/0.35
Glucose	34.9±1.52	35.4	33.7	
Xylose	22.8±0.85	17.8	23.6	
Arabinose	2.37±0.09	2.70		

*Composition percentages are oven-dry-weight basis and values are average of duplicate measurements. **Acid soluble lignin and lignin ash included.

2.4.2. Hot Water Pre-Extractions

The loss percentages of weight, glucose, xylose, and lignin in the wheat straw pre-extracted with hot water at varying treatment temperatures are shown in Figure 2.1. The results revealed that treatment temperature had a major effect and that an increase in treatment temperature removed more material from the structure. The weight loss in the straw resulting from the removal of hemicellulose, lignin, and other extraneous materials was greater for higher treatment temperatures, and when the material was pre-extracted at 150 °C, up to 38.5% of the material was removed from the structure. A material loss of almost 17% was reported by Resalati et al. (2012) when the straw was treated at 150 °C for 90 min.

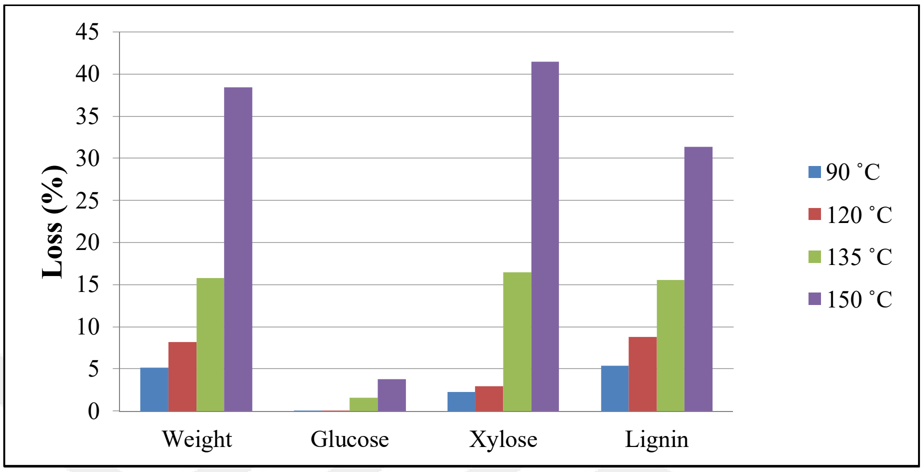

Figure 2.1. Glucose, xylose, lignin, and weight loss for hot water pre-extracted wheat straw at varying treatment temperatures.

For pre-extraction temperatures of 90 and 120 °C, greater lignin removal was observed compared to xylan removal. Similar amounts of xylan and lignin removal were obtained when the treatment temperature was raised to 135 °C. Above this treatment temperature (150 °C), a large amount of xylose (41.5%) was removed from the structure; however, the xylose removal ratio was higher than that of lignin. The removal of lignin with hemicelluloses could be attributed to the covalent bond chains of xylan and lignin in the straw structure. The removal of both components was almost linear. For a similar H factor (794), the hot water extraction of loblolly pine (Yoon et al. 2008) resulted in a weight loss of 15.0%, which is less than half as much as the wheat straw treated in this study (38.5%). This finding could be due to the lower degree of polymerization (DP) of the hemicelluloses of the straw (Antongiovanni and Sargentini 1991).

As can be seen in Figure 2.1, the cellulose fraction was undamaged up to the treatment temperature of 135 °C. When the material was pre-extracted with hot water at 150 °C, 3.79% of the glucan was removed from the structure. The small amount of cellulose degradation observed in this study could be explained by the low cellulose crystallinity of straw (Liu et al. 2005). In addition, statistical analysis showed that the weight, glucose, xylose, and lignin losses obtained with hot water pre-extraction were statistically significant ($p<0.001$).

2.4.3. Alkali (NaOH) Pre-Extractions

The major effect of alkali (NaOH) pre-extraction of hemicelluloses is the removal of lignin and acetyl as well as the various uronic acid groups. Figures 2.2a through 2.2d show the alkali pre-extraction results for removal of weight, glucan, xylan, and lignin from the wheat straw structure. The alkali used in this study was capable of reducing the contents of both hemicellulose and lignin from the straw structure under the applied treatment conditions. The alkali pre-extraction under various conditions resulted in weight losses ranging from 34.5% to 52.4% (Table 2.2). The results indicated that treatment temperature and alkali charge had a noticeable effect on the pre-extraction yield. An increase in both alkali charge and treatment temperature extracted more material from the straw structure. The rate of material extraction was lower for alkali concentrations of 26.7% to 33.3% compared to concentrations of 16.7% to 26.7% (Figure 2.2a).

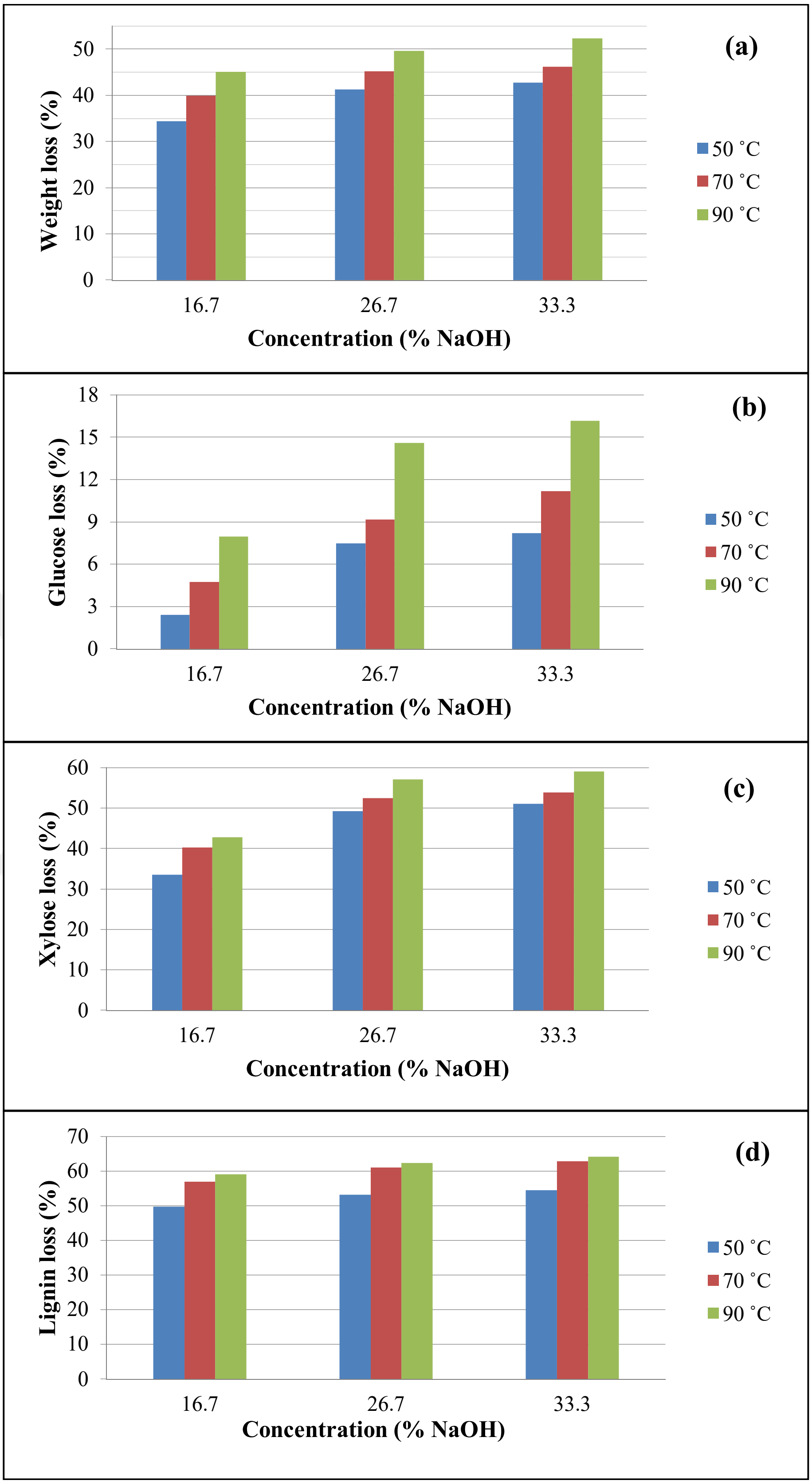

Figure 2.2. (a) Weight loss, (b) glucose loss, (c) xylose loss, and (d) lignin loss in NaOH pre-extracted wheat straw at various treatment temperatures and chemical concentrations.

The pre-extraction also dissolved some xylan in the straw structure. The results showed that both alkali charges and treatment temperatures greatly affected the xylan extraction (Figure 2.2c) and increases in both resulted in higher xylan extraction from the structure. An increase in the alkali charge from 16.7% to 26.7% resulted in extraction of more xylan; however, the xylan extraction was lower for a higher (33.3%) alkali charge. The maximum xylan extraction was 59.2%, i.e., approximately 13.5% based on the original straw, when the wheat straw was treated at 90 °C and 33.3% alkali (NaOH) charge. In the literature, ground extracted wheat straw treated with 12% NaOH for 4 h at 80 °C resulted in the removal of 90% of the pentosans and 80% of the lignin (Cunningham et al. 1986). The lower xylan extractions in this study could be due to the straw size. In addition, nearly linear xylan extraction was observed when the treatment temperature was increased from 50 to 70 and 90 °C. One objective in this study was to optimize the pulping yield and paper properties after pre-extraction, and for that reason, some hemicelluloses had to be preserved in the material structure. The purpose was to collect pulp from pre-extracted material having almost equal pulp and paper properties of control soda pulp.

Table 2.2. Glucose, xylose, lignin, and weight loss of NaOH pre-extracted wheat straw at varying treatment temperatures and chemical concentrations.

Temp. (°C)	NaOH conc. (%)	Weight loss (%)	Glucose loss (%)	Xylose loss (%)	Lignin loss (%)
50	16.7	34.5±0.67 a*	2.41±0.71 a	33.6±0.71 a	49.9±0.06 a
	26.7	41.3±0.83 c	7.49±0.07 c	49.3±0.04 d	53.3±0.05 b
	33.3	42.8±0.30 d	8.22±0.42 d	51.1±0.21 e	54.5±0.20 c
70	16.7	40.0±0.09 b	4.76±0.22 b	40.3±0.29 b	57.0±0.12 d
	26.7	45.3±0.01 e	9.25±0.04 e	52.5±0.27 f	61.1±0.54 f
	33.3	46.3±0.32 f	11.2±0.07 f	54.0±0.14 g	63.0±0.10 g
90	16.7	45.1±0.83 e	7.98±0.14 cd	42.9±0.35 c	59.1±0.14 e
	26.7	49.7±0.36 g	14.6±0.17 g	57.1±0.33 h	62.5±0.28 g
	33.3	52.4±0.67 h	16.2±0.04 h	59.2±0.21 i	64.3±0.99 h

*Within each column, the factors followed by this letter were not significantly different according to the Duncan test ($p<0.05$)

As with xylan extraction, increases in treatment temperature and alkali charge removed more glucan from the straw structure. Compared with the original wheat straw, consisting of 34.9% glucose, glucan degradation ranged from 2.41% to 16.2% (based on OD straw), depending on the treatment temperature and alkali charge (Table 2.2). Even with alkali extractions performed at low treatment temperatures (lower than 100 °C), results in this study indicated that some delignification and degradation reactions occurred. The mild extraction temperatures could explain this lower glucan removal. Taherzadeh and Karimi (2008) observed similar results. Pulping potential and paper properties are all affected by cellulose degradation, and the lower glucan degradation in this study could improve paper properties produced from extracted solid materials.

The results displayed higher lignin removal from the straw structure for higher treatment temperatures and alkali charges (Figure 2.2d). In this study, the rate of lignin removal was greater than that of xylan removal.

When the extraction rates of glucan, xylan, and lignin under varying treatment conditions were compared, it may be observed that the treatment temperature, as opposed to the alkali (NaOH) charge, was more effective. The improved solubility of the structural straw components with high alkali concentrations most likely occurred because the delignification reactions were more complete at higher alkali concentrations. On the other hand, alkaline pre-extractions had a significant ($p<0.001$) effect on weight, glucose, xylose, and lignin losses.

Optimized pre-extraction conditions could thus be set as a guide regarding hemicellulose extraction and pulp properties. The optimum treatment condition was determined for the rate of lignin and xylan removal from the structure; the optimum alkali pre-extraction charge was found to be 16.7% at 50 °C.

2.4.4. $NaBH_4$-modified Alkali Extraction

The addition of a small amount of $NaBH_4$ in alkali pulping improves pulping selectivity by preventing peeling reactions and hemicelluloses degradation (Çöpür et al. 2012). The $NaBH_4$ utilized in this study was expected to preserve more glucan and xylan and to degrade lignin more selectively (Copur and Tozluoglu 2008). To gain a more efficient delignification, the alkali extraction was modified by adding 0.1%, 0.5%, 2%, and 4% (OD material) of $NaBH_4$ at the optimum NaOH extraction conditions (16.7% NaOH at 50 °C). The data in Table 2.3 and Figure 2.3 show that modifying the alkali extraction with $NaBH_4$ had only a slight effect on xylan degradation. The resulting glucan loss after modification was very small and thus was not reported in this study. On the other hand, the xylan loss was much higher than that of glucan. This finding could be explained by the chemical bonds of xylan and lignin in the straw structure (Yoon et al. 2008). The effect of modification on lignin delignification was remarkable; the addition of 0.1% $NaBH_4$ removed 6.40% of the lignin from the straw structure. While the treatment with 2% $NaBH_4$ led to a slight increase in xylan solubilization (1.60%), it resulted in more lignin delignification (10.2%) when compared to the weight loss of the straw without the addition of $NaBH_4$. Statistical analysis indicated that adding $NaBH_4$ to 16.7% NaOH had significant effect on weight ($p<0.001$), lignin ($p<0.001$), and xylose ($p<0.01$) losses.

Table 2.3. Weight and chemical loss for NaOH+$NaBH_4$ pre-extracted wheat straw.

Temp. (°C)	NaOH-$NaBH_4$ conc. (%)	Weight loss (%)	Xylose loss (%)	Lignin loss (%)
50	16.7	34.5±0.67 a*	33.6±0.71 a	49.9±0.06 a
	16.7-0.1	34.8±0.03 ab	33.9±0.49 a	56.3±0.70 b
	16.7-0.5	35.2±0.27 bc	34.4±0.00 ab	58.9±0.76 c
	16.7-2.0	35.5±0.19 c	35.2±0.42 bc	60.1±0.64 d
	16.7-4.0	36.5±0.14 d	35.9±0.28 c	60.4±0.73 d

*within each column, the factors followed by this letter were not significantly different according to the Duncan test ($p<0.05$)

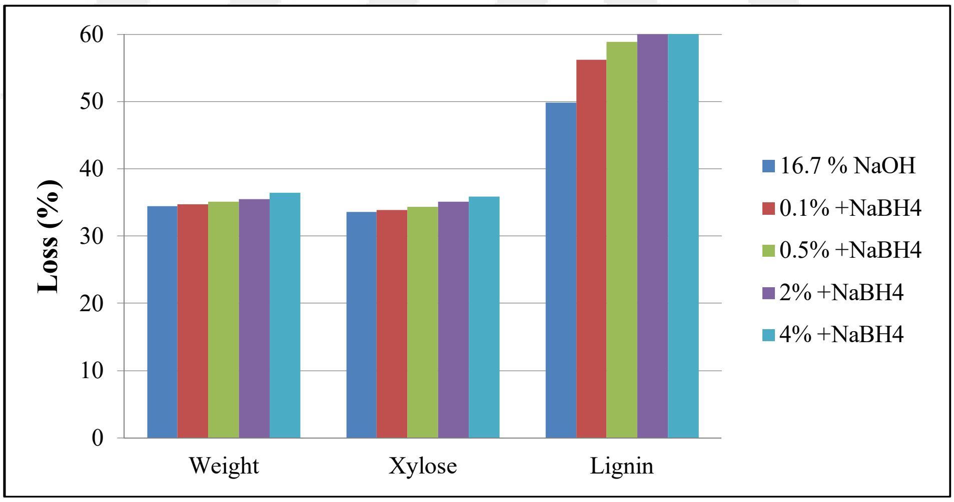

Figure 2.3. Xylose, lignin, and weight loss of NaOH+$NaBH_4$ pre-extracted wheat straw at varying $NaBH_4$ concentrations.

2.4.5. Composition of Liquids and Ethanol Production

The liquid portion of some pre-extracted straw (hot water, alkali, and $NaBH_4$- modified) was further examined for this study. The liquid portion had some oligomers, including glucan, xylan, arabinan, and water-soluble lignin. The yield of glucan in the liquid portion was very low, which indicated that the glucan was unhydrolyzed in pre-extraction. This could be explained by the mild treatment conditions utilized in this study.

Almost 94.1% of the xylan was recovered as monomeric sugar (xylose) when the straw was treated with hot water at 135 °C. The higher treatment temperature (150 °C) resulted in lower (81.3%) monomeric sugar recovery. The lowest xylose recovery (41.2%) was observed when the straw was treated with 16.7% alkali (Table 2.4). This variability in xylose recovery could be related to lignin solubilization, which impedes the recovery of xylose sugars (Mok and Antal 1992). Alkali treatment under mild conditions may prevent lignin condensation and result in high lignin solubility. In addition, mild treatment conditions inhibit conversion of degradation sugars to furfural, HMF, and organic acids (Chang and Holtzapple 2000), as was the case in this study. For this investigation, the xylose and arabinose sugars in the extraction liquids were converted to ethanol (Figures 2.4a-d). The theoretical ethanol yield for both 135 °C and 150 °C hot water pre-extracted straw was found to be 98%.

Table 2.4. Sugars in pre-extracted solids and liquids.

Pre-extraction conditions	Solids				Liquids		
	Sample	Untreated straw (g)	Extracted solids (g)	Loss (%)	Sample	Extracted liquids (%)	Recovery (%)
135 °C	Yield	100	84.2	15.8			
	Xylan	22.8	19.0	16.5	Xylose	15.6	94.1
	Arabinan	2.37	1.52	36.1	Arabinose	6.70	18.5
150 °C	Yield	100	61.5	38.5			
	Xylan	22.8	13.3	41.5	Xylose	33.7	81.3
	Arabinan	2.37	0.00	100	Arabinose	7.40	7.40
16.7% NaOH	Yield	100	65.5	34.5			
	Xylan	22.8	15.1	33.6	Xylose	13.9	41.2
	Arabinan	2.37	1.64	30.9	Arabinose	2.90	9.40
16.7% NaOH +0.1% $NaBH_4$	Yield	100	65.2	34.8			
	Xylan	22.8	15.1	33.7	Xylose	14.0	41.6
	Arabinan	2.37	1.53	35.4	Arabinose	3.50	9.80

In the current study, ethanol yields of 7.79% and 16.9% (g/100 g soluble material) were obtained, respectively. The liquid portion of the alkali-extracted straw (16.7% and 50 °C) had a theoretical ethanol yield of 96.5%, and 6.81% (g/100 g soluble material) ethanol was obtained.

The lowest theoretical ethanol yield (59.2%) was observed when the straw was pre-extracted with 0.1% $NaBH_4$, and 4.22% (g/100 g soluble material) of ethanol was obtained for that sample. The lower xylose recovery and the alkali media in the samples could explain the lower ethanol yield in the alkali and $NaBH_4$-modified pre-extracted samples. Another possible reason is the presence of lignin, which inhibits fermentation.

