UNIVERSITI
MALAYSIA
KELANTAN

EXTRACTION OF CELLULOSE FIBER FROM BANANA PSEUDO-STEM USING CHEMICAL PRETREATMENT AND ULTRASONIC

GUAN CHOI YEE
F15A0046

A thesis submitted in fulfillment of the requirements for the degree of Bachelor of Applied Science (Bioindustrial Technology) with Honours

FACULTY OF BIOENGINEERING AND TECHNOLOGY
UNIVERSITI MALAYSIA KELANTAN

2019

DECLARATION

I declare that this thesis entitled "Extraction of Cellulose Fiber from Banana Pseudo-Stem Using Chemical Pretreatment and Ultrasonic" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : _____

Name : _____

Date : _____

Verified by,

_____

Signature of Supervisor

Name of Supervisor : Dr. Siti Roshayu Binti Hassan

Cop :

Date :

ACKNOWLEDGEMENT

First of all, I would like to send my biggest gratitude to Faculty of Bioengineering and Technology, University Malaysia Kelantan Jeli Campus which giving me this wonderful golden opportunity to learn and utilize the laboratory facilities and instruments in order to complete my research successfully.

Besides, I would like to express my appreciation and gratitude to my supervisor, Dr. Siti Roshayu binti Hassan for all kinds of support and guidance. She had scarified her precious time to guide me for my research. I would like to thanks her for provides me useful opinions and suggestions throughout the research.

Moreover, I would like to dedicate these sincere thanks to lab technician in UMK Jeli Campus for their cooperation and assistance by helping me for samples analysis.

Last but not least, I would like to expand my special thanks to all of my lectures and friends for giving all support and motivation when I am in the process to complete my research and laboratory work.

TABLE OF CONTENTS

CONTENTS	PAGE
DECLARATION	ii
ACKNOWLEDGEMENT	iii
TABLE OF CONTENTS	xi
LIST OF TABLES	xiiii
LIST OF FIGURES	ivi
LIST OF SYMBOLS	ix
LIST OF ABBREVIATIONS	viii
ABSTRACT	ixi
ABSTRAK	ixi
CHAPTER 1 INTRODUCTION	
1.1 Background of study	1
1.2 Problem statement	4
1.3 Objectives	4
1.4 Scope of study	5
1.5 Significance of study	5
CHAPTER 2 LITERATURE REVIEW	
2.1 Cellulose	6
2.2 Agricultural waste fiber	7
2.3 Extraction method on cellulose nanofiber	10
2.3.1 Conventional method	10
(a) Chemical method	10
(b) Mechanical method	11
(c) Enzymatic method	13
2.3.2 Chemomechanical method	14
2.3.4 Extraction of cellulose by ultrasonic technique	15
2.4 Characterization of cellulose	17
2.4.1 Functional group determination	17

2.4.2 Morphology analysis 18
2.4.3 Crystallinity 19
2.4.4 Thermal stability 20
CHAPTER 3 MATERIALS AND METHODS
3.1 Materials 21
3.1.1 Reagents 21
3.2 Methods 21
3.2.1 Raw material preparation 21
3.2.2 Chemical pretreatment 22
3.2.3 Ultrasound technique 22
3.2.4 Yield of cellulose nanofiber 23
3.3 Sample preparation for characterization 23
3.3.1 Functional group analysis 23
3.3.2 Morphology analysis 23
3.3.3 Crystallinity Determination 24
3.3.4 Thermal analysis 24
CHAPTER 4 RESULTS AND DISCUSSION
4.1 Physical appearance of cellulose 26
4.2 Chemical composition of banana pseudo-stem 27
4.3 Yield of extraction of cellulose fiber from banana pseudo stem by ultrasonic 28
4.4 Characterization of cellulose fiber from banana pseudo-stem 30
4.4.1 Functional group analysis of untreated, chemical treated and chemical treated followed by ultrasonic cellulose 30
4.5.2 Morphology of cellulose fiber 32
4.5.3 X-Ray Diffraction 37
4.5.4 Thermal stability analysis 40
CHAPTER 5 CONCLUSION AND RECOMMENDATIONS
5.1 Conclusion 44
5.2 Recommendations 45
REFERENCES 47

APPENDIX

A The yield of chemical treated cellulose extraction by ultrasonic 53

B The average diameter of cellulose fiber 54

C The crystallinity index of cellulsoe 55

D Raw materials preparation 55

E Apparatus used 56

F Chemical treated and ultrasonic of cellulose 57

LIST OF TABLES

	PAGE
2.1 Dimension of agricultural waste fiber.	8
2.2 Chemical composition of agricultural waste.	8
2.3 Physical and mechanical properties of agricultural waste.	9
4.1 The average chemical composition of banana pseudo-stem	28
4.2 The average yield of extraction of cellulose fiber from banana pseudo-stem by ultrasonic.	29
4.3 The vibration bands of components corresponding to lignin, hemicellulose and cellulose	31
4.4 Average diameter of cellulose.	35
4.5 Crystallinity and amorphous of cellulose with different parameter.	39
4.6 Thermal stability of untreated and treated cellulose.	41

LIST OF FIGURES

	PAGE
2.1 Chemical structure of cellulose	6
2.4 Ultrasonic principle	16
3.1 Research flow chart	25
4.1 The banana pseudo stem changes colour from dark brown to light brown as a result of alkali treatment.	26
4.2 The colour transition from brown and gradual discoloration to white due to bleaching treatment.	27
4.3 FTIR spectra of untreated, chemical treated, chemical treated followed by ultrasonic of cellulose fiber.	31
4.4 SEM image of (a) chemical treated at 200x magnification, (b) chemical treated followed by ultrasonic with 30 minute and amplitude 25 % at 200x magnification, (c) chemical treated followed by ultrasonic with 30 minute and amplitude 75 % at 300x magnification, (d) chemical treated followed by ultrasonic with 30 minute and amplitude 75 % at 2400x magnification, (e) chemical treated followed by ultrasonic with 30 minute and amplitude 75 % at 5000x magnification, (f) chemical treated followed by ultrasonic with 45 minute and amplitude 50 % at 1000x magnification.	34
4.5 SEM image of diameter of (a) chemical treated cellulose at 200x magnification, (b) chemical treated followed by ultrasonic with 15 minute and amplitude 75 % at 200x magnification, (c) chemical treated followed by ultrasonic with 30 minute and amplitude 25 % at 200x magnification, (d) chemical treated and ultrasonic with 30 minute and amplitude 75 % at 10,000x magnification.	36
4.6 SEM image of (a) cellulose after ball milling at 800x magnification, (b) cellulose after ball milling at 120,000x magnification.	37
4.7 XRD diffraction pattern of cellulose from banana pseudo stem.	38
4.8 TGA curve of untreated, chemical treated and chemical treated with ultrasonic banana pseudo stem.	41

LIST OF SYMBOLS

%	Percentage
°	Degrees
θ	Delta
°C	Degree Celsius
β	Beta

LIST OF ABBREVIATIONS

AFM	Atomic force microscopy
C	Carbon
cm	Centimeter
CrI	Crystallinity index
FTIR	Fourier transform infrared spectroscopy
g	Gram
GPa	Gigapascal pressure unit
H	Hydrogen
HPH	High pressure homogenizer
kHz	Kilohertz
mg	Milligram
min	Minute
ml	Milliliter
mL/min	Milliliter per minute
mm	Millimeter
MPa	Megapascal pressure unit
nm	Nanometer
O	Oxygen
OH	Hydroxyl group
psi	