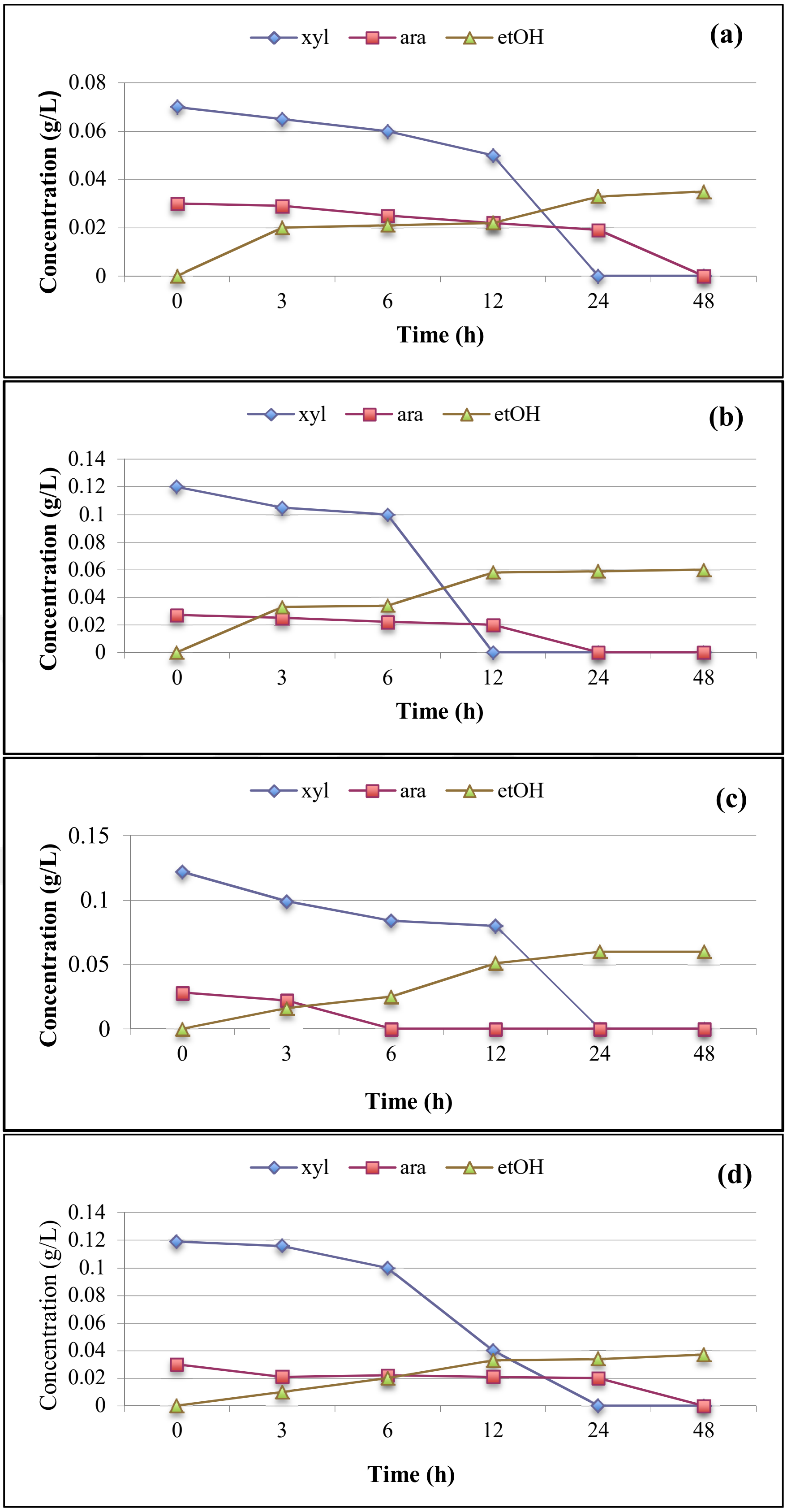

Figure 2.4. The change in xylose, arabinose, and ethanol concentrations of (a) 135 °C hot water pre-extracted, (b) 150 °C hot water pre-extracted, (c) 16.7% NaOH pre-extracted, and (d) 16.7% NaOH + 0.1% $NaBH_4$ pre-extracted wheat straw liquids fermented with *Pichia stipitis*.

2.4.6. Biodegradable Films

Films produced using only xylan developed cracks (Figure 2.5), as also observed in the literature (Gabrielii and Gatenholm 1998, Göksu 2005). To eliminate this problem, some xylitol (Seber 2010, Goncalves 2011) was added, resulting in self-supporting films (Figure 2.5). The mechanical properties of the obtained films were low. However, they were improved by adding some gluten and nanocellulose (Table 2.5). Results indicated that even gluten addition improved the tensile strength. A dramatic increase was observed when small amounts (0.025 g) of nanocellulose were added to the solution (Table 2.5).

Table 2.5. Thickness and mechanical properties of biodegradable films.

Sample	Thickness (mm)	Elastic modulus (N/mm^2)	Tensile strength (N/mm^2)
Control (Xylan + Xylitol)	0.141±0.01	49±2.74	1.72±0.05
Control + Gluten	0.162±0.02	145±12.4	2.88±0.30
Control + Nanocellulose	0.127±0.01	160±6.03	5.24±0.21

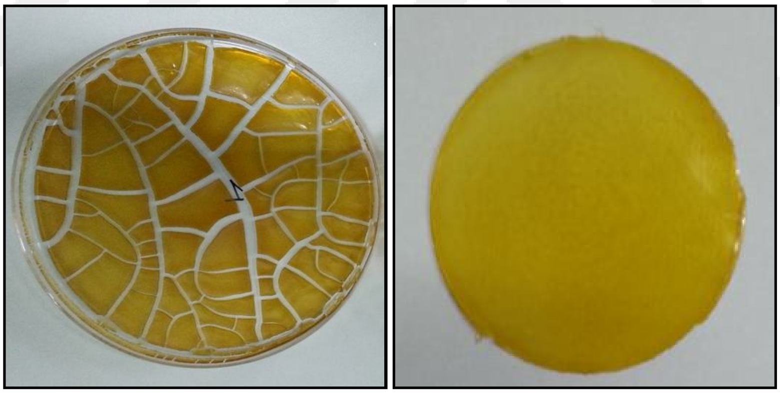

Figure 2.5. Films obtained using only extracted xylan (left) and extracted xylan+xylitol (right).

2.4.7. Pulp and Paper Properties

Mill scale pulp of wheat straw is usually made using the soda method. The main problem after cooking is the high silica content in recovery (Schal et al. 2009). Mustajoki et al. (2010) pointed out that hot water pre-treated soda pulps resulted in better pulp bleachability. In the present study, to minimize the disadvantages of pulp production from wheat straw, the results obtained for the pre-extracted and the control soda pulps were evaluated. The properties of the control and the hot water (135 °C) pre-extracted soda pulps, as well as the properties of the 16.7 % (OD straw) alkali pre-extracted and 0.1 % (OD straw) $NaBH_4$-modified mechanical pulps are given in Table 2.6.

The hot water pre-extracted pulp resulted in almost same unscreened pulp yield (47.9% OD straw) as compared to the control soda pulp (47.6% OD straw). On the other hand, Resalati et al. (2012) found a higher pulp yield for pre-extracted straw soda-AQ pulps. As seen in Table 2.6, the control pulp had a much higher rejection rate (6.40% OD straw). The hot water pre-extracted soda pulp had slightly less xylose, but more lignin in the structure; this could be caused by the removal of some hemicelluloses with the lignin (LCC complexes) (Yoon et al. 2008). Similar to results reported by Zhang and Yang (2011), the hot water pre-extracted (135 °C) soda pulp had lower viscosity than the control pulp; this could be due to the straw structure being more open to cooking chemicals that cause more cellulose degradation (Yoon et al. 2008). The hot water pre-extracted straw resulted in higher kappa pulp. Hot water pre-extraction could improve lignin reactivity and holes because hemicellulose removal from the structure may increase the ability of the cooking chemicals to reach the inner structures. The kappa and yield for the soda pulps obtained in this study were higher than the results in the literature (Resalati et al. 2012); this may be a consequence of the mild cooking conditions applied. The observed values for kappa, viscosity, xylose, glucose, and lignin were all statistically significant ($p<0.001$).

The alkali (16.7% OD straw) pre-extracted and $NaBH_4$ (0.1% OD straw) modified mechanical pulps had similar yields. The results showed that $NaBH_4$ modification preserved cellulose (55.7% OD pulp) and decreased lignin (13.0% OD pulp) content.

The material after alkali (16.7% OD straw) pre-extraction and $NaBH_4$ (0.1% OD straw) modification resulted in lower glucose and xylose contents compared to the hot water (135 °C) pre-extraction. On the other hand, there was a much higher lignin content in the structure. The reason for this may be that the pre-extraction temperature and the lower temperature (50 °C) in the alkali and $NaBH_4$ modifications dissolved less lignin from the straw structure. In contrast, the alkali utilized in pre-extraction as well as the $NaBH_4$ modification dissolved more cellulose and xylose.

The soda pulps (control and hot water pre-extracted) were refined to 0, 2, and 4 min and the mechanical pulps (16.7% alkali pre-extracted and 0.1% $NaBH_4$-modified) were fiberized for 10 min prior to hand-sheet preparation. The freeness, mechanical (tensile, tear and burst), and physical (brightness and opacity) properties of the pulps were measured, and the results are given in Table 2.7.

Table 2.6. Data on pulps.

Pre-extractions	Yield (% of o.d. straw)	Reject (% of o.d. straw)	Kappa (ml/g)	Viscosity (cm^3/g)	Glucose (% of o.d. pulp)	Xylose (% of o.d. pulp)	Lignin (% of o.d. pulp)
Control	47.6	6.40	20.3±0.09 a[*]	979±0.07 b	67.0±0.28 c	26.0±0.14 c	4.32±0.16 a
Hot water 135 °C	47.9	1.98	21.9±0.00 b	798±0.11 a	68.0±1.70 c	24.8±0.71 b	5.54±0.28 b
16.7% NaOH	65.5				55.2±0.71 a	23.1±0.71 a	15.0±0.06 d
+0.1% $NaBH_4$	65.2				55.7±0.71 a	23.2±0.07 a	13.0±0.26 c

[*] within each column, the factors followed by this letter were not significantly different according to the Duncan test ($p<0.05$)

Table 2.7. Paper properties.

Pre-extractions	Refining (min)	SR (mL)	Tensile index (Nm/g)	Tear index (mNm^2/g)	Burst index ($kPam^2/g$)	Brightness (% ISO)	Opacity (% ISO)
Control[*]	0	25	42.4±0.09 b[***]	7.35±0.08 ab	2.44±0.24 b	37.4±0.32 g	94.4±1.16 c
	2	44	63.7±0.23 f	7.41±0.07 ab	3.82±0.25 f	35.7±0.57 f	93.7±0.43 c
	4	57	65.0±0.19 f	6.68±0.04 a	4.01±0.18 g	35.0±0.26 e	93.5±0.17 c
Hot water 135 °C[*]	0	27	40.4±0.09 a	7.23±0.05 ab	2.30±0.23 a	33.8±0.17 d	98.5±0.32 f
	2	45	58.0±0.19 d	7.43±0.08 ab	3.27±0.24 d	32.1±0.26 c	97.6±0.49 df
	4	51	60.6±0.10 e	6.96±0.03 ab	3.48±0.17 e	31.4±0.53 b	97.4±0.28 d
16.7% NaOH[**]	1	45	55.7±0.30 c	7.64±0.04 b	2.64±0.22 c	30.7±0.72 a	89.2±0.53 a
+0.1% $NaBH_4$[**]	1	45	54.0±0.18 c	7.20±0.04 ab	2.54±0.25 c	33.4±0.49 d	90.1±1.03 b

[*] Pre-extracted soda chemical pulps

[**] Pre-extracted mechanical pulps

[***] within each column, the factors followed by this letter were not significantly different according to the Duncan test ($p<0.05$)

The refining response of the hot water pre-treated pulp was lower than that of the control soda pulp. This could be due to the lower hemicellulose content of the hot water pre-extracted straw, causing lower swellability of the fibers (Yoon and Heiningen 2008; Helmerius et al. 2010, Çöpür 2007).

Refining resulted in better mechanical properties (tensile and burst) for the soda pulps, which may be due to the fibrillation of the fibers during refining. On the other hand, the tear index value slightly improved at first, but then decreased with refining intensity. This decrease might be attributed to the damage to individual fibers during refining. The control soda pulp exhibited higher tensile and burst properties compared to the hot water pre-extracted soda pulp. These higher tensile and burst strength values could be explained by better fiber flexibility because of improved inter-fiber bonding of the higher hemicellulose content of the control soda pulp (Çöpür 2007). When the chemical and mechanical pulps were compared, the mechanical pulps had lower tensile and burst index values. This finding could be explained by the higher lignin content of the mechanical pulps, which had lower fiber flexibility and inter-fiber bonding (Çöpür 2007).

The tear value usually depends on individual fiber strength, but infrequently can depend on the degree of inter-fiber bonding. Refining decreases the tear strength of pulps after reaching an initial maximum. The control soda pulp with higher xylose content was expected to exhibit lower tear strength; however, similar tear index values for the control and the hot water pre-extracted soda pulps were observed in this study.

When the physical properties of the paper were considered, the control soda pulp was brighter than the hot water pre-extracted soda pulp. Although refining appeared to have had little effect on pulp brightness and opacity, both properties were diminished slightly. The statistical analysis implied that the pre-extractions had a significant ($p<0.001$) effect on the tensile index, burst index, opacity, and brightness, while no significant differences ($p>0.05$) were found for the tear indices.

The control soda pulp gave better brightness than the hot water pre-extracted chemical pulp and 16.7% NaOH pre-extracted mechanical pulp. This finding could be due to the higher lignin content of mechanical pulp and the effect of heat in hot water pre-extracted pulp.

2.4.8. Mass Distribution of Pre-Extracted Compounds

The mass balance was performed on the basis of 1000 kg OD wheat straw, which was extracted with hot water under the conditions of 135 °C for 4 h (Figure 2.6). The results indicated that approximately 26 kg of xylan could be extracted from 1000 kg of OD wheat straw. Conversely, 2.48 kg of glucan was lost and 25 kg of lignin was delignified during the pre-extraction. The dissolved sugars were fermented to obtain bioethanol; 12.3 kg of ethanol was produced in this study.

The extracted solid wheat straw was cooked with the soda method, and 455 kg of straw pulp was produced. The pulp consisted of 309 kg of glucan, 113 kg of xylan, and 25 kg of lignin. In contrast, 412 kg of soda pulp was obtained when the control cook was accomplished for the equal H factor of the pre-extracted soda cook. The control soda pulp had 276 kg of glucan, 107 kg of xylan, and 18 kg of lignin in the structure. Compared to the control pulp, the hot water pre-extracted pulp had almost similar xylan in the structure. Furthermore, the hot water pre-extraction resulted in 455 kg of soda pulp in addition to the 12.3 kg of ethanol.

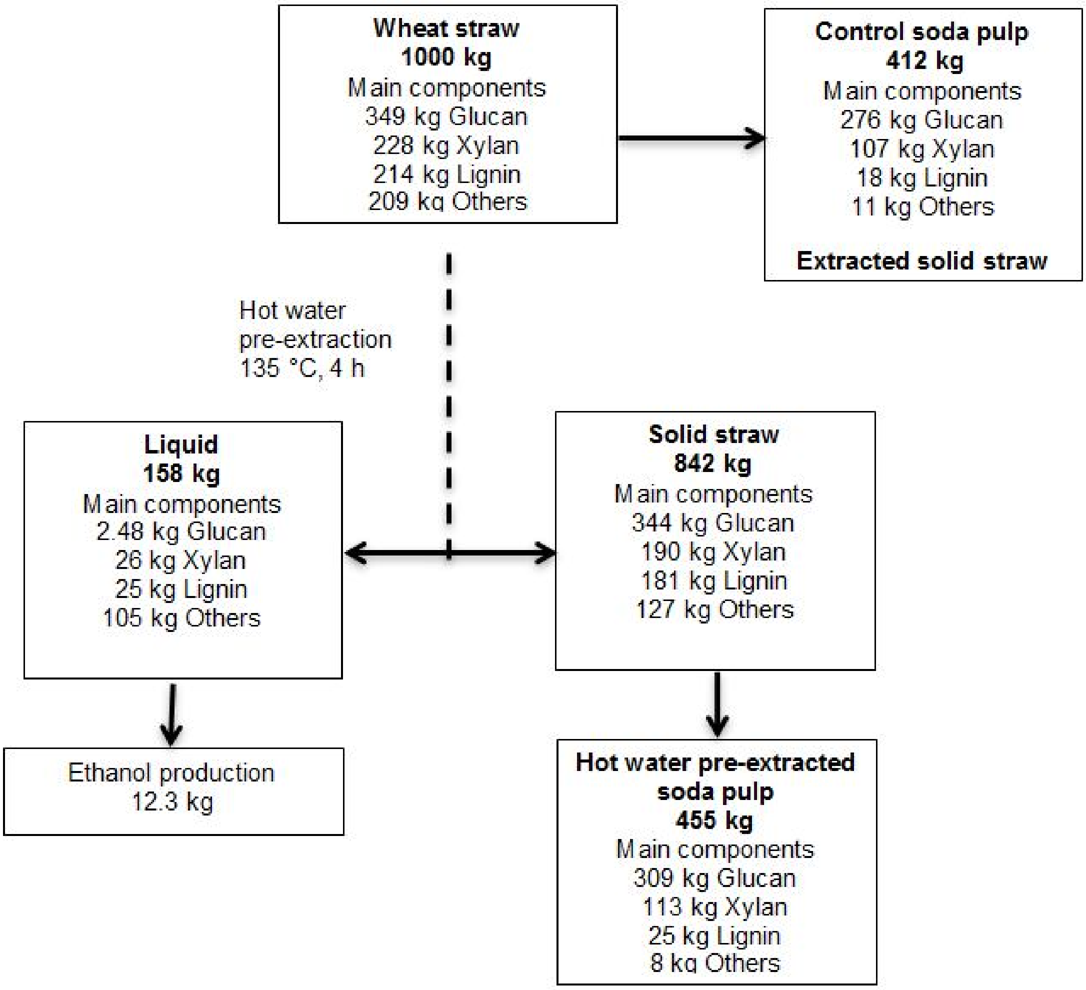

Figure 2.6. Mass balance for hot water pre-extracted wheat straw

2.4.9. Conclusions

The results indicated that cellulose fraction was undamaged up to the treatment temperature of 135 °C. Consequently, the hot water pre-extraction at 135 °C for 4 h provided the optimum pre-extraction conditions. Under other conditions carried out in this study, more than 30% of the xylan in the structure was dissolved. The hot water pre-extracted pulp gave same pulp yield and exhibited comparable mechanical and physical properties. On the other hand, ethanol (12.3 kg/1000 kg of straw) was produced from the hot water pre-extracted hydrolyzate. Biodegradable films produced using only pre-extracted xylan failed the film formation. Addition of xylitol caused self-supporting film formation. On the other hand, addition of gluten and nanocellulose significantly improved the mechanical properties of the films.

3. AN INTEGRATED BIOREFINERY TO PRODUCE BIODEGRADABLE FILM, BIOETHANOL, AND SODA PULP FROM CORN STALKS

3.1. ABSTRACT

Corn stalks have a significant carbohydrate content (75% w/w), and thus were examined in this study in terms of the integrated biorefinery concept. The hemicelluloses were pre-extracted with hot water, alkali (NaOH), and modified alkali ($NaBH_4$+NaOH). The extracts were utilized to produce bioethanol and biodegradable films, and papermaking pulps were produced from the solid fractions. The analysis of variance was used to identify significant differences and the differences between groups were determined by the Duncan test. The results showed that the alkaline pre-extraction (26.7% NaOH at 50 °C) removed 35.6% of the xylan from the stalk structure. The liquid fraction collected from the hot water pre-extraction (150 °C) gave a 14.7% (g/100 g soluble material) yield of bioethanol. Good-quality biodegradable films were produced when gluten and nano cellulose were added to the xylan. Furthermore, the pulps produced from the hot water pre-extracted solid fractions were comparable in yield and pulp properties to the control soda pulp.

3.2. INTRODUCTION

The importance of lignocellulosic biomass has been increasing due to its promising potential for the production of economically valuable chemicals and biofuels along with papermaking pulp. In conventional pulping, black liquor includes mostly lignin and some hemicelluloses which are burned in the alkali recovery system to produce steam and electricity. On the other hand, the heat value of hemicelluloses is only half that of lignin (van Heiningen 2006) and the hemicelluloses can be pre-extracted to produce chemicals and biofuels. Therefore, in the newly developed concept of the integrated forest products biorefinery (IFBR), some hemicelluloses are pre-extracted prior to pulping (van Heiningen 2006). Moreover, this approach is expected to provide additional income for pulp and paper mills.

Previous studies have examined hemicellulose pre-extractions along with pulp production, and several techniques have been applied in the hemicellulose pre-extraction process. Mendes et al. (2009) examined an acid-catalyzed pre-hydrolysis of eucalyptus, with the pre-extracted hemicelluloses being further fermented to produce bioethanol. Furthermore, the pulp produced had lower bleaching requirements and brightness reversion. Al-Dajani and Tschirner (2008) pre-extracted poplar chips with NaOH and obtained a comparable yield and higher pulp quality when the alkaline techniques was compared with acidic pre-extraction. The alkaline technique extracted more hemicelluloses from the structure and resulted in less degradation to furfural substances compared to the acidic one. Alkaline techniques are carried out under much milder treatment conditions (temperature, pressure, and chemical dosage). In addition, neither adjustment of the pH nor washing of the chips is required before alkaline pulping. Copur and Tozluoglu (2008) and Çöpür et al. (2012) modified the alkaline pre-extraction technique by adding a small amount of sodium borohydrate ($NaBH_4$), and results showed that, thanks to this modification, the pulping selectivity was improved by preventing peeling reactions during cooking (lowering hemicelluloses degradation), and the pulp yield was increased by providing a higher rate of delignification.

It should be noted that hot water extraction, also called autohydrolysis, could be considered as an effective and environmentally friendly technique because no chemicals are used. Yoon et al. (2011) examined hot-water pre-extraction of pine and observed nearly the same pulp yield as that of the control cook.

Techniques for pre-extracting hemicelluloses before pulping were evaluated for the woody biomass of eucalyptus by Mendes et al. (2009), for aspen by Al-Dajani and Tschirner (2008), and for loblolly pine by Yoon et al. (2011). However, studies with wheat straw and corn stalks/stover in this context are more limited (Jahan and Rahman, 2012, Resalati et al. 2012, Cheng et al. 2014). Studies of agricultural residues have mainly focused on hemicellulose pre-extraction but have not examined the subsequent pulping process and pulp properties. Wheat straw (Feng et al. 2014), corn stalks (Egüés et al. 2012), and sugarcane bagasse (Batalha et al. 2015) have only been studied for hemicellulose extraction.

Corn stalks have little economic value and are a cheap and abundant feedstock in Turkey. They are usually burnt or left in the field after harvest and can create environmental pollution. As corn stalks have a high carbohydrate content (75% w/w), they present potential for both bioethanol and papermaking pulp production. Therefore, the aim of this study was to determine whether an integrated biorefinery is technically feasible when corn stalks are used as a feedstock. Furthermore, hot water and alkali (NaOH) methods as well as modified alkali (NaOH and $NaBH_4$) pre-extraction techniques were applied to assess the benefits.

3.3. MATERIALS AND METHODS

3.3.1. Materials

Corn stalks (*Zea mays indurata* Sturt.) were locally obtained in Düzce province, Turkey. The material was sized to 2-3 cm using a garden chopper and the chopped material was then air dried at room temperature. The moisture content of the material was determined (TAPPI T 412 om-11) and the material was sealed in plastic bags and stored at room temperature.

3.3.2. Methods

Hot water and alkali (NaOH) pre-extraction techniques were performed for this study. In addition, the alkaline extraction method was modified with the addition of a small amount of $NaBH_4$. The extracted liquids were hydrolyzed and fermented to produce ethanol. The solid fractions were further chemically or mechanically pulped.

3.3.2.1. Pre-extractions

All pre-extractions were made in a 3-L rotating digester. The equivalent of 150 g of oven-dry (OD) stalks was extracted with hot water and alkali. In the hot water pre-extractions, the liquid-to-stalks ratio (L/S) was 10 L/kg oven-dry (OD). The stalks were treated at the maximum temperatures of 90, 120, 135, and 150 °C for 4 h, with the maximum temperature being reached in 30 min.

In the alkaline pre-extractions, the L/S was 4 L/kg OD. Varying NaOH concentrations (16.7%, 26.7%, and 33.3% w/w) and treatment temperatures (50, 70, and 90 °C) were investigated. The optimum alkaline pre-extraction conditions were determined regarding the ratio of carbohydrates to lignin in the solid residues. In addition, the alkaline technique was modified by adding 0.1%, 0.5%, 2%, and 4% $NaBH_4$ for the optimum condition. After all pre-extractions, the liquid and residual solids were collected by filtration using a cloth filter, and the liquid and solid materials were collected for further testing. All pre-extractions were performed in duplicate.

3.3.2.2. Analysis of pre-extracted liquids

The pre-extracted liquors were first centrifuged (5000×g) to precipitate the solids. To hydrolyze the oligomers in the liquid portion into monomeric sugars, 50 mL of liquid was incubated in an autoclave with 1000 mL of 4% sulfuric acid at 121 °C for 1 h (Li et al. 2010). The liquids were neutralized with NaOH to pH 7 and the monomeric sugar contents in the liquids were analyzed using high performance liquid chromatography (HPLC) (Agilent 1200 series, USA). The above procedure was conducted to determine the total sugar content of the liquids before fermentation.

3.3.2.3. Fermentation and ethanol production

The obtained liquids were further fermented in the bioreactor (Sartorius Stedim Biotech Fermenter) to produce bioethanol. The pH was maintained at 6.0 throughout the fermentation by the automatic addition of 0.5 M NaOH and 0.5 M HCl. The medium was supplemented with 1.70 g/L $(NH_4)_2SO_4$, 0.96 g/L K_2HPO_4, 0.17 g/L $MgSO_4.7H_2O$, 0.23 g/L $CaCl_2.2H_2O$, and 0.02 g yeast extract. For fermentation, *Pichia stipitis* from overnight cultures was added and incubated in the bioreactor. Fermentation was conducted for 48 h at 30 °C (100 rpm). Samples taken after 3, 6, 12, 24, and 48 h were centrifuged at 10,000 × g for 10 min. The supernatants were then filtered through 0.45-µm filters and stored at -20 °C until HPLC analysis. The fermentation experiments were also performed in duplicate.

3.3.2.4. Biodegradable film production

To produce biodegradable film, the liquids obtained after pre-extractions were first filtered and then centrifuged (5000 × g) for 5 min to completely remove solid particles. Hemicelluloses in the liquid were precipitated with 2500 mL of refrigerated acetic acid-ethanol (1:10 v/v). The precipitates were desalted by washing with deionized water (200 mL) and ethanol (600 mL) three times. The residue was then dried at room temperature.

Mixtures of 0.7 g of precipitated material (xylan) and 0.4 g of xylitol were stirred in deionized water (15 mL), and the solutions were then poured into polystyrene petri dishes (9 cm). The samples were dried in a conditioned room (50% relative humidity and 23 °C) for three days. Additional biodegradable films were produced using 0.10 g of gluten and 0.025 g of nanocellulose to improve the mechanical properties.

The produced films were conditioned according to the TAPPI standard method (T 402 sp-08). The thickness of the samples was determined with a digital micrometer (40 EXL, Mahr GmbH, Esslingen, Germany) with a sensitivity of 0.1 µm. The tensile strength of the samples was also tested (Zwick/Roell Z250 Strength Testing Device with 100 N/10 kN load cell). Samples for testing were prepared according to the TAPPI standard method (T494 om-96) and the elastic modulus and tensile strength values were recorded.

3.3.2.5. Pulping

The control soda pulp was cooked from corn stalks to compare with the hot water (120 °C) pre-extracted pulps. The alkaline and modified alkaline ($NaBH_4$) pre-extracted stalks resulted in lower yields and therefore, mechanical pulps were produced from them according to the method explained by Chang et al. (2012).

The soda pulps were cooked at 16.7% alkali charge. The liquid-to-solid ratio was 10 to 1 L/kg OD. The samples were then cooked to a target H factor of 104 at 140 °C for 90 min. Maximum temperature was reached in 30 min. The H factor of the hot water pre-extraction was calculated and excluded from the total H factor. Pulps obtained from each cook were defibrated in a laboratory disintegrator and thoroughly washed with tap water on a 200 mesh screen.

To determine paper properties, the control and hot water pre-extracted pulps were subjected to refining. A Waring blender (Chang et al. 2012, Chang et al. 2010, French and Maddern 1994, Shaw 1984) was used to evaluate refining response, and the pulps were refined for 0, 2, and 4 min. The freeness levels of the pulps were determined according to ISO Standard 5267-1. Handsheets were produced in a Rapid Kothen machine in accordance with relevant ISO standard 5269-2 methods. Paper physical properties were determined according to the TAPPI standard method (T 220). The optical properties of the pulps were tested according to ISO standard (2469 and 2471) methods.

3.3.2.6. Analytical Tests

The corn stalks for the study were sampled and prepared according to TAPPI standard method (T 257 sp-14) for analytical tests. The ash content was determined by TAPPI standard method (T 211 om-12) and the solubility properties were examined according to the methods of alcohol-benzene (TAPPI T 204 cm-07), hot-water (TAPPI T 207 cm-08) and 1% NaOH (TAPPI T 212 om-12).

The yield determined by gravimetric measurements was calculated from the dry weight of the stalks and the weight of the initial sample. The dry solid contents were determined by drying the samples to constant weight at 105 °C. The kappa number and the viscosity of the pulps were determined according to the TAPPI standard method (T 236 om-99) and SCAN standard method cm 15:88, respectively.

The laboratory analytical procedures from the National Renewable Energy Laboratory (NREL) were used to determine sugar (glucose, xylose, and arabinose) and lignin (acid-insoluble and acid-soluble) contents of the samples (Sluiter et al. 2011). The sugar and ethanol contents were determined by using HPLC (Agilent 1200 system) equipped with a Shodex SH1011 sugar column (mobile phase: 5 mM H_2SO_4, flow rate: 0.5 ml/min, column temperature: 60 °C) and refractive index detector. The acid-insoluble lignin was determined by weighing the solid samples. The acid-soluble lignin was analyzed at the adsorption of 320 nm against blank deionized water.

The percentage of solids recovered was calculated on an oven-dry basis as follows in Equation 1.

$$\text{The percentage of solids recovered} = (W_2 \div W_1) \times 100 \qquad (1)$$

where W_1 is the dry weight of the sample before pretreatment (g), and W_2 is the dry weight of the treated sample (g).

The reduction in lignin was calculated in terms of the initial dry weight of the lignin in the untreated material (LU) and the dry weight of the lignin in the remaining solids after treatment (LP). The percentage of lignin reduction was calculated with Equation 2.

$$\text{The percentage of lignin reduction} = [(LU\text{-}LP) \div LU)] \times 100 \quad (2)$$

where LP is the dry weight of lignin in the pre-treated sample and LU is the dry weight of lignin in the untreated biomass.

In addition, the solubilization of glucan and xylan in the pre-treated samples was calculated in the same manner by substituting the appropriate percentages for glucan and xylan.

During fermentation, the ethanol yield was calculated as a percentage of the theoretical maximum yield (Kim and Lee 2005) using Equation 3.

$$\text{The percentage of theoretical ethanol yield} = E \div (X \times 0.511) \times 100 \quad (3)$$

where E and X represent the ethanol (g) produced during fermentation and the xylose (g) in the liquids, respectively. The constant 0.511 is the theoretical yield of ethanol produced from xylose.

3.3.2.7. Statistical analyses

The data obtained were statistically analyzed via the SPSS packet program. The analysis of variance (ANOVA) was used to identify significant differences and the differences between groups were determined according to the Duncan test.

3.4. RESULTS AND DISCUSSION

3.4.1. Chemical Composition of Corn Stalks

The chemical composition determined for corn stalks and the literature values for hardwood and softwood (Fengel and Wegener 1989) and corn stalks (Barbash et al. 2012, Latibari et al. 2011) are given in Table 3.1. Corn stalks were higher in carbohydrate and extractive contents but lower in lignin compared to hard/softwood. In addition, all solubility values of corn stalks were significantly higher compared to those of hard/softwood.

The total carbohydrate content of the biomass is important for the IFBR concept and results showed that the corn stalks had 47.7% glucose and 25.0% xylose. The total polysaccharide content was 74.5%. The total carbohydrate content observed in this study was higher than that reported in the literature (Li et al. 2015; Shi et al. 2014; Varga et al. 2002) for corn stalks/stover. Although the total lignin content (22.1%) was similar, the ash content (2.61%) was lower (Li et al. 2015; Shi et al. 2014; Varga et al. 2002). The differences in these values could be due to the growth environment, growing season, growing location, soil conditions, analysis methods, etc. (Utne and Hegbom 1992; Amores et al. 2013).

Table 3.1. Chemical composition of corn stalks and hard/softwood.

Chemical composition	Raw material (%)*			
	Corn stalks (Current work)	Corn stalks (Latibari et al. 2011)	Corn stalks (Barbash et al. 2012)	Hard/Softwood (Fengel and Wegener 1989)
Extractives	10.2±0.11	15.0	3.50	2-6/2-8
Hot water solubility	11.2±0.14		11.8	2-7/3-6
1% NaOH solubility	43.0±0.07	45.9	19.6	14-20/9-16
Lignin	22.1±0.10**	23.0	17.9	30-35/25-35
Ash	2.61±0.03	2.06	4.70	0.35/0.35
Glucose	47.7±0.23	51.2	41.6	
Xylose	25.0±0.11		23.4	
Arabinose	1.84±0.17		2.21	

*Composition percentages are dry-weight basis and values are average of duplicate measurements. **Acid soluble lignin and lignin ash included.

3.4.2. Hot Water Pre-Extractions

The weight, glucose, xylose, and lignin losses of corn stalks in hot water pre-extractions for varying treatment temperatures are shown in Figure 3.1. Results showed that the treatment temperature had a major effect, and that an increase in temperature removed more material from the structure. Up to 33.3% of material was removed from the structure when the material was pre-extracted at 150 °C. Results indicated that the weight loss in the hot water pre-extraction was mainly due to the removal of hemicelluloses with some of the lignin and extraneous materials. Jahan and Rahman (2012) also reported a 12.2% weight loss when corn stalks were pre-extracted with hot water (150 °C) for 1 h. Tozluoğlu et al. (2015) reported a slightly higher weight loss (38.5%) when wheat straw was pre -extracted at 150 °C for 4 h. Consequently, the high amount of material removal observed in this study could be due to the lower degree of polymerization (DP) of hemicelluloses in the stalk structure and the extensive treatment time (240 min).

Compared to lignin, significantly higher xylose removal was observed for treatment temperatures of up to 135°C. The highest xylose removal of 44.8% was observed when stalks were extracted at 150 °C. It should be noted that the removal of both components (xylose and lignin) was almost linear even though the removal ratio of xylose was higher than that of lignin. The removal of lignin with hemicelluloses could be attributed to the covalent bond chains of xylan and lignin in the corn stalks structure.

The HPLC results indicated that this was not a case of the hydrolyzation of xylan to hydrolyzed xylose and its further degradation products of furfural, hydroxymethyl furfural, organic acids, etc. This could be due to the mild treatment temperatures applied in this study.

Results showed that the highest treatment temperature (150 °C) damaged the cellulose fraction and removed 16.5% of the glucose from the stalk structure (Figure 3.1). The high amount of glucose removal may have been the result of the lower DP and the more amorphous regions of the cellulose in the stalk structure. In addition, statistical analysis showed that the weight, glucose, xylose, and lignin losses obtained with hot water pre-extraction were statistically significant ($p<0.001$).

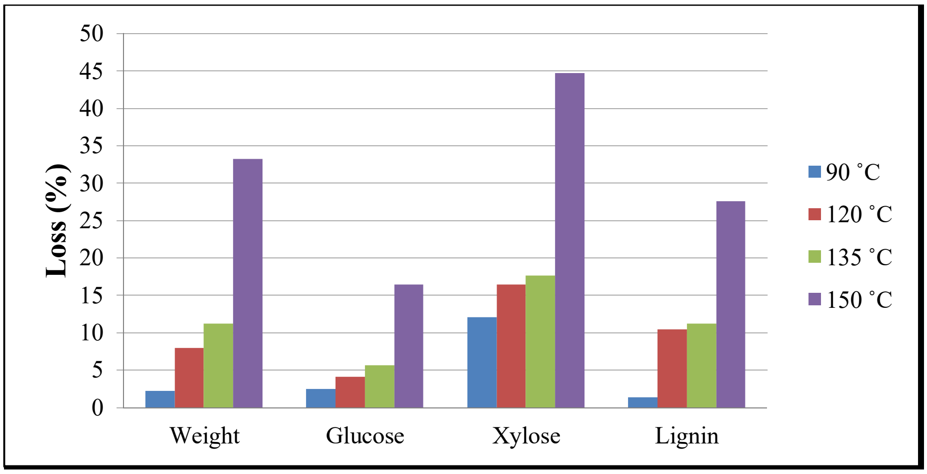

Figure 3.1. Glucose, xylose, lignin, and weight loss for hot water pre-extracted corn stalks at varying treatment temperatures.

3.4.3. Alkali (NaOH) Pre-Extractions

The major effect of alkali (NaOH) pre-extraction could be the removal of lignin and some hemicelluloses as well as acetyl and the various uronic acid groups from the stalk structure. The amounts of alkaline pre-extraction weight, glucose, xylose, and lignin loss from the corn stalks structure are tabulated in Table 3.2. Results showed that the alkali used in this study was capable of reducing the lignin and hemicelluloses under the applied treatment conditions. In this study, the alkaline pre-extraction under various conditions resulted in weight losses ranging from 28.7 to 43.5% (Table 3.2). The results indicated that treatment temperature and alkali charge had a significant effect on the pre-extraction yield. An increase in both alkali charge and treatment temperature resulted in greater material removal (Figure 3.2). There was less of a difference in the extraction rates (weight loss) between 26.7 and 33.3% concentrations than between 16.7 and 26.7% (Figure 3.2a). Statistical analysis indicated that the change in alkali concentration had a significant effect on weight losses ($p<0.001$).

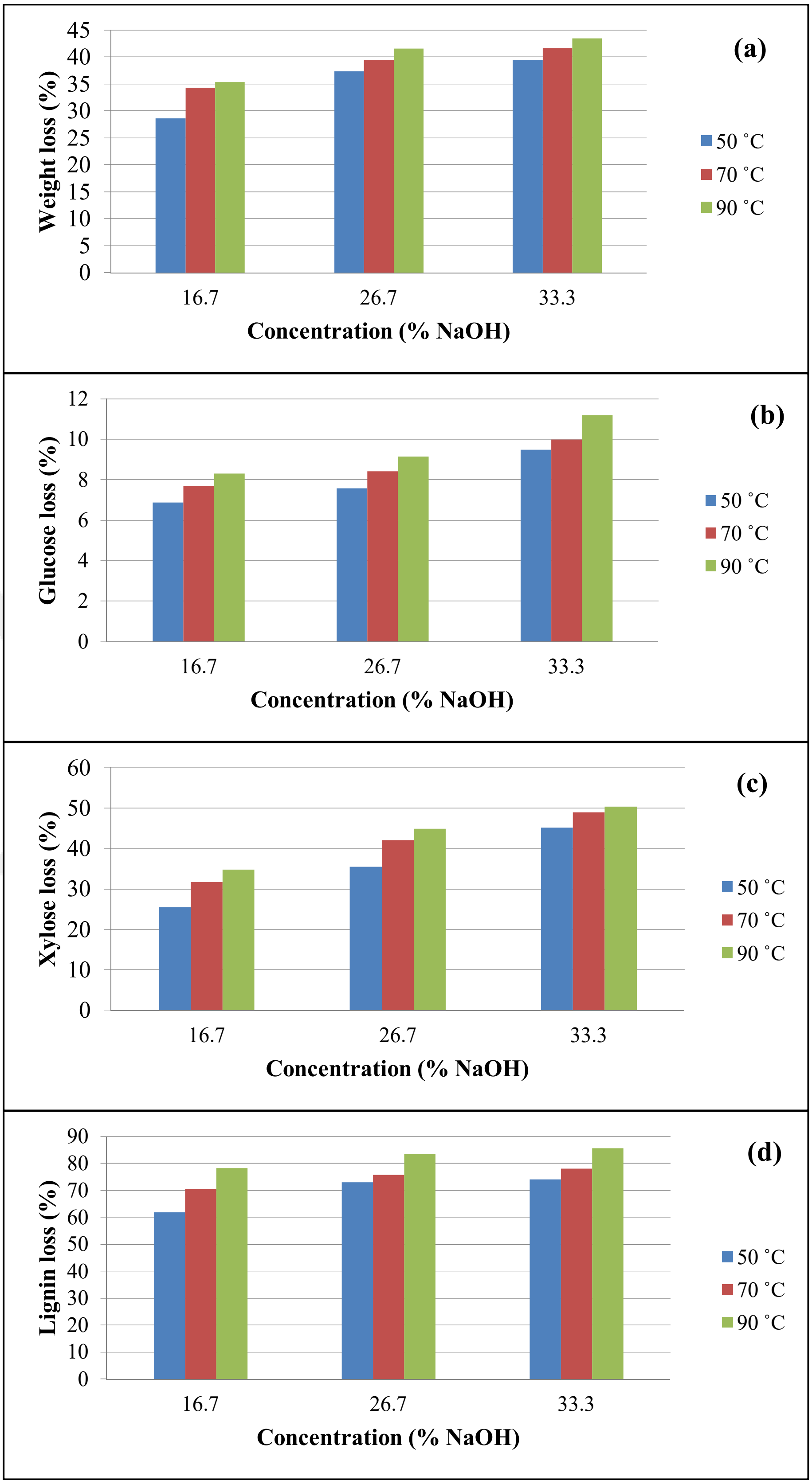

Figure 3.2. (a) Weight loss, (b) glucose loss, (c) xylose loss, and (d) lignin loss in NaOH pre-extracted corn stalks at various treatment temperatures and chemical concentrations.

The pre-extraction in this study also dissolved some hemicelluloses (xylan). The results showed that both alkali charges and treatment temperatures affected the xylan extraction (Figure 3.2c) and increases in both variables resulted in higher xylan extraction. An increase in alkali charge from 16.7 to 26.7% resulted in a higher xylan extraction ratio; however, the extraction ratio was lower for the higher (33.3%) alkali charge. The maximum xylan extraction of 50.5% (i.e., approximately 12.6% based on the original stalks) was observed when the stalks were pre-extracted with 33.3% alkali (NaOH) charge at 90 °C.

Varga, et al. (2002) reported the removal of 88.2% of the hemicelluloses and 95.9% of the lignin when ground corn stalks were extracted with 10% (w/w) NaOH for 1 h at 120 °C. In this study, the lower xylan extractions could have to do with the stalk size and the mild treatment temperature.

When the effect of the extraction temperature was considered, almost linear xylan removal was observed by raising the treatment temperature from 50 to 90 °C. One aim in this study was to optimize the pulping yield after pre-extraction. Therefore, some hemicelluloses had to be preserved in the solid portion in order to produce pulp and paper having properties at least equal to those of the un-extracted control soda pulp.

Table 3.2. Glucose, xylose, lignin, and weight loss of NaOH pre-extracted corn stalks at varying treatment temperatures and chemical concentrations.

Temp. (°C)	NaOH conc. (%)	Weight loss (%)	Glucose loss (%)	Xylose loss (%)	Lignin loss (%)
50	16.7	28.7±0.44 a*	6.88±0.13 a	25.6±0.49 a	62.0±0.26 a
	26.7	37.4±0.73 d	7.60±0.77 b	35.6±0.73 c	73.2±0.12 c
	33.3	39.5±0.59 e	9.50±0.65 d	45.2±0.49 e	74.2±0.18 d
70	16.7	34.3±0.58 b	7.70±0.63 b	31.7±0.42 b	70.6±0.14 b
	26.7	39.5±0.29 e	8.43±0.27 c	42.2±0.35 d	75.9±0.36 e
	33.3	41.7±0.19 f	10.0±0.07 e	49.0±0.14 f	78.1±0.22 f
90	16.7	35.4±0.16 c	8.31±0.71 c	34.9±0.28 c	78.4±0.00 f
	26.7	41.6±0.28 f	9.16±0.07 d	44.9±0.35 e	83.7±0.43 g
	33.3	43.5±0.13 g	11.2±0.14 f	50.5±0.17 g	85.8±0.82 h

*within each column, the factors followed by this letter were not significantly different according to the Duncan test ($p < 0.05$)

As with xylan, the increase in treatment temperature and alkali charge during pre-extraction removed more glucan from the stalk structure. Un-extracted corn stalks had 47.7% glucose, and during pre-extraction, 6.88 to 11.2% (based on OD stalks) of the glucose was removed, depending on the treatment temperature and alkali charge (Table

3.2). Alkaline pre-extractions performed at lower than 100 °C caused some delignification and degradation reactions. The mild pre-extraction temperatures applied in this study may explain the lower glucan removal. The results obtained were comparable to the findings of Taherzadeh and Karimi (2008). Cellulose degradation may significantly affect pulp and paper properties, and thus, the lower glucan degradation seen in this study could be applied in the production of paper with better properties.

Higher lignin removal was observed when the stalks were pre-extracted at higher temperatures and alkali charges (Figure 3.2d). The rate of lignin removal was significantly greater than that of xylan during alkaline pre-extraction. When the extraction rates of glucan, xylan, and lignin under varying extraction conditions were compared, it may be concluded that the treatment temperature, as opposed to the alkali (NaOH) charge, was more effective. In addition, the HPLC detected a trace amount of furfural, possibly due to the relatively lower treatment temperatures applied in this study. Higher alkali concentrations removed more material as a possible result of the more complete delignification reactions at higher alkali concentrations. On the other hand, alkaline pre-extractions had a significant ($p<0.001$) effect on weight, glucose, xylose, and lignin losses.

Optimized pre-extraction conditions could further be established as parameters for the dissolution of hemicelluloses, which may significantly affect paper properties. Therefore, the optimum treatment condition for lignin and xylan removal rates was determined for stalks which were extracted with 26.7% alkali at 50 °C.

3.4.4. Modified Alkaline Extraction ($NaBH_4$)

Adding a small amount of $NaBH_4$ in alkali pulping improves the pulping selectivity by preventing peeling reactions and hemicelluloses degradation (Copur and Tozluoglu 2008). The modification of alkaline pre-extraction using $NaBH_4$ was expected to preserve more glucan and xylan and to result in selective lignin degradation (Çöpür et al. 2012). Therefore, in order to gain a more efficient delignification, the alkali extraction was modified by adding 0.1%, 0.5%, 2%, and 4% (OD material) of $NaBH_4$ for the optimum alkaline pre-extraction condition (26.7% NaOH at 50 °C). Results showed that this modification affected the delignification and xylan degradation (Table

3.3 and Figure 3.3). Xylan degradation could be due to the chemical bonds of xylan and lignin in the stalks structure (Yoon et al. 2008). The glucan loss after modification was insignificant and therefore was not reported in this study. On the other hand, the effect of the modification on delignification was notable, and the addition of 0.5% $NaBH_4$ removed 2.30% of the lignin from the straw structure. Moreover, modifying the extraction with 2% $NaBH_4$ led to 4.50% delignification; however, the xylan dissolution was higher (6.60%). The weight, lignin, and xylose losses for $NaBH_4$ modified pre-extraction were all statistically significant ($p<0.001$).

Table 3.3. Weight and chemical loss for $NaOH+NaBH_4$ pre-extracted corn stalks.

Temp. (°C)	$NaOH-NaBH_4$ conc. (%)	Weight loss (%)	Xylose loss (%)	Lignin loss (%)
50	26.7	37.4±0.73 a*	35.6±0.73 a	73.2±0.12 a
	26.7-0.1	40.0±0.50 b	37.5±0.49 b	73.9±0.19 a
	26.7-0.5	41.0±0.27 b	39.9±0.21 c	75.5±0.35 b
	26.7-2.0	43.5±0.16 c	42.2±0.14 d	77.7±0.12 c
	26.7-4.0	44.2±0.42 c	43.4±0.07 e	80.2±0.24 d

*within each column, the factors followed by this letter were not significantly different according to the Duncan test ($p < 0.05$)

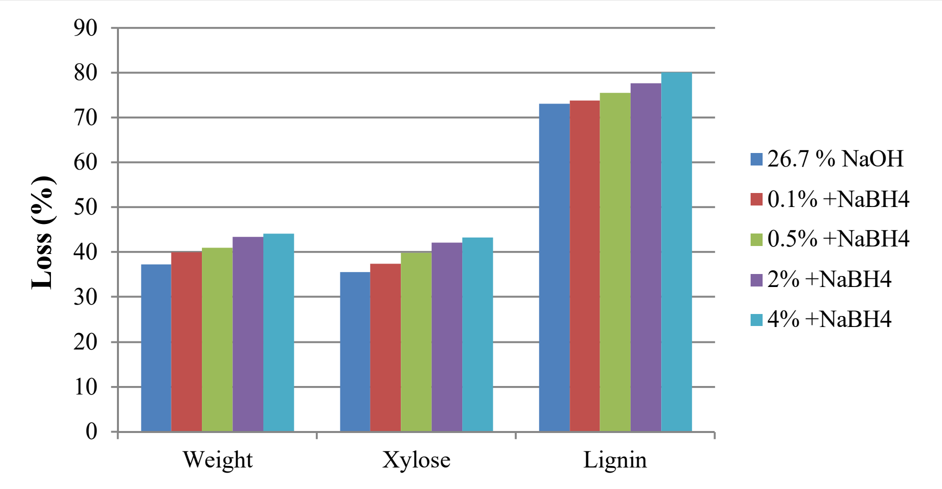

Figure 3.3. Xylose, lignin, and weight loss of $NaOH+NaBH_4$ pre-extracted corn stalks at varying $NaBH_4$ concentrations.

3.4.5. Composition of Liquids and Ethanol Production

When the liquid portion of the pre-extracted stalks (hot water, alkali, and modified $NaBH_4$) was examined, it contained oligomers of glucan, xylan and arabinan, and lignin. Results showed that the glucan in the liquid was very low. This may be explained by the mild extraction conditions applied in this study, which produced unhydrolyzed glucan.

When stalks were pre-extracted with hot water at 150 °C and 120 °C, nearly 71.8% and 69.1% of the xylan was recovered, respectively, as monomeric sugar (xylose). On the other hand, the lowest xylose recovery (21.0%) was observed when the stalks were treated with 26.7% alkali (Table 3.4). This variability in xylose recovery could be related to the lignin solubilization in the alkali and hot water pre-extractions. According to Mok and Antal (1992), the higher delignification in alkaline pre-extraction may impede the recovery of xylose sugars. In addition, mild alkaline extraction conditions may prevent lignin condensation and result in high lignin dissolution. This may in turn inhibit the conversion of the degraded sugars to furfural, hydroxymethylfurfural (HMF), and organic acids (Chang and Holtzapple 2000), which may have been the case in this study.

Table 3.4. Sugars in pre-extracted solids and liquids.

Pre-extraction conditions	Solids				Liquids		
	Sample	Untreated stalks (g)	Extracted solids (g)	Loss (%)	Sample	Extracted liquids (%)	Recovery (%)
120 °C	Yield	100	92.0	8.00			
	Xylan	25.0	20.8	16.8	Xylose	11.6	69.1
150 °C	Yield	100	66.7	33.3			
	Xylan	25.0	13.8	44.8	Xylose	32.2	71.8
26.7% NaOH	Yield	100	62.6	37.4			
	Xylan	25.0	16.1	35.6	Xylose	7.50	21.0

For this study, the xylose in the extraction liquids was fermented to produce bioethanol (Figures 3.4a-c). Consequently, the theoretical ethanol yield was found to be 83%, 89.4%, and 80.7% for 120 °C, 150 °C hot water, and 26.7 % alkaline pre-extracted stalks, respectively.

In this investigation, liquids obtained from hot water pre-extracted stalks (120 °C and 150 °C) resulted in ethanol yields of 4.91 g (g/100g soluble material) and 14.7 g (g/100g soluble material), respectively. The liquid portion of the alkaline-extracted stalks (26.7% NaOH at 50 °C) yielded 7.66 g (g/100g soluble material) of ethanol. The lower ethanol yield in the alkaline pre-extracted samples could be explained by the lower xylose recovery and the alkali media of the samples. Another possible reason is the presence of lignin, which inhibits fermentation. $NaBH_4$ modified samples had low xylose recovery and bioethanol production was not conducted in this study. Low xylose recovery may be due to the lignin and other inhibitors in the solutions.

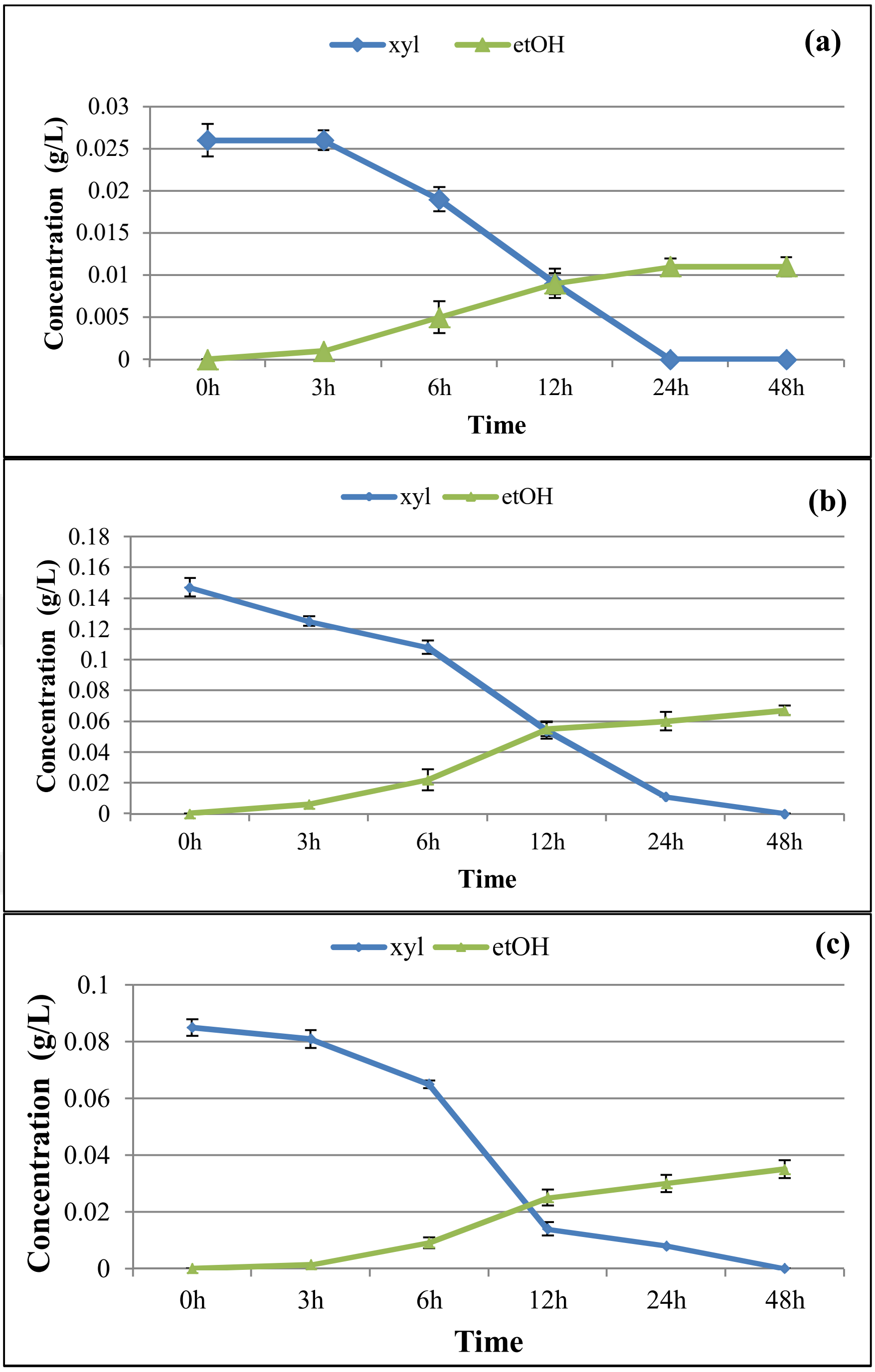

Figure 3.4. The change in xylose and ethanol concentrations of (a) 120 °C hot water pre-extracted, (b) 150 °C hot water pre-extracted, and (c) 26.7% NaOH pre-extracted corn stalks liquids fermented with *Pichia stipitis*

3.4.6. Biodegradable Films

In addition to bioethanol, biofilm production was examined in this study. Biofilm samples produced using solely xylan resulted in crack formation (Figure 3.5). This finding was also observed by Gabrielii and Gatenholm (1998) and Göksu (2005). To eliminate this problem, some xylitol (Seber 2010; Goncalves 2011) was added to the mixture and this resulted in a good biofilm construction (Figure 3.5). The produced biofilms were tested to determine the mechanical properties, and the results showed the mechanical properties to be very poor. To improve the mechanical properties, some gluten and nano cellulose were added to the mixture (Table 3.5). Results showed that even the addition of gluten improved the tensile strength, while a dramatic increase (more than two-fold) in tensile strength was observed when small amounts of nano cellulose were added to the mixture (Table 3.5). The results also indicated that biofilms produced from corn stalks, when compared to wheat straw (Tozluoğlu et al. 2015), gave better (almost 2-3 times greater) mechanical strength values. In the litreature, corncob xylan added films had a more uniform surface than the other films, which explains the high stretchability of corncob xylan films. However, films with grass xylan resulted in little globular formation on the surface and birchwood xylan resulted in more heterogeneous films. (Göksu 2005).

Table 3.5. Thickness and mechanical properties of biodegradable films.

Sample	Thickness (mm)	Elastic modulus (N/mm²)	Tensile strength (N/mm²)
Control (Xylan + Xylitol)	0.151±0.02	171±17.5	3.60±0.22
Control + Gluten	0.141±0.02	214±10.7	4.07±0.28
Control + Nanocellulose	0.133±0.01	341±33.6	7.78±0.37

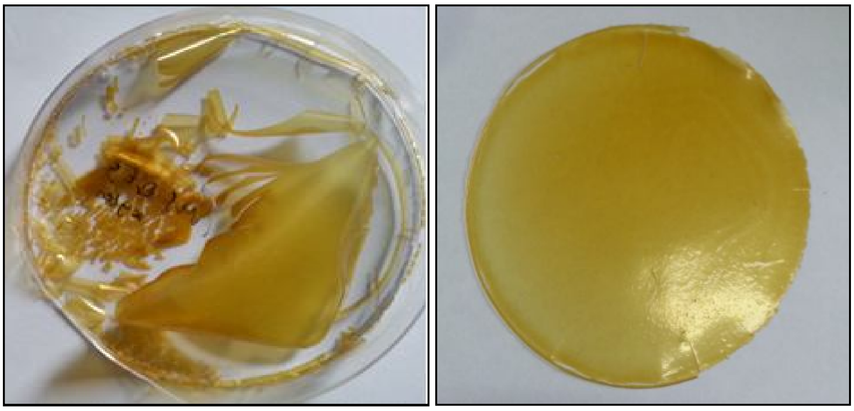

Figure 3.5. Films obtained using only extracted xylan (left) and extracted xylan+xylitol (right).

3.4.7. Pulp and Paper Properties

This study included chemically pulped control soda and hot water (120 °C) pre-extracted pulps and mechanically pulped alkaline (26.7% NaOH) and modified alkaline (0.1% $NaBH_4$) pre-extracted pulps (Table 3.6).

Similar to the findings of Yoon et al. (2011), the hot water pre-extracted samples gave a slightly lower pulp yield (52.9% OD stalks) compared to the control soda pulp (53.6% OD stalks). However, Jahan and Rahman (2012) reported lower pulp yields (around 49%) when hot water pre-extracted corn stalks were cooked using the soda-AQ method. On the other hand, a significant reduction in the pulp yield of pre-extracted corn stover was observed by Cheng et al. (2014). Furthermore, the hot water pre-extracted pulp had slightly higher reject content (3.34% OD stalks) (Table 3.6). The chemical compositions of both pulps were analyzed and the results showed that both pulps had similar amounts of glucose and xylose. The hot water pre-extracted soda pulp had a slightly higher kappa number compared to the control soda pulp. Cheng et al. (2014) also observed similar results. However, Jahan and Rahman (2012) found a lower kappa number compared to our findings. In contrast to the control, the hot water pre-extracted soda pulp exhibited slightly lower viscosity, which could be due to the stalk structure being more open to the cooking chemicals, thus causing more cellulose degradation (Yoon et al. 2011). Consequently, the hot water pre-extraction created a more porous structure that may have increased the ability of the cooking chemicals to reach the inner structures, resulting in improved lignin reactivity and hemicelluloses degradation. The higher yield reported in this study compared to the findings of Cheng et al. (2014) could be due to the mild cooking conditions applied in this study. The observed values for kappa ($p<0.001$), viscosity ($p<0.001$), xylose ($p<0.05$), glucose, ($p<0.01$) and lignin ($p<0.001$) were all statistically significant.

The alkaline (26.7% OD stalks) and modified alkaline (0.1% $NaBH_4$ OD stalks) mechanical pulps had similar yields. The results showed that the $NaBH_4$ modified pulp had higher cellulose (63.7% OD pulp) but lower lignin (8.77% OD pulp) contents.

Prior to handsheet preparation, the chemical soda pulps of the control and the hot water pre-extracted pulps were refined for 0, 2, and 4 min. Additionally, the mechanical pulps were fiberized for 1 min using a Waring blender. The freeness, mechanical (tensile, tear, and burst) and physical (brightness and opacity) properties of the pulps were determined and are tabulated in Table 3.7.

Table 3.6. Data on pulps.

Pre-extractions	Yield (% of o.d. stalks)	Reject (% of o.d. stalks)	Kappa (ml/g)	Viscosity (cm^3/g)	Glucose (% of o.d. pulp)	Xylose (% of o.d. pulp)	Lignin (% of o.d. pulp)
Control	53.6	3.00	12.2±0.05 a*	1031±0.04 b	64.2±1.19 a	24.5±0.85 ab	3.47±0.44 a
Hot water 120 °C	52.9	3.34	13.1±0.00 b	967±0.07 a	64.7±1.20 a	24.9±0.42 b	6.33±0.24 b
26.7% NaOH	62.6				60.3±0.77 b	25.7±0.73 b	8.94±0.12 c
+0.1% NaBH$_4$	60.0				63.7±0.49 a	22.7±0.49 a	8.77±0.19 c

*within each column, the factors followed by this letter were not significantly different according to the Duncan test ($p<0.05$)

Table 3.7. Paper properties.

Pre-extractions	Refining (min)	SR (mL)	Tensile index (Nm/g)	Tear index (mNm^2/g)	Burst index (kPam^2/g)	Brightness (% ISO)	Opacity (% ISO)
Control*	0	24	58.4±0.51 b***	8.25±0.03 b	2.73±0.51 b	35.3±0.33 e	90.7±0.89 d
	2	55	95.0±0.34 e	8.17±0.06 b	5.67±0.25 f	33.8±1.07 d	84.4±0.63 a
	4	72	100±0.17 f	7.41±0.04 a	5.79±0.56 g	32.9±0.20 c	80.6±0.27 b
Hot water 120 °C*	0	22	46.1±0.13 a	8.11±0.04 b	2.04±0.19 a	33.9±0.44 d	94.4±0.56 e
	2	52	80.5±0.20 c	7.84±0.04 b	4.77±0.11d	31.8±0.50 b	88.8±0.40 c
	4	66	81.4±0.18 c	7.76±0.05 b	4.84±0.43 d	30.2±0.27 a	84.2±0.81 a
26.7% NaOH**	1	33	93.1±0.31 d	9.83±0.03 c	4.93±0.41e	30.6±0.47 a	87.4±0.27 b
+0.1% NaBH$_4$**	1	37	81.9±0.22 c	12.2±0.04 d	3.80±0.50 c	30.0±0.64 a	87.0±0.42 b

* Pre-extracted soda chemical pulps

**Pre-extracted mechanical pulps

***within each column, the factors followed by this letter were not significantly different according to the Duncan test ($p<0.05$)

The refining response of the hot water pre-extracted pulp was lower than that of the control soda pulp. This may be attributed to the lower hemicellulose content, resulting in lower swellability of the fibers (Yoon and Heiningen 2008, Helmerius et al. 2010, Çöpür 2007).

The refining improved the mechanical properties (tensile and burst) of the chemical pulps, possibly due to the fibrillation of the fibers during refining. On the other hand, the tear index value decreased slightly, depending on refining intensity. The damage done to individual fibers may have been responsible for the higher tensile and burst properties exhibited by the control soda pulp compared to the hot water pre-extracted soda pulp. The higher tensile and burst index values could be explained by better fiber flexibility and improved inter-fiber bonding (Çöpür 2007). When the chemical and mechanical pulps were compared, the mechanical pulps displayed lower tensile and burst index values. Mechanical pulps had a higher lignin content, which produced stiff fibers, and thus caused lower inter-fiber bonding (Çöpür 2007).

The tear value chiefly depends on individual fiber strength, but rarely depends on the degree of inter-fiber bonding. Therefore, compared to the chemical pulps, the mechanical pulps presented a higher tear index. The control soda pulp, with a relatively undamaged structure, resulted in a better tear index value compared to the hot water pre-extracted soda pulp.

When the physical properties of the pulps were compared, the control pulp yielded a slightly better brightness value, whereas the opacity value was the reverse. The refining diminished both brightness and opacity due to the larger optical contact area between the fibers (i.e., higher fiber bonding). The statistical analysis implied that the pre-extractions had a significant ($p<0.001$) effect on the tensile index, burst index, opacity, and brightness, while no significant differences ($p>0.05$) were found for the tear indices.

3.4.8. Conclusions

The pre-extraction of corn stalks with hot water (150 °C) removed up to 44.8% of the xylose from the structure. The ratio of xylose removal was higher than that of lignin for all hot water pre-extractions. The increase in alkali concentration removed more material from the structure. Modified alkali ($NaBH_4$+NaOH) pre-extraction increased the lignin removal. Bioethanol was obtained from the extracts, and the stalks pre-extracted with hot water at 150 °C produced 14.7% (g/100g OD) of ethanol. Moreover, the theoretical ethanol yield was calculated to be 89.4%. The pre-extracted xylan was used to produce biodegradable films but pure xylan was not formed self-supporting biofilm. The results showed that adding xylitol, gluten, and nano cellulose resulted in self-supporting film formation with sufficient mechanical properties. Lastly, the pulp properties and yield of the hot water pre-extracted and control soda pulps were comparable.

4. FORMIC ACID REINFORCED AUTOHYDROLYSIS OF WHEAT STRAW FOR PRODUCTION OF A HIGH YIELD MONOSUGARS AND MINIMAL LIGNIN PRECIPITATION

4.1. ABSTRACT

Wheat straw is the second largest agricultural residue in the world. With a significant carbohydrate content of about 70% w/w, wheat straw is a potential cheap and abundant feedstock for production of fermentable sugars for bioethanol production. However a cost efficient pretreatment technology is required for competitive sugar production at industrial implementation. Autohydrolysis would be a cost-effective process because only water/steam is needed. Unfortunately the formation of "sticky lignin" precipitates in the hydrolysate leads to severe plugging in the hydrolysis reactor and downstream equipment in a continuous process. Yasukawa et al. (2014) found that the presence of formic acid (FA) at relatively low concentrations (5-20 g/L) during autohydrolysis (160 °C) of mixed hardwood chips significantly reduced the amount of lignin precipitates in the pre-hydrolysate. In addition, the FA reinforced hydrolysate contained mostly monomeric hemicellulose sugars than that without FA supplementation. In the present study wheat straw was pretreated at 150°C for 100 min at a liquor-to-straw ratio of 6 L/kg (OD) straw. Different FA concentrations ranging from 0 to 15 g/L were used. The results showed excellent sugar and lignin mass balances for pretreatment were obtained, and precipitated lignin in the hydrolysate decreased from 0.94 g/100g straw at autohydrolysis conditions (no FA) to 0.31 g/100g straw at 15 g/L of FA. The average molecular weight of precipitated lignin decreased from 1970 g/mole for autohydrolysis to 710 g/mole at 15 g/L of FA. The monomeric sugar yield during pretreatment improved dramatically with increasing FA concentration relative to autohydrolysis. Without FA the sugar yield in the hydrolysate was only 3.83 g/100g. However at a FA concentration of 15 g/L, the dissolved sugar yield increased to 23.5 g/100g. The results showed that FA reinforced autohydrolysis not only minimize the formation of "sticky lignin" but also significantly increased dissolved sugar yield.

4.2. INTRODUCTION

Lignocellulosic biomass which is the main feedstock of the forest product industry consists of almost 35-45% cellulose, 25-35% hemicellulose, 15-25% lignin and other components. Traditionally, paper mills burn the woody material dissolved during pulping, i.e. lignin, hemicelluloses and others components for energy. Although the raw material contains approximately 25-35% hemicelluloses, pulp consists of only 5-10% hemicelluloses after chemical pulping. Therefore the dissolved hemicelluloses, consisting of 15-30% based on the original woody feedstock mass, could be utilized for higher value applications. Recent research has focused on integrated production of pulp and chemicals (such as acetic acid, furfural, lactic acid, etc.), biofuels (ethanol, methanol, etc.) and biomaterials from biomass. This approach, called integrated forest products biorefinery (IFBR), is expected to change traditional pulp and paper production. Also the IFBR concept addresses the social need to use renewable resources rather than fossil fuels to produce commodity products, chemicals, biomaterials and electricity (van Heiningen 2006, Amidon and Liu 2009, Jahan and Rahman 2012).

Publications on research effort to implement the IFBR concept have usually involved wood as raw material (van Heiningen 2006, Ragauskas et al. 2006, Al Dajani and Tschirner 2008, Yoon et al. 2008, Li et al. 2010, Helmerius et al. 2010, Yoon et al. 2011), with limited studies dealing with agricultural residues as feedstock (Jahan and Rahman, 2012, Jahan et al. 2013, Cheng et al. 2014).

Wheat straw is the second largest agricultural residue in the world (Talebnia et al. 2010) after sugarcane straw at 867 million MT/year in 2008/09. Turkey, having a rich agricultural potential and large agricultural tradition, is an important wheat producer in the world and hence there are huge quantities of underutilized and low-cost wheat straw available. Approximately 40-53 million tons of straw is produced in Turkey per year (Ergudenler and Isigigur 1994). The amount of this up to 18 million tons per year is wheat straw (Bostancı 1987). Wheat straw with low-no economic value, burnt in the field after harvest creates environmental pollution. Burning wheat straw has been done for a long time and burning results in large amount of air pollutants (Li et al. 2008) and health problems. Similar to other biomass, wheat straw consists of cellulose (33-40% w/w), hemicelluloses (20-25% w/w) and lignin (15-20% w/w) and a small amount of extractives and mostly silica-containing ash (Prasad et al. 2007).The variation in composition depends on the wheat species, soil, climate conditions, etc. (Utne and Hegbom 1992). With a significant carbohydrate content of about 70% w/w, wheat straw is a potential cheap and abundant feedstock for production of fermentable sugars for bioethanol. However a cost efficient pretreatment technology and low enzymatic charge is required for competitive sugar production (≤ \$0.30/kg) at industrial scale.

In this research, we used wheat straw as part of the IFBR approach to generate value-added products with the objective to contribute to our country's economy. In this approach, hemicelluloses and some part of the lignin are extracted from wheat straw by pre-treating the biomass with hot water and FA at varying concentrations. Autohydrolysis would be a good pretreatment process because only water is needed. Unfortunately the presence of "sticky lignin" precipitates in the hydrolysate leads to severe plugging in the hydrolysis reactor and downstream equipment in a continuous process. For this reason we used FA reinforced autohydrolysis to minimize the formation of sticky precipitates and convert the remaining precipitates in non-sticky particles. Massive problems related to the precipitation of resin-like materials have also been reported during sulfuric acid post-hydrolysis of autohydrolysates to convert the dissolved hemicelluloses into xylose (Stanciu and Ciurea, 2008, Stanciu 1974). Leschinsky et al. (2009) found that during autohydrolysis, lignin is degraded through cleavage of aryl-ether bonds resulting in a reduced molecular weight. As a result the degraded lignin is characterized by an increased content of phenolic hydroxyl groups and a reduced content of aliphatic hydroxyl groups. In parallel to the cleavage reactions, condensation reactions of lignin take place which leads to increased molecular weight lignin and precipitation. The formation of these sticky lignin precipitates explains why hot-water pre-extraction is not commercially applied. In recent research Yasukawa et al. (2014) found that the presence of FA present at relatively low concentrations (5-20 g/L) during hot water (160 °C) treatment of mixed hardwood chips significantly reduced the amount of lignin precipitates in the pre-hydrolysate. In addition, the FA reinforced hydrolysate contained much more monomeric hemicellulose sugars than that without FA supplementation. In the present study we quantified the effect of low FA concentrations during pretreatment of wheat straw on hemicellulose dissolution and lignin precipitate formation. In addition to FA 1 g/L sulfuric acid (SA) was added to determine the effect of higher acidity on FA assisted pre-hydrolysis.

4.3. MATERIALS AND METHODS

4.3.1. Materials

Wheat straw samples locally obtained in the State of Maine, US, were cut to suitable sizes (1-2 cm) using a grinder Munson Mill. The chopped material was air dried at room temperature. The moisture content was determined according to the TAPPI (TAPPI T 412 om-11) test method and the material was sealed in plastic bags and stored at room temperature before FA reinforced pre-hydrolysis.

4.3.2. Methods

4.3.2.1. Pre-hydrolysis of Wheat Straw

For this study, the pre-hydrolysis of 15 g (OD) wheat straw was performed at 150 °C, for 100 min (includes heat-up time) in a polyethylene glycol (PEG) bath laboratory reactor with 8 rotating digesters. The required amount of water, formic acid (FA) and sulfuric acid (SA) at different concentrations taking into account the moisture content of the wheat straw were added to the 220 ml stainless steel pressure vessels to obtain different liquor-to-straw (L/S) ratios L/kg. The equivalent heat-up time of the content of the vessels was about 8 minutes. The vessels were submersed in the PEG bath and exposed to a rotating motion to ensure uniform mixing between the straw and liquor. The used FA concentrations accounting for dilution by straw moisture are 0 (Control), 2, 5, 10, and 15 g/L. Another variable was the (L/S) which was studied at control (0 g/L FA) and 10 g/L FA and 4, 6, 8, and 10 L/kg. The (L/S) was selected as 6 L/kg based on a set of experiments at 0 g/L FA (i.e. control) and 10 g/L FA at different (L/S) ratios (4, 6, 8, and 10). Finally, at a FA concentration of 15 g/L and L/S ratio of 6 L/kg 1 g/L sulfuric acid (SA) was added to determine the effect of higher acidity on FA assisted pre-hydrolysis. All pre-hydrolysis experiments were performed in duplicate. After the pre-hydrolysis time of 100 min was reached the vessels were immersed into ice-water to stop the pre-hydrolysis reaction. The pre-hydrolysate liquor and residual solids were collected by filtration using 200 mesh nylon filter and the liquid part weighed and stored at -20 °C for further analysis at a later date. The pre-treated solid residues were stored in a zip locked plastic bag and placed in a refrigerator at 4°C for further testing.

4.3.2.2. Analytical Methods

Before subjecting the wheat straw to chemical tests, the samples were ground in a Wiley Laboratory Mill using 0.5 mm screen. The moisture content was determined according to the TAPPI (T 412 om-11) test method and the material was sealed in zip locked plastic bags and stored at room temperature. The ash (TAPPI T 211 om-12) content and the acetone solubility were examined according to method (TAPPI T 204 cm-07). The pre-hydrolysis yield determined by gravimetric measurements was calculated from the dry weight of the prehydrolysed straw and the weight of initial straw.

Sugar content of the wheat straw, pre-hydrolysis solids and pre-hydrolysate liquor were determined using high performance anion exchange chromatography (HPAEC) with pulsed amperometric detection (PAD). The wheat straw and pre-hydrolysis solid residues were subjected to a two-step sulfuric acid hydrolysis (72 and 4%). Total sugar content of the pre-hydrolysate was determined by HPAEC-PAD after 4% sulfuric acid hydrolysis. Laboratory Analytical Procedures (LAP) from the National Renewable Energy Laboratory (NREL) was used to determine lignin (acid insoluble and soluble) contents of the samples (Sluiter et al. 2011). The acid-insoluble lignin was obtained by weighing the solid samples remaining after the two-step sulfuric acid hydrolysis. The acid-soluble lignin was analyzed by UV-Vis analysis at a wavelength of 320 nm against blank 4% sulfuric acid with an absorptivity of 30 L/g.cm.

Hemicellulose components or degradation products in the wheat straw, pre-hydrolysis solid parts and pre-hydrolysate liquor such as acetyl groups (or acetic acid), formic acid, HMF and furfural were determined by HPLC using a refractive index detector and BIO-RAD Aminex HPX-87H column. 5 mM sulfuric acid was used as mobile phase. Flow rate and column temperature were 0.6 ml/min and 60 °C respectively. The acetyl group (AcG) content in the original straw and pre-hydrolysis residual solids was calculated based on the amount of acetic acid.

Uronic anhydride (UA) content of the original straw was determined using the chromophoric group analysis after a two-step acid hydrolysis treatment of the secondary hydrolysate according to the method developed by Scott (1979). Dimethylphenol was used as standard, and the content of UA was calculated from the difference in UV absorption at 400 and 450 nm. 4-O-methyl glucuronic anhydride was calculated from uronic anhydride content using the formula (4-O-methyl glucuronic anhydride = UA x (190/176))

The amount of precipitate in the pre-hydrolysate liquor was determined after centrifugation at 14000 g for 15 min at room temperature. After removal of supernatant, the precipitate was subjected to vacuum drying at room temperature and its weight determined. The molecular weight of the precipitates was measured by SEC with UV and RI detectors using DMSO/0.5% w/w LiBr as eluent at flow rate of 0.5 mL min^{-1} at 60 °C. The lignin fractions were dissolved without derivatization at room temperature in the SEC eluent and filtered through a 0.45 μm PTFE syringe filter prior to injection. Standard calibration was performed using pullulan standards of MW range 342 – 3.44×10^5 g mol^{-1}.

4.4. RESULTS AND DISCUSSION

4.4.1. Chemical Composition of Wheat Straw

The results of chemical composition of wheat straw obtained in this study are given in Table 4.1. As shown in Table 4.1, wheat straw mostly consist of glucan and xylan, 40.5% and 22.9%, respectively for a total carbohydrate content (sum of glucan, xylan, arabinan, mannan plus acetyl groups (1.48%) and 4-O-methyl glucuronic anhydride (1.82%)) of 70.1% on oven dry basis of wheat straw. This value is important because, for the IFBR concept, a cheap and abundant raw material with high carbohydrate content is a potential feedstock for production of fermentable sugars for bioethanol. The total amount of wheat straw components determined is 98.6%, which is an indication of the good accuracy of the chemical analyses. The main part of glucan originates from cellulose and xylan represents most of the hemicelluloses. The total amount of carbohydrates is slightly higher than that reported in literature for wheat straw. Although the total lignin content (24.1%) was similar, the ash content (3.02%) was lower than the literature reported results (Ertas et al. 2014, Mustajoki et al. 2010). Slight differences in the values could be due to the growth environment, growing season, growing location, soil conditions, analysis methods, etc. (Utne and Hegbom 1992, Amores et al. 2013).

Table 4.1. Chemical composition of wheat straw on oven-dry basis.

Component	g/100g od straw
Arabinan	2.17±0.01*
Galactan	0.64±0.02
Glucan	40.5±0.41
Xylan	22.9±0.06
Mannan	0.62±0.01
Acetyl groups (AcG)	1.48±0.02
4-O-methyl glucuronic anhydride	1.82±0.03
Acid soluble lignin (ASL)	0.81±0.09
Klason lignin (KL)	23.3±0.01
Ash	3.02±0.03
Extractives	1.30±0.05
Total	98.6±0.74

*The range and average of duplicate measurement is reported.

4.4.2. Effect of L/S Ratio on the Yield of Wheat Straw

Table 4.2 shows the pre-hydrolysis yield of wheat straw at different L/S ratios (L/kg) and FA concentrations in g/L. As shown in Table 4.2 and Figure 4.1, the yield of the solid residue decreases from 93.6% to 74% at increasing L/S ratios and FA concentrations of 0 (autohydrolysis) and 10 g/L.

Table 4.2. The pre-hydrolysis yield of wheat straw.

Variables	Yield (%)*			
L/S ratio (L/kg)	4	6	8	10
0g/L FA (Control)	93.6±0.05	91.7±0.43	90.4±0.09	89.4±0.03
10g/L FA	82.3±0.21	79.2±0.25	76.4±0.18	74.0±0.16

*The range and average of duplicate measurement is reported.

Figure 4.1 shows the effect of L/S ratio on the residual straw yield at 0 g/L (control) and 10 g/L FA. It can be seen that the yield decreases significantly for autohydrolysis and 10 g/L FA when the L/S ratio increases from 4 to 10 L/kg, and that the amount of straw dissolved is about 2.5 to 3 times larger with 10 g/L FA compared to autohydrolysis. In both cases the largest incremental decrease in yield occurs when the L/S ratio is increased from 4 to 6 L/kg. Because it is not practical from an industrial point of view to use too high L/S ratios it was decided to adopt a L/S ratio of 6 L/kg for further experimentation in the present study.

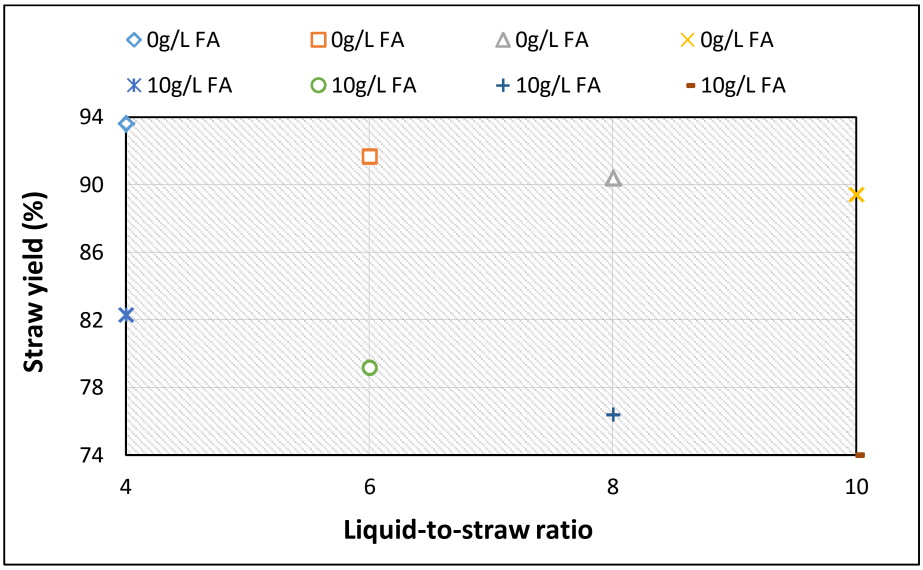

Figure 4.1. Yield of the control and 10 g/L FA versus different L/S ratios.

4.4.3. Effect of Pre-hydrolysis Conditions on the Composition of Wheat Straw

Table 4.3 and Figure 4.2 shows the residual straw yield versus FA concentrations at L/S ratio of 6 L/kg. It can be seen in Figure 4.2 that the yield of the prehydrolysed solid residue decreases from 91.7% at autohydrolysis to 76.2% at the highest FA concentration of 15 g/L, while the yield decreased further to 67.3% when 1 g/L sulfuric acid added to 15 g/L FA. The amount of straw dissolved more than doubles with 2 g/L FA compared to autohydrolysis. The obvious reason for the decrease in solid yield with increasing FA concentration and finally with the addition of 1 g/L SA is that the increasing acidity solubilizes more hemicelluloses.

Table 4.3. The effect of FA concentration on the straw yield at L/S ratio of 6 L/kg.

FA+SA conc. (g/L)	Yield (%)*
0 (Control)	91.7±0.43
2	84.2±0.01
5	80.7±0.08
10	79.2±0.25
15	76.2±0.23
15+1	67.3±0.44

*The range and average of duplicate measurement is reported.

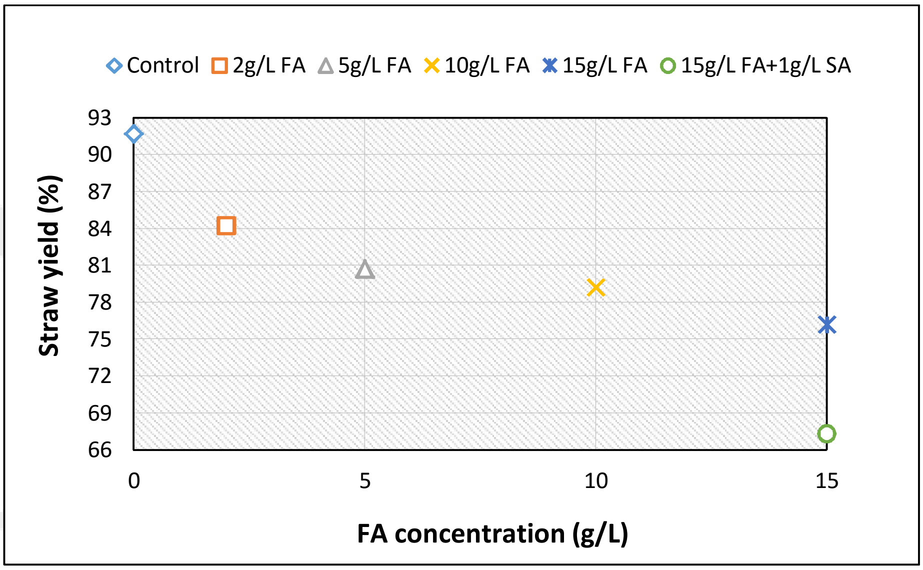

Figure 4.2. Straw yield versus different FA concentrations at L/S ratio of 6 L/kg.

The sugar content in the original straw and the pre-extracted residues based the dry mass of the original straw is listed in Table 4.4. The sugar content is given in the form of the different anhydrosugars, i.e. arabinan, galactan, glucan, xylan and mannan, as well as the total sugar content. As can be seen that all sugars decrease with increasing FA concentration, except for the glucan content which remains unchanged within experimental error. The latter is due to the crystalline nature and high degree of polymerization (DP) of cellulose (Ruigang et al. 2005). On the other hand the xylan, arabinan, galactan and mannan contents of the solid residues based on original straw decrease steadily from 20 .1% to 4.15%, 1.50% to 0.11%, 0.61% to 0.04% and 0.64% to 0.10% as the FA concentration increases from 0 g/L (Control) to 15 g/L+1 g/L SA, respectively.

The large dissolution of the most important hemicellulose component, xylan, can be explained by the fact that the glycosidic linkages in xylan are degraded 1500 times faster than that of cellulose (Huseman et al. 1942). The remaining xylan in the pre-extracted solid parts is probable more strongly bound to residual lignin, which makes it less vulnerable to hydrolysis under the increasing FA concentration and adding SA in this study. Similar results were reported by others (Lawther et al. 1995, Ertas et al. 2014).

Table 4.4. Sugar content of wheat straw and pre-extracted residues at different FA concentrations and L/S ratio of 6 L/kg.

Solid part	Arabinan	Galactan	Glucan	Xylan	Mannan	Total sugar
Original straw	2.17±0.01*	0.64±0.02	40.5±0.41	22.9±0.06	0.62±0.01	66.8
0g/L FA (Control)	1.50±0.04	0.61±0.03	39.7±0.51	20.1±0.30	0.64±0.02	62.6
2g/L FA	0.83±0.06	0.28±0.02	40.5±0.53	15.2±0.24	0.51±0.03	57.4
5g/L FA	0.65±0.04	0.23±0.04	39.9±0.54	11.4±0.23	0.48±0.04	52.6
10g/L FA	0.53±0.04	0.21±0.01	41.1±0.66	9.08±0.18	0.46±0.01	51.4
15g/L FA	0.47±0.03	0.16±0.01	38.8±0.57	7.30±0.28	0.36±0.05	47.1
15g/L FA+1g/L SA	0.11±0.07	0.04±0.05	39.7±0.94	4.15±0.55	0.10±0.01	44.1

* All values based on g/100g on straw and the range and average of duplicate measurement is reported.

The residual Klason lignin and acid soluble lignin in the solid residues based on original straw are listed in Table 4.5. It can be seen that about 1.5-2% of Klason lignin is removed during autohydrolysis or FA-assisted pre-hydrolysis. With the addition of sulfuric acid to 15 g/L FA the Klason lignin removal increases to about 3% on original straw weight. The acid soluble lignin behavior is different with the lowest amount remaining in the autohydrolysed solids and for 15 g/L+1 g/L SA. The total amount of lignin removed from the original straw of only about 2-3% is significantly smaller than the amount of sugars removed of up to 23.5% at 15 g/L FA+1 g/L SA. This implies that with increasing FA concentration the ratio of dissolved lignin and sugar decreases with increasing FA concentration compared to autohydrolysis.

Table 4.5. Klason and acid soluble lignin content of wheat straw and pre-extracted residue at different FA concentrations.

Solid part	Klason lignin	Acid soluble lignin	Total lignin
Original straw	23.3±0.35*	0.81±0.09	24.1
0g/L FA (Control)	21.7±0.66	0.67±0.02	22.4
2g/L FA	21.7±0.08	0.74±0.03	22.5
5g/L FA	21.6±0.10	0.85±0.00	22.4
10g/L FA	21.4±0.06	0.91±0.01	22.3
15g/L FA	21.2±0.65	0.89±0.01	22.1
15g/L FA+1g/L SA	20.5±0.00	0.60±0.01	21.1

*All values based on g/100g on straw and the range and average of duplicate measurement is reported

The acetyl groups (AcG), 4-O-methylglucuronic anhydride (4-O-MeGA) and ash content remaining in the original and pre-extracted straws are shown in

Table **4.6** and Figure 4.3. As can be seen in

Table **4.6**, the ash, AcG, and 4-O-MeGA in remaining the straw decreases from 2.21% to 1.53%, 0.88% to 0.16% and 1.24% to 0.18%, respectively with increasing FA concentration. The ash content after autohydrolysis is the same (within experimental error) as that after FA pre-hydrolysis at the different FA concentrations. However with sulfuric acid addition the ash content decreases further. The 4-O-MeGA decreases nicely with increasing acidity.

Table 4.6. Acetyl groups, 4-O-methylglucuronic acid, and ash composition of original and pre-extracted wheat straw on oven dry basis.

Solid part	Ash	4-O-methylglucuronic anhydride	Acetyl groups (AcG)
Original straw	3.02±0.03*	1.82±0.03	1.48±0.02
0g/L FA (Control)	2.21±0.11	1.24±0.23	0.88±0.02
2g/L FA	2.21±0.02	0.78±0.09	0.82±0.00
5g/L FA	2.12±0.01	0.58±0.01	0.59±0.00
10g/L FA	2.35±0.00	0.38±0.07	0.47±0.02
15g/L FA	2.19±0.01	0.23±0.01	0.38±0.02
15g/L FA+1g/L SA	1.53±0.03	0.18±0.02	0.16±0.00

*All values based on g/100g on straw and the range and average of duplicate measurement is reported.

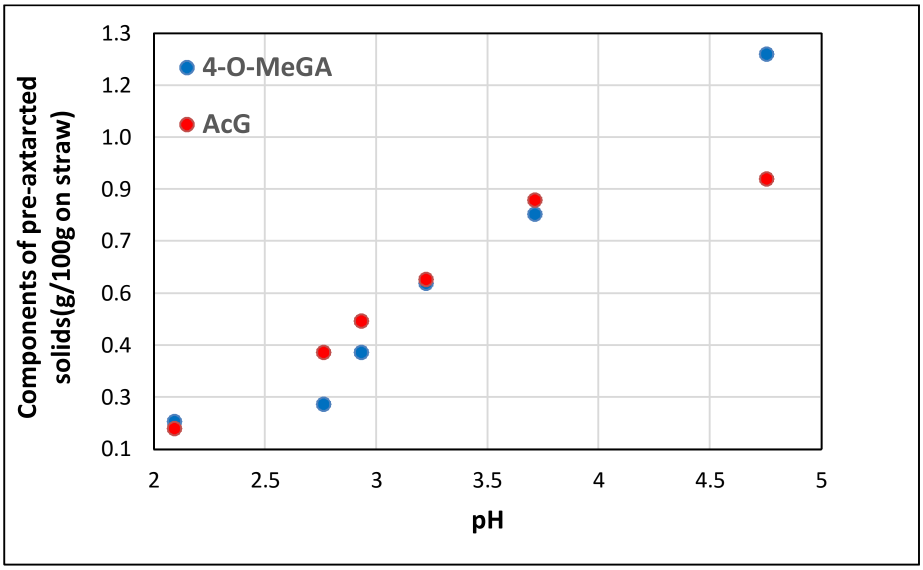

Figure 4.3. The 4-O-MeGA and AcG amount versus pH.

4.4.4. Sugars in the Pre-hydrolysate Liquor

The total content of the different sugars (so the sum of both monomers and oligomers) in the pre-hydrolysate liquor at different operating conditions are given Table 4.7. All values are reported in grams based on 100 g of raw straw. During the pre-hydrolysis, cellulose, lignin, and especially hemicelluloses are solubilized from the biomass structure because of the chemical penetration under the high pressure and temperature (Ertas et al. 2014). The hemicelluloses are dissolved from the straw leading to increased dissolution of the different sugars (xylan, arabinan, galactan, and mannan) increasing from 1.98 g to 18.2 g, 0.60 g to 1.68 g, 0.26 g to 0.68 g and 0.14 g to 0. 33 g as the FA concentration increases from 0 g/L (Control) to 15 g/L+1 g/L SA, respectively. As expected the highest total sugar dissolution (23.5 g) is obtained at 15g/L FA+1g/L SA which means that around 35% of all sugars are dissolved. At this condition xylan has the highest concentration (18.2 g) in the pre-hydrolysate (Table 4.7), which means that around 77% of all sugars.

Table 4.7. Sugar contents of pre-hydrolysate liquor at different FA concentrations.

Liquid part	Arabinan	Galactan	Glucan	Xylan	Mannan	Total sugar
0g/L FA (Control)	0.60±0.18*	0.26±0.02	0.84±0.20	1.98±0.71	0.14±0.00	3.83
2g/L FA	0.92±0.07	0.42±0.03	1.30±0.16	7.06±0.72	0.20±0.11	9.89
5g/L FA	0.99±0.15	0.47±0.02	1.58±0.18	9.82±1.62	0.26±0.05	13.1
10g/L FA	1.06±0.02	0.52±0.01	1.73±0.04	11.9±0.22	0.30±0.00	15.5
15g/L FA	1.30±0.07	0.62±0.01	2.16±0.04	14.7±0.30	0.39±0.01	19.2
15g/L FA+1g/L SA	1.68±0.11	0.68±0.21	2.65±0.17	18.2±0.33	0.33±0.01	23.5

*All values based on g/100g on straw and the range and average of duplicate measurement is reported.

4.4.5. Sugar Mass Balances

Table 4.8 shows the overall sugar mas balance at the different operating conditions. It can be seen that the mass balance is excellent with the total sugar amount detected in the extracted solids and in the pre-hydrolysate adding up to 66-67% based on original straw weight, which is the same as that present in the original straw. This indicates that there are no large sugar degradation losses, and that the analytical techniques are good.

Table 4.8. Sugar mass balance of wheat straw and pre-extracted residue.

Treatment	Total sugar in solids	Total sugar in liquids	Total sugar
Original straw	66.8*	0	66.8
0g/L FA (Control)	62.6	3.83	66.4
2g/L FA	57.4	9.89	67.3
5g/L FA	52.6	13.1	65.7
10g/L FA	51.4	15.5	66.9
15g/L FA	47.1	19.2	66.3
15g/L FA+1g/L SA	44.1	23.5	67.6

*All values based on g/100g on straw and the range and average of duplicate measurement is reported.

4.4.6. Lignin in the Pre-hydrolysate Liquor

The concentration and amount of precipitated lignin (PL) and soluble lignin (SL) in g/L and g/100 g as a function of straw yield are given in Figure 4.4 and Table 4.9, respectively. The amount of PL decreases from 0.94 to 0.31 when the FA concentration is increased from 0 to 15 g/L, while that of the SL determined by UV spectroscopy increases from 0.39 to 1.64 based on g/100 g of raw straw. The average molecular weight of precipitated lignin determined by GPC decreased from 1970 g/mol at autohydrolysis to 710 g/mole at 15 g/L of FA. This supports the earlier described mechanism that FA minimizes condensation of dissolved lignin fragments by formylation. The same behavior was observed in the literature (Yasukawa et al. 2014). The decrease in the amount of PL with increasing FA concentration is important because the PL is considered to be responsible for operational problems due to equipment and piping plugging with sticky lignin.

Table 4.9. Lignin content of pre-hydrolysate liquor at different FA concentrations.

Liquid part	Soluble lignin	Precipitated lignin	Total lignin
0g/L FA (Control)	0.39±0.00*	0.939±0.00	1.33
2g/L FA	0.95±0.00	0.705±0.00	1.66
5g/L FA	1.01±0.00	0.501±0.00	1.51
10g/L FA	1.09±0.00	0.441±0.00	1.53
15g/L FA	1.29±0.00	0.312±0.00	1.60
15g/L FA+1g/L SA	1.64±0.32	0.309±0.00	1.94

*All values based on g/100g on straw and the range and average of duplicate measurement is reported.

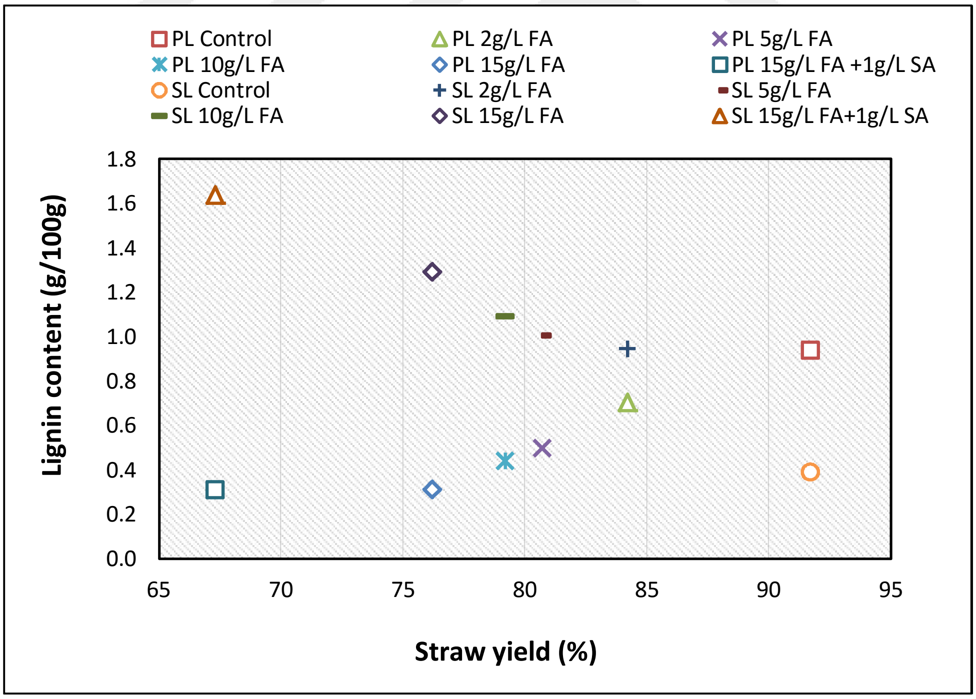

Figure 4.4. The amount of precipitated lignin (PL) and soluble lignin (SL) based on g/100g on straw as function of straw yield.

FA in pre-hydrolysate of wheat straw reduces lignin precipitate. The decrease in PL lignin is also seen in Figure 4.5 with the FA assisted pre-hydrolysates being much more transparent than the autohydrolysate.

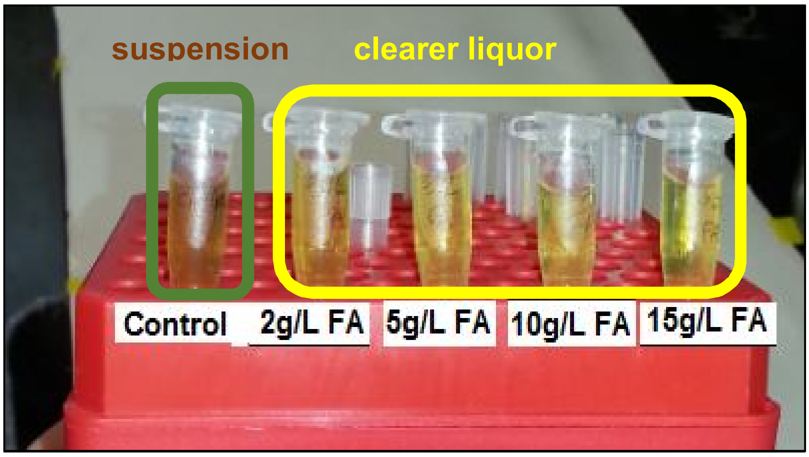

Figure 4.5. Pre-hydrolysate samples at different FA concentrations.

The quantities of all 4 forms of lignin (insoluble lignin, soluble lignin, acid soluble lignin, and Klason lignin) as a function of pre-hydrolysis conditions are shown in Figure 4.6. The graph suggests that with increasing FA concentration less soluble lignin is converted in insoluble/precipitated lignin.

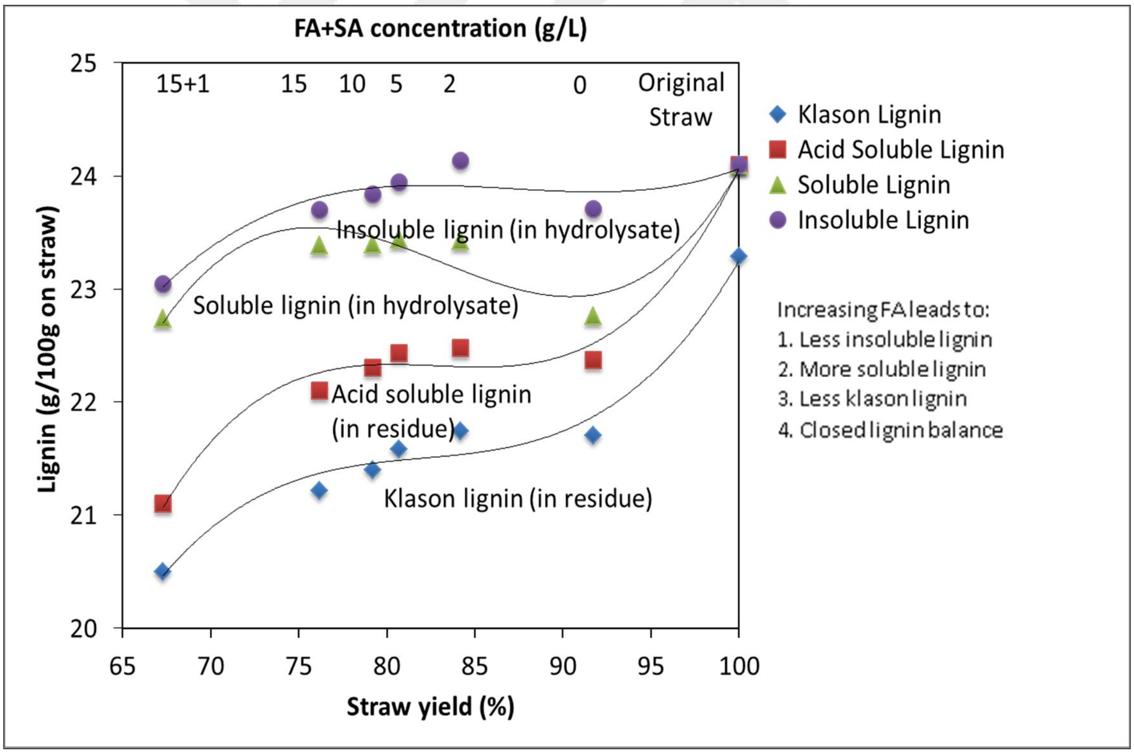

Figure 4.6. Lignin mass balance of wheat straw and pre-extracted residue at different FA concentrations.

4.4.7. Lignin Mass Balance

Table 4.10 shows the lignin mass balance at the different operating conditions. It can be seen that the lignin mass balance is nicely closed for autohydrolysis and FA assisted pre-hydrolysis, while the lignin yield is about 1 % down based on original straw for the experiment performed at 15 g/L FA+1 g/L SA.

Table 4.10. Lignin mass balance of wheat straw and pre-extracted residue.

Treatment	Total lignin in solids	Total lignin in liquids	Total lignin
Original straw	24.1*	0	24.1
0g/L FA (Control)	22.4	1.33	23.7
2g/L FA	22.5	1.66	24.1
5g/L FA	22.4	1.51	23.9
10g/L FA	22.3	1.53	23.9
15g/L FA	22.1	1.60	23.7
15g/L FA+1g/L SA	21.1	1.94	23.1

*All values based on g/100g on straw and the range and average of duplicate measurement is reported

4.4.8. Effect of Pre-Hydrolysis Conditions on pH and Solid Content of the Drained Pre-hydrolysate Liquor of Wheat Straw

Table 4.11 shows the pH and dry solids content of the drained pre-hydrolysate liquor and residual straw yield based on original straw. Figure 4.7 shows that the pH of the drained hydrolysate decreases significantly as the residual straw yield decreases. During the autohydrolysis, acetyl groups present in wheat straw are cleaved to produce acetic acid which further induces hemicellulose depolymerization (Lee et al. 2010). The data in Table 4.11 and Figure 4.7 also shows the pH during FA assisted pre-hydrolysis decreases from 4.75 at autohydrolysis to 2.76 at 15 g/L FA. This much lower pH is to be expected because the pKa of formic acid is 3.8, and thus is an order of magnitude more acidic than acetic acid pKa of 4.8.

Table 4.11. The dry solid content and pH of the pre-hydrolysate liquor and yield of residue wheat straw at L/S of 6 L/kg and 100 minutes pre-hydrolysis.

Pre-extracted liquor	pH	Solid content (g/100 g on straw)	Straw yield (%)
0g/L FA (Control)	4.75	2.21±0.00	91.7±0.43
2g/L FA	3.71	3.57±0.00	84.2±0.01
5g/L FA	3.22	4.08±0.00	80.7±0.08
10g/L FA	2.93	4.08±0.00	79.2±0.25
15g/L FA	2.76	3.99±0.00	76.2±0.23
15g/L FA+1g/L SA	2.09	4.77±0.11	67.3±0.44

Figure 4.8 shows the dry solids content of the drained pre-hydrolysate liquor versus residual straw yield for the control experiment (autohydrolysis) and the different FA concentration trials. As shown in Table 4.11 and Figure 4.8, the solids content of the pre-hydrolysate increases from 2.21 to 4.77% as the FA concentration increases from 0 g/L to 15 g/L+1 g/L SA. A slight decrease in solids content from 4.08 to 3.99 from 5, to 15g/L FA concentration while the straw yield decreases from about 81 to 76% shows that the dissolved solids do not correspond to the dissolved straw mass. The reason is mostly due to increasing losses of volatile hemicellulose degradation components, such as straw derived formic acid, acetic acid, furfural, HMF, etc., during drying as part of the dry solids content method. This also confirms that solids content of pre-hydrolysate liquor is not a good measure of the degree of extraction (Yasukawa et al. 2014).

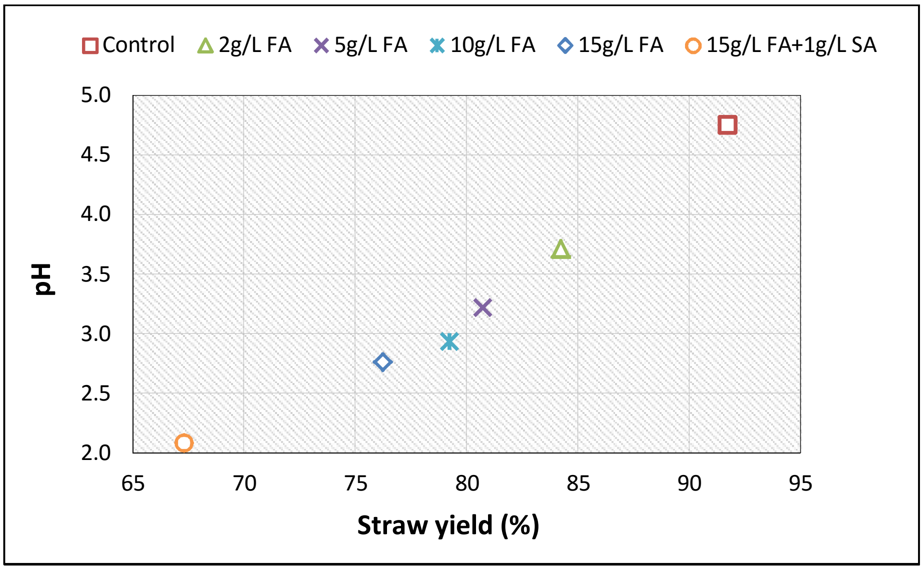

Figure 4.7. pH of the pre-hydrolysate liquor versus straw yield at different FA concentrations.

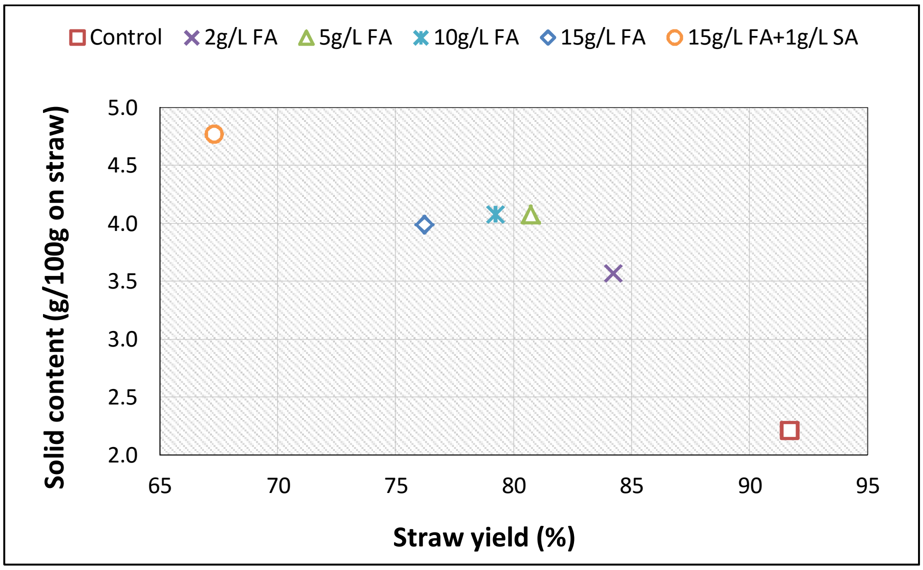

Figure 4.8. Solid content of the pre-hydrolysate liquor versus straw yield at different FA concentrations.

4.4.9. Effect of Pre-hydrolysis Conditions on Byproducts

Figure 4.9 and Table 4.12 show concentration of acetic acid and furfural versus straw yield and the byproduct content of the pre-hydrolysate liquor at the different operating conditions. Sugars degradation products from pre-hydrolysis, such as acetic acid, formic acid, furfural and HMF (5-hydroxymethyl furfural), are known inhibitors during the ethanol fermentation process and also decrease the overall sugar yield (Çöpür et al. 2012, Ertas et al. 2014). The formation of inhibitory products during pre-hydrolysis of lignocellulosic materials depends both on the feedstock and pre-hydrolysis conditions such as temperature, pH, time, pressure and addition of chemicals. During the pre-hydrolysis, five carbon (xylose and arabinose) and six carbon (glucose, mannose and galactose) sugars may be degraded to furfural and HMF, respectively. Also, HMF can be further degraded to levulinic acid and formic acid. Acetic acid is formed from acetyl groups linked to xylan (Batalha et al. 2015). Acetic acid and furfural are the main byproducts produced during the pre-hydrolysis. As shown is Table 4.12, furfural and acetic acid are produced in the pre-hydrolysate liquor ranging from 0.21 to 1.68 g/100 g straw and 0.47 to 2.12 g/100 g straw, respectively as the FA concentration increases from 0 g/L (Control) to 15 g/L+1 g/L SA, in agreement with the increasing acidity from pH 4.75 to 2.09 (Table 4.11), respectively (Yasukawa et al. 2014, Ertas et al. 2014, Batalha et al. 2015). On the other hand, the HMF concentration is too low to be determined. The highest acetic acid concentration in Fig. 9 of 3.53 g/L at 15 g/L FA is equivalent to deacetylation of 1.52% acetyl groups, which is the same within experimental error as the acetyl group content of the original straw of 1.48% (see Table 4.1). Thus at this condition all acetyl groups are cleaved off. The highest furfural concentration of 2.80 g/L at the same condition is stoichiometrically equivalent to 2.3% (on original straw) of xylan (or arabinan). Since the amount of xylan+arabinan dissolved at this condition is 20.8% (see Table 4.4) while 19.9% xylan+arabinan is detected in the pre-hydrolysate (see Table 4.7), the amount of furfural measured at this condition appears reasonable.

Table 4.12. The byproduct content of the pre-hydrolysate liquor at the different operating conditions.

Liquid part	HMF (g/L)	Furfural (g/L)	AcOH (g/L)	Furfural (g/100g straw)	AcOH (g/100g straw)
0g/L FA (Control)	nd	0.35	0.79	0.21	0.47
2g/L FA	nd	0.71	1.12	0.43	0.67
5g/L FA	nd	0.99	1.49	0.58	0.89
10g/L FA	nd	1.38	1.99	0.83	1.19
15g/L FA	nd	2.05	2.26	1.21	1.35
15g/L FA+1g/L SA	nd	2.80	3.53	1.68	2.12

[*] All values based on g/100g on straw and the range and average of duplicate measurement is reported.

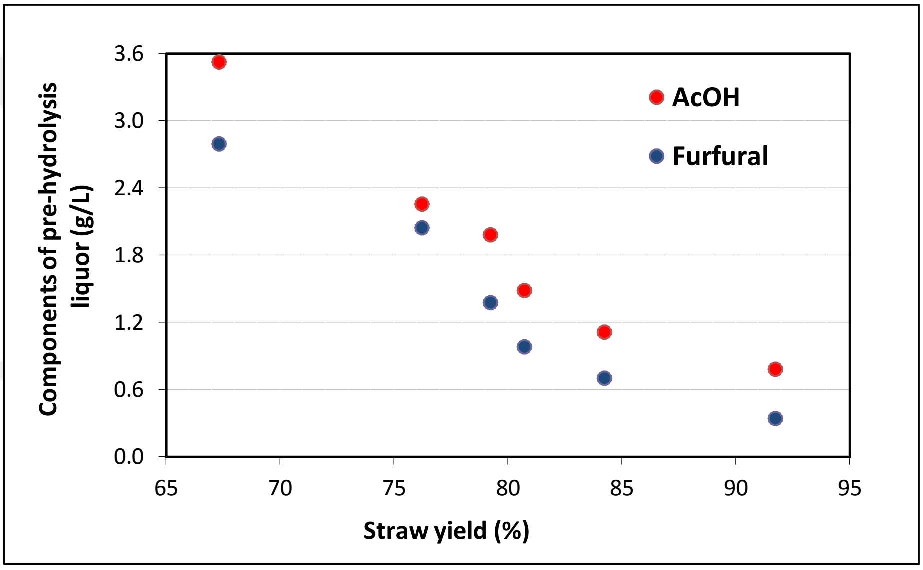

Figure 4.9. Concentration of acetic acid and furfural versus straw yield.

4.4.10. Conclusions

The FA reinforced autohydrolysis is an effective process to obtain high yield monosugar from wheat straw, while minimizing the problem of lignin precipitates. The sugar yield improves dramatically with increasing FA concentration relative to autohydrolysis. The results showed excellent sugar and lignin mass balances during pretreatment, and that the suspended ("sticky lignin") concentration decreased from 0.94 g/100g straw at autohydrolysis to 0.31 g/100g straw at FA concentration of 15 g/L. The average molecular weight of precipitated lignin determined by GPC decreased from 1970 g/mole at autohydrolysis to 710 g/mole at 15 g/L of FA. This supports the earlier described mechanism that FA minimizes condensation of dissolved lignin fragments by formylation. The monomeric sugar yield during pretreatment improved dramatically with increasing FA concentration relative to autohydrolysis. Without FA the sugar yield in the hydrolysate was only 3.8 g/100g. However at a FA concentration of 15 g/L, the dissolved sugar yield increased to 23.5 g/100g. The results showed that FA reinforced autohydrolysis not only minimize the formation of "sticky lignin" but also significantly increased dissolved sugar yield.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. CONCLUSIONS

The results indicated that cellulose fraction of wheat straw was undamaged up to the treatment temperature of 135 °C and this provided the optimum pre-extraction condition. The other conditions resulted more than 30% of the xylan dissolution. Results indicated that the hot water pre-extracted pulp gave same pulp yield and showed comparable mechanical and physical properties. On the other hand, ethanol (12.3 kg/1000 kg of straw) was produced from the hot water pre-extracted hydrolyzate. Biodegradable films produced using only pre-extracted xylan failed the film formation. Addition of xylitol provided self-supporting film formation. In addition, gluten and nanocellulose significantly improved the mechanical properties of the films.

The pre-extraction of corn stalks with hot water (150 °C) removed up to 44.8% of the xylose from the structure. The ratio of xylose removal was higher than that of lignin for all hot water pre-extractions. The increase in alkali concentration removed more material from the structure. Modified alkali ($NaBH_4$+NaOH) pre-extraction increased the lignin removal. Bioethanol was obtained from the extracts, and the stalks pre-extracted with hot water at 150 °C produced 14.7% (g/100g OD) of ethanol. Moreover, the theoretical ethanol yield was calculated to be 89.4%. The pre-extracted xylan was used to produce biodegradable films but pure xylan was not formed self-supporting biofilm. The results showed that adding xylitol, gluten, and nanocellulose resulted in quality film formation with sufficient mechanical properties. Lastly, the pulp properties and yield of the hot water pre-extracted and control soda pulps were comparable.

Results showed that the FA reinforced autohydrolysis is an effective process to obtain high yield monosugars from wheat straw. The reinforcement in addition minimized the lignin precipitation. Increase in FA concentration dramatically improved the sugar yield. A significant mass balances for sugars and lignin was observed taking into calculation of liquids and solid fractions. The precipitated lignin in the hydrolysate decreased from 0.94 g/100g (without FA) to 0.31 g/100g straw at 15 g/L of FA. The average molecular weight of precipitated lignin decreased from 1970 g/mole (without FA) to 710 g/mole at 15 g/L of FA. The monomeric sugar yield during pretreatment improved dramatically when FA concentration was increased. The sugar yield in the hydrolysate was only 3.83 g/100g (without FA). On the other hand, the dissolved sugar yield was 23.5 g/100g when the samples were pretreated with FA (15 g/L). Consequently, FA reinforced autohydrolysis minimized the formation of “sticky lignin” and significantly increased the dissolved sugar yield.

5.2. FUTURE WORK

- Industrially important resources utilized for pulp and paper production should be pre-extracted with different/new methods to extract hemicelluloses, which could be converted to much higher value-added products.

- The materials in pre-extracted liquids should be recovered with the highest possible yields and low-costs.

- Pre-extracted liquors include some lignin which could also be converted to value-added products.

- Biodegradable films could be produced from the liquid part of FA reinforced autohydrolyzed wheat straw. Sticky lignin is a problem in production and the process should be improved to overcome the problem.

- In FA reinforced autohydrolysis, the enzymatic hydrolysis should be optimized to achieve optimum cellulose conversion.

- The FA reinforced autohydrolysis could be combined with refining to optimize the enzymatic hydrolysis.

- Parameters of time, enzyme, and solid loadings could be extensively studied to minimize the processing costs and maximizing the glucose yield.

- The economic feasibility of ethanol production from wheat straw and corn stalks should be investigated. It could not be addressed in this study.

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7. APPENDICES

7.1. APPENDIX-1. BIODEGRADABLE FILMS PRODUCTIONS

Figure A1.1. Xylan solutions obtained wheat straw (left) and corn stalks (right) from pre-extracted liquor.

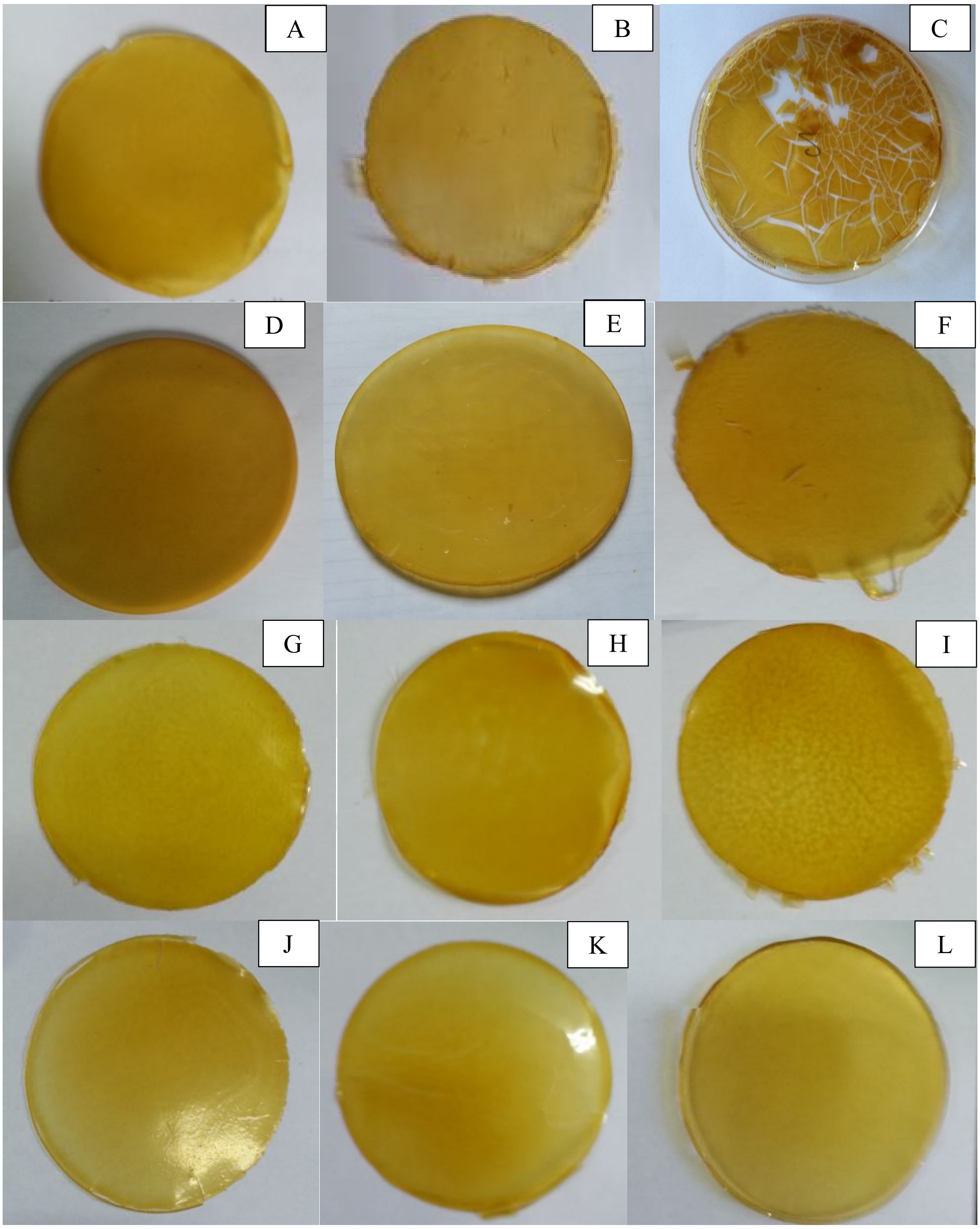

Figure A1.2. Biodegradable films obtained from pre-extracted liquors of wheat straw (WS) and corn stalks (CS).

(A) CS xylan (0.6 g)+0.7 g xylitol, (B) WS xylan (0.6 g)+0.4 g xylitol+0.05 g nanocellulose, (C) CS xylan (0.85 g)+0.4 g xylitol, (D) CS xylan (0.6 g)+0.7 g xylitol+0.4 g gluten, (E) CS xylan (0.4 g)+0.2 g xylitol+0.25 g gluten, (F) CS xylan (0.85 g)+0.7 g xylitol, (G) WS xylan (0.6 g)+0.5 g xylitol, (H) WS xylan (0.675 g)+0.4 g xylitol+0.025 g nanocellulose, (I) WS xylan (0.6 g)+0.4 g xylitol+0.1 g gluten, (J) CS xylan (0.7 g)+0.4 g xylitol, (K) CS xylan (0.675 g)+0.4 g xylitol+0.025 g nanocellulose, (L) CS xylan (0.6 g)+0.7 g xylitol+0.1 g gluten.

7.2. APPENDIX-2. STATISTICAL DATA

Table A2.1. Variation analysis results (ANOVA) of weight, glucose, xylose, and lignin loss for wheat straw hot water pre-extraction at different temperatures.

Material loss	Variation Source	Sum of Squares	Degree of Freedom	Mean Square	F	Significance
Weight loss	Between Groups	2041.043	3	680.348	2741.955	0.000
	Within Groups	1.985	8	0.248		
	Total	2043.028	11			
Glucose loss	Between Groups	28.174	3	9.391	4795.557	0.000
	Within Groups	0.016	8	0.002		
	Total	28.190	11			
Xylose loss	Between Groups	3024.595	3	1008.198	15090.906	0.000
	Within Groups	.534	8	0.067		
	Total	3025.129	11			
Lignin loss	Between Groups	1200.519	3	400.173	1148.328	0.000
	Within Groups	2.788	8	0.348		
	Total	1203.307	11			

Table A2.2. Duncan test results of weight, glucose, xylose, and lignin loss for wheat straw hot water pre-extraction at different temperatures.

Temperature (°C)	Weight loss				Glucose loss			Xylose loss				Lignin loss			
	Subset For Alpha = 0.05														
	1	2	3	4	1	2	3	1	2	3	4	1	2	3	4
90	5.20				0.04			2.29				5.38			
120		8.20			0.05				2.97				8.80		
135			15.8			1.57				16.5				15.6	
150				38.5			3.77				41.5				31.4

Table A2.3. Variation analysis results (ANOVA) of weight, glucose, xylose, and lignin loss for corn stalks hot water pre-extraction at different temperatures.

Material loss	Variation Source	Sum of Squares	Degree of Freedom	Mean Square	F	Significance
Weight loss	Between Groups	1657.103	3	552.368	36824.500	0.000
	Within Groups	0.120	8	0.015		
	Total	1657.223	11			
Glucose loss	Between Groups	360.399	3	120.133	216.720	0.000
	Within Groups	4.435	8	0.554		
	Total	364.834	11			
Xylose loss	Between Groups	1992.563	3	664.188	1321.766	0.000
	Within Groups	4.020	8	0.502		
	Total	1996.583	11			
Lignin loss	Between Groups	1068.145	3	356.048	2637.395	0.000
	Within Groups	1.080	8	0.135		
	Total	1069.225	11			

Table A2.4. Duncan test results of weight, glucose, xylose, and lignin loss for corn stalks hot water pre-extraction at different temperatures.

Temperature (°C)	Subset For Alpha = 0.05														
	Weight loss				Glucose loss				Xylose loss			Lignin loss			
	1	2	3	4	1	2	3	4	1	2	3	1	2	3	4
90	2.30				2.50				12.1			1.42			
120		8.00				4.14				16.5			10.5		
135			11.3				5.72			17.7				11.3	
150				33.3				16.5			44.8				27.6

Table A2.5. Interactions between temperatures and concentrations on weight, glucose, xylose, and lignin for wheat straw.

Source		Type III Sum of Squares	df	Mean Square	F	P
Weight loss	Temperature	413	2	207	1234	0.000
	Concentration	261	2	131	780	0.000
	Temperature * Concentration	6.16	4	1.50	9.21	0.000
Glucose loss	Temperature	220	2	110	803	0.000
	Concentration	233	2	117	849	0.000
	Temperature * Concentration	6.33	4	1.58	11.5	0.000
Xylose loss	Temperature	318	2	159	252	0.000
	Concentration	1353	2	676	1072	0.000
	Temperature * Concentration	13.9	4	3.47	5.50	0.004
Lignin loss	Temperature	382	2	191	104.0	0.000
	Concentration	165	2	82,5	44.8	0.000
	Temperature * Concentration	3.15	4	0.79	0.43	0.787

Table A2.6. Interactions between temperatures and concentrations on weight, glucose, xylose, and lignin for corn stalks.

Source		Type III Sum of Squares	df	Mean Square	F	P
Weight loss	Temperature	115	2	57.3	624	0.000
	Concentration	379	2	190	2066	0.000
	Temperature * Concentration	13.1	4	3.26	35.5	0.000
Glucose loss	Temperature	11.0	2	5.51	128	0.000
	Concentration	32.4	2	16.2	376	0.000
	Temperature * Concentration	0.28	4	0.07	1.64	0.207
Xylose loss	Temperature	300	2	150	219	0.000
	Concentration	1390	2	695	1013	0.000
	Temperature * Concentration	16.8	4	4.19	6.11	0.003
Lignin loss	Temperature	753	2	377	1153	0.000
	Concentration	419	2	210	642	0.000
	Temperature * Concentration	37.8	4	9.45	28.9	0.000

Table A2.7. Variation analysis results (ANOVA) of weight, glucose, xylose, and lignin loss for wheat straw alkali pre-extraction at different concentrations and temperatures.

Material loss	Variation Source	Sum of Squares	Degree of Freedom	Mean Square	F	Significance
Weight loss	Between Groups	680.853	8	85.107	508.381	0.000
	Within Groups	3.013	18	0.167		
	Total	683.867	26			
Glucose loss	Between Groups	459.901	8	57.488	418.633	0.000
	Within Groups	2.472	18	0.137		
	Total	462.372	26			
Xylose loss	Between Groups	1684.047	8	210.506	333.548	0.000
	Within Groups	11.360	18	0.631		
	Total	1695.407	26			
Lignin loss	Between Groups	550.339	8	68.792	37.312	0.000
	Within Groups	33.187	18	1.844		
	Total	583.525	26			

Table A2.8. Duncan test results of weight loss for wheat straw alkali pre-extraction at different concentrations and temperatures.

Methods	Subset For Alpha = 0.05							
	1	2	3	4	5	6	7	8
16.7% NaOH 50 °C	34.5							
16.7% NaOH 70 °C		40.0						
26.7% NaOH 50 °C			41.3					
33.3% NaOH 50 °C				42.8				
16.7% NaOH 90 °C					45.1			
26.7% NaOH 70 °C					45.3			
33.3% NaOH 70 °C						46.3		
26.7% NaOH 90 °C							49.7	
33.3% NaOH 90 °C								52.4

Table A2.9. Duncan test results of glucose loss for wheat straw alkali pre-extraction at different concentrations and temperatures.

Methods	Subset For Alpha = 0.05							
	1	2	3	4	5	6	7	8
16.7% NaOH 50 °C	2.41							
16.7% NaOH 70 °C		4.76						
26.7% NaOH 50 °C			7.49					
16.7% NaOH 90 °C			7.98	7.98				
33.3% NaOH 50 °C				8.22				
26.7% NaOH 70 °C					9.25			
33.3% NaOH 70 °C						11.2		
26.7% NaOH 90 °C							14.6	
33.3% NaOH 90 °C								16.2

Table A2.10. Duncan test results of xylose loss for wheat straw alkali pre-extraction at different concentrations and temperatures.

Methods	Subset For Alpha = 0.05								
	1	2	3	4	5	6	7	8	9
16.7% NaOH 50 °C	33.6								
16.7% NaOH 70 °C		40.3							
16.7% NaOH 90 °C			42.9						
26.7% NaOH 50 °C				49.3					
33.3% NaOH 50 °C					51.1				
26.7% NaOH 70 °C						52.5			
33.3% NaOH 70 °C							54.0		
26.7% NaOH 90 °C								57.1	
33.3% NaOH 90 °C									59.2

Table A2.11. Duncan test results of lignin loss for wheat straw alkali pre-extraction at different concentrations and temperatures.

Methods	Subset For Alpha = 0.05							
	1	2	3	4	5	6	7	8
16.7% NaOH 50 °C	49.9							
26.7% NaOH 50 °C		53.3						
33.3% NaOH 50 °C			54.5					
16.7% NaOH 70 °C				57.0				
16.7% NaOH 90 °C					59.1			
26.7% NaOH 70 °C						61.1		
26.7% NaOH 90 °C							62.5	
33.3% NaOH 70 °C							63.0	
33.3% NaOH 90 °C								64.3

Table A2.12. Variation analysis results (ANOVA) of weight, glucose, xylose, and lignin loss for corn stalks alkali pre-extraction at different concentrations and temperatures.

Material loss	Variation Source	Sum of Squares	Degree of Freedom	Mean Square	F	Significance
Weight loss	Between Groups	507.110	8	63.389	690.119	0.000
	Within Groups	1.653	18	0.092		
	Total	508.763	26			
Glucose loss	Between Groups	43.666	8	5.458	126.891	0.000
	Within Groups	0.774	18	0.043		
	Total	44.440	26			
Xylose loss	Between Groups	1707.219	8	213.402	311.116	0.000
	Within Groups	12.347	18	0.686		
	Total	1719.565	26			
Lignin loss	Between Groups	1210.087	8	151.261	463.043	0.000
	Within Groups	5.880	18	0.327		
	Total	1215.967	26			

Table A2.13. Duncan test results of weight loss for corn stalks alkali pre-extraction at different concentrations and temperatures.

Methods	Subset For Alpha = 0.05						
	1	2	3	4	5	6	7
16.7% NaOH 50 °C	28.7						
16.7% NaOH 70 °C		34.3					
16.7% NaOH 90 °C			35.4				
26.7% NaOH 50 °C				37.4			
33.3% NaOH 50 °C					39.5		
26.7% NaOH 70 °C					39.5		
26.7% NaOH 90 °C						41.6	
33.3% NaOH 70 °C						41.7	
33.3% NaOH 90 °C							43.5

Table A2.14. Duncan test results of glucose loss for corn stalks alkali pre-extraction at different concentrations and temperatures.

Methods	Subset For Alpha = 0.05					
	1	2	3	4	5	6
16.7% NaOH 50 °C	6.88					
26.7% NaOH 50 °C		7.60				
16.7% NaOH 70 °C		7.70				
16.7% NaOH 90 °C			8.31			
26.7% NaOH 70 °C			8.43			
26.7% NaOH 90 °C				9.16		
33.3% NaOH 50 °C				9.50		
33.3% NaOH 70 °C					10.0	
33.3% NaOH 90 °C						11.2

Table A2.15. Duncan test results of xylose loss for corn stalks alkali pre-extraction at different concentrations and temperatures.

Methods	Subset For Alpha = 0.05						
	1	2	3	4	5	6	7
16.7% NaOH 50 °C	25.6						
16.7% NaOH 70 °C		31.7					
16.7% NaOH 90 °C			34.9				
26.7% NaOH 50 °C			35.6				
26.7% NaOH 70 °C				42.2			
26.7% NaOH 90 °C					44.9		
33.3% NaOH 50 °C					45.2		
33.3% NaOH 70 °C						49.0	
33.3% NaOH 90 °C							50.5

Table A2.16. Duncan test results of lignin loss for corn stalks alkali pre-extraction at different concentrations and temperatures.

Methods	Subset For Alpha = 0.05							
	1	2	3	4	5	6	7	8
16.7% NaOH 50 °C	62.0							
16.7% NaOH 70 °C		70.6						
26.7% NaOH 50 °C			73.2					
33.3% NaOH 50 °C				74.2				
26.7% NaOH 70 °C					75.9			
33.3% NaOH 70 °C						78.1		
16.7% NaOH 90 °C						78.4		
26.7% NaOH 90 °C							83.7	
33.3% NaOH 90 °C								85.8

Table A2.17. Variation analysis results (ANOVA) of weight, glucose, xylose, and lignin loss for wheat straw modified alkali pre-extraction at different concentrations.

Material loss	Variation Source	Sum of Squares	Degree of Freedom	Mean Square	F	Significance
Weight loss	Between Groups	7.140	4	1.785	15.388	0.000
	Within Groups	1.160	10	0.116		
	Total	8.300	14			
Xylose loss	Between Groups	10.740	4	2.685	6.581	0.007
	Within Groups	4.080	10	0.408		
	Total	14.820	14			
Lignin loss	Between Groups	225.523	4	56.381	159.870	0.000
	Within Groups	3.527	10	0.353		
	Total	229.049	14			

Table A2.18. Duncan test results of weight, xylose, and lignin loss for wheat straw modified alkali pre-extraction at different concentrations.

$NaOH-NaBH_4$ Concentrations (%)	Subset For Alpha = 0.05										
	Weight loss				Xylose loss			Lignin loss			
	1	2	3	4	1	2	3	1	2	3	4
16.7	34.5				33.6			49.9			
16.7-0.1	34.8	34.8			33.9				56.3		
16.7-0.5		35.2	35.2		34.4	34.4				58.9	
16.7-1.0			35.5			35.2	35.2				60.1
16.7-2.0				36.5			35.9				60.3

Table A2.19. Variation analysis results (ANOVA) of weight, xylose, and lignin loss for corn stalks modified alkali pre-extraction at different concentrations.

Material loss	Variation Source	Sum of Squares	Degree of Freedom	Mean Square	F	Significance
Weight loss	Between Groups	90.624	4	22.656	69.497	0.000
	Within Groups	3.260	10	0.326		
	Total	93.884	14			
Xylose loss	Between Groups	124.884	4	31.221	124.884	0.000
	Within Groups	2.500	10	0.250		
	Total	127.384	14			
Lignin loss	Between Groups	99.263	4	24.816	45.395	0.000
	Within Groups	5.467	10	0.547		
	Total	104.729	14			

Table A2.20. Duncan test results of weight, xylose, and lignin loss for corn stalks modified alkali pre-extraction at different concentrations.

NaOH-NaBH$_4$ Concentrations (%)	Subset For Alpha = 0.05											
	Weight loss			Xylose loss					Lignin loss			
	1	2	3	1	2	3	4	5	1	2	3	4
16.7	37.4			35.6					73.2			
16.7-0.1		40.0			37.5				73.9			
16.7-0.5		41.0				39.9				75.5		
16.7-1.0			43.5				42.2				77.7	
16.7-2.0			44.2					43.4				80.2

Table A2.21. Variation analysis results (ANOVA) of tensile, tear, burst index, brightness and opacity for wheat straw pretreated with different methods.

Paper properties	Variation Source	Sum of Squares	Degree of Freedom	Mean Square	F	Significance
Tensile index	Between Groups	2943.694	7	420.528	233.367	0.000
	Within Groups	57.664	32	1.802		
	Total	3001.358	39			
Tear index	Between Groups	3.153	7	0.450	1.634	0.162
	Within Groups	8.822	32	0.276		
	Total	11.975	39			
Burst index	Between Groups	15.626	7	2.232	285.225	0.000
	Within Groups	.250	32	0.008		
	Total	15.876	39			
Brightness	Between Groups	179.350	7	25.621	131.392	0.000
	Within Groups	6.240	32	0.195		
	Total	185.590	39			
Opacity	Between Groups	391.608	7	55.944	137.624	0.000
	Within Groups	13.008	32	0.407		
	Total	404.616	39			

Table A2.22. Duncan test results of tensile index for wheat straw pretreated with different methods.

Pre-reatment and refining (min)	Subset For Alpha = 0.05					
	1	2	3	4	5	6
HW 135 °C, 0 min	40.4					
Control, 0 min		42.4				
+0.1% $NaBH_4$, 1 min			54.2			
16.7% NaOH, 1 min			55.7			
HW 135 °C, 2 min				58.0		
HW 135 °C, 4 min					60.6	
Control, 2 min						63.7
Control, 4 min						65.0

Table A2.23. Duncan test results of tear index for wheat straw pretreated with different methods.

Pre-treatment and refining (min)	Subset For Alpha = 0.05	
	1	2
Control, 4 min	6.68	
HW 135 °C, 4 min	6.96	6.96
+0.1% $NaBH_4$, 1 min	7.20	7.21
HW 135 °C, 0 min	7.23	7.23
Control, 0 min	7.35	7.35
Control, 2 min	7.41	7.41
HW 135 °C, 2 min	7.43	7.43
16.7% NaOH, 1 min		7.64

Table A2.24. Duncan test results of burst index for wheat straw pretreated with different methods.

Pre-reatment and refining (min)	Subset For Alpha = 0.05						
	1	2	3	4	5	6	7
HW 135 °C, 0 min	2.30						
Control, 0 min		2.44					
+0.1% $NaBH_4$, 1 min		2.54	2.54				
16.7% NaOH, 1 min			2.64				
HW 135 °C, 2 min				3.27			
HW 135 °C, 4 min					3.48		
Control, 2 min						3.82	
Control, 4 min							4.01

Table A2.25. Duncan test results of brightness for wheat straw pretreated with different methods.

Pre-reatment and refining (min)	Subset For Alpha = 0.05						
	1	2	3	4	5	6	7
16.7% NaOH, 1 min	30.7						
HW 135 °C, 4 min		31.4					
HW 135 °C, 2 min			32.1				
+0.1% $NaBH_4$, 1 min				33.4			
HW 135 °C, 0 min				33.8			
Control, 4 min					35.0		
Control, 2 min						35.7	
Control, 0 min							37.4

Table A2.26. Duncan test results of opacity for wheat straw pretreated with different methods.

Pre-reatment and refining (min)	Subset For Alpha = 0.05				
	1	2	3	4	5
16.7% NaOH, 1 min	89.2				
+0.1% $NaBH_4$, 1 min		90.1			
Control, 4 min			93.5		
Control, 2 min			93.7		
Control, 0 min			94.4		
HW 135 °C, 4 min				97.4	
HW 135 °C, 2 min				97.6	97.6
HW 135 °C, 0 min					98.5

Table A2.27. Variation analysis results (ANOVA) of tensile, tear, burst index, brightness and opacity for corn stalks pretreated with different methods.

Paper properties	Variation Source	Sum of Squares	Degree of Freedom	Mean Square	F	Significance
Tensile index	Between Groups	12104.190	7	1729.170	1127.413	0.000
	Within Groups	49.080	32	1.534		
	Total	12153.270	39			
Tear index	Between Groups	92.437	7	13.205	78.090	0.000
	Within Groups	5.411	32	0.169		
	Total	97.849	39			
Burst index	Between Groups	64.098	7	9.157	2925.492	0.000
	Within Groups	0.100	32	0.003		
	Total	64.198	39			
Brightness	Between Groups	136.252	7	19.465	64.963	0.000
	Within Groups	9.588	32	0.300		
	Total	145.840	39			
Opacity	Between Groups	633.798	7	90.543	188.630	0.000
	Within Groups	15.360	32	0.480		
	Total	649.158	39			

Table A2.28. Duncan test results of tensile index for corn stalks pretreated with different methods.

Pre-reatment and refining (min)	Subset For Alpha = 0.05					
	1	2	3	4	5	6
HW 120 °C, 0 min	46.0					
Control, 0 min		58.4				
HW 120 °C, 2 min			80.5			
HW 120 °C, 4 min			81.4			
+0.1% $NaBH_4$, 1 min			81.9			
26.7% NaOH, 1 min				93.1		
Control, 2 min					95.0	
Control, 4 min						100.0

Table A2. 29. Duncan test results of tear index for corn stalks pretreated with different methods.

Pre-treatment and refining (min)	Subset For Alpha = 0.05			
	1	2	3	4
Control, 4 min	7.41			
HW 120 °C, 4 min	7.76	7.84		
HW 120 °C, 2 min	7.84	7.88		
HW 120 °C, 0 min		8.11		
Control, 2 min		8.17		
Control, 0 min		8.25		
26.7% NaOH, 1 min			9.83	
+0.1% $NaBH_4$, 1 min				12.2

Table A2.30. Duncan test results of burst index for corn stalks pretreated with different methods.

Pre-reatment and refining (min)	Subset For Alpha = 0.05						
	1	2	3	4	5	6	7
HW 120 °C, 0 min	2.04						
Control, 0 min		2.73					
+0.1% $NaBH_4$, 1 min			3.80				
HW 120 °C, 2 min				4.77			
HW 120 °C, 4 min				4.84			
26.7% NaOH, 1 min					4.93		
Control, 2 min						5.67	
Control, 4 min							5.79

Table A2.31. Duncan test results of brightness for corn stalks pretreated with different methods.

Pre-reatment and refining (min)	Subset For Alpha = 0.05				
	1	2	3	4	5
+0.1% $NaBH_4$, 1 min	30.0				
HW 120 °C, 4 min	30.2				
26.7% NaOH, 1 min	30.6				
HW 120 °C, 2 min		31.8			
Control, 4 min			33.0		
Control, 2 min				33.8	
HW 120 °C, 0 min				33.9	
Control, 0 min					35.3

Table A2.32. Duncan test results of opacity for corn stalks pretreated with different methods.

Pre-reatment and refining (min)	Subset For Alpha = 0.05					
	1	2	3	4	5	6
Control, 4 min	80.6					
HW 120 °C, 4 min		84.2				
Control, 2 min		84.4				
+0.1% $NaBH_4$, 1 min			87.0			
26.7% NaOH, 1 min			87.4			
HW 120 °C, 2 min				88.8		
Control, 0 min					90.7	
HW 120 °C, 0 min						94.4

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Education

Degree	**University- Faculty/Institue**	**Graduation Year**
M.Sc.	Düzce University Graduate School of Natural and Applied Sciences Department of Forest Industry Engineering	2011
B.Sc.	İstanbul University Forestry Faculty	2007

Professional Experience

Date	**Employer**	**Posistion**
2010-	Düzce University	Graduate Research Assistant
2014-2015	The University of Maine	Visiting Graduate Researcher

Language

English (The Foreign Language Examination for Civil Servants in Turkey-2010: 62.5)

Publications

Articles in SCI, SSCI, AHCI index

1. Tozluoglu A., **Özyürek Ö.**, Çöpür Y., and Özdemir H., Integrated production of biofilm, bioethanol, and papermaking pulp from wheat straw. *Bioresources*, 10 (4) (**2015**) 7834-7853.
2. Çöpür Y., **Özyürek Ö.**, Tozluoglu A., Kütük S., Enzymatic digestibility of tomato, pepper, and eggplant stalks mixture, *Bioresources*, 7 (3) (**2012**) 3252-3261.
3. Copur Y., Tozluoglu A., **Özyürek Ö.**, Sodium borohydrate ($NaBH_4$) pretreatment for efficient enzymatic saccharification of wheat straw, *Bioresour. Technol.*, 107 (**2012**) 258-266.

Papers in National journals

1. Tozluoglu A., Çöpür Y., **Özyürek Ö.,** Çıtlak S., Nanoselüloz üretim teknolojisi, *Turkish Journal of Forestry*, 16 (2) (**2015**) 204-220.
2. Copur Y., Tozluoglu A., **Özyürek Ö.,** Selülozik biyoetanol üretim teknolojisi, Düzce University, *Journal of Forestry*, 7 (1) (**2011**) 10-37.

Conferences and Symposiums

1. **Omer Ozyurek** and Adriaan van Heiningen, "Formic acid reinforced autohydrolysis of wheat straw for high yield enzymatic sugar production and minimal lignin precipitation during pretreatment", 'Global Biotechnology Congress 2015', July 22nd-25th, (**2015**), Boston-USA.

2. Çöpür Y., **Özyürek Ö.**, Özdemir H., Tozluoğlu A., "Integrated forest product bio-refinery of wheat straw", *Biorefinery analytics – Outcomes from COST Action Final Meeting FP0901* Åbo/Turku, Finland, September 17th-18th, (**2013**).

3. Yalçın ÇÖPÜR, **Ömer ÖZYÜREK,** Ayhan Tozluoğlu, "Sebze saplarından biyoetanol üretiminde enzimatik etkinliğin araştırılması", *I. Ulusal Akdeniz Orman ve Çevre Sempozyumu* 26-28 Ekim 2011, Kahramanmaraş, (**2011**).

4. Çöpür Y., Tozluoglu A., **Özyürek Ö.**, Enzymatic digestibility of vegetable stalks, *6th Italian meeting on lignocellulosic chemistry Science & Technology of Biomasses: Advances and Challenges,* Viterbo-Italy, (**2011**).

5. Çöpür Y., Tozluoglu A., **Özyürek Ö.**, Bioethanol production via enzymatic hydrolysis of lignocellulosic biomass: hazelnut husk, corn stalks and wheat straw, *The Fourth Annual Workshop of Cost FP0602 Biotechnical Processing of Lignocellulosic Raw Materials*, Izmir-Turkey, (**2010**).

International Education Programs

1. **Ozyurek, O.,** Fermentation Short Course July 04-08 2012 at Bilkent University UNAM, Institue of Materials Science and Nanotechnology, Turkey (**2012**).
2. **Ozyurek, O.,** PhD Course on industrial biotechnology for lignocellulose based processed, October, 16-21 2011 at Chalmers University of Technology, Sweden (**2011**).

Projects

1. Producing biodegradable film, bioethanol and pulp with integrated forest product biorefinery of pre-extraction of hemicelluloses from crop stalks, TUBITAK 111O502, Yalçın ÇÖPÜR (Project Leader), Hasan ÖZDEMİR (Researcher), **Ömer ÖZYÜREK (Scholarship Holder) 2012-2015**.

2. Bioethanol production via enzymatic hydrolysis of lignocellulosic biomass: wheat straw, corn stalks, and hazelnut husks, TUBITAK 109O200, Melek ÖZKAN (Researcher), Ayhan TOZLUOĞLU, (Scholarship Holder), **Ömer ÖZYÜREK (Scholarship Holder)**, Selva KÜTÜK (Scholarship Holder), **2009-2012**.

3. Sebze saplarının biyoyakıt üretiminde kullanım olanakları, BAP Projesi, Yalçın ÇÖPÜR (Project Leader), Mehmet AKGÜL (Researcher), **Ömer ÖZYÜREK (Researcher), 2012-2014**.

Awards

3rd of Faculty- İstanbul University, TURKEY

Encouragement Award- TUBITAK, ULAKBIM, TURKEY (3 times)

TUBITAK 2214-A-Doctoral Research Fellowship, The University of Maine, USA, (**08/09/2014-08/09/2015**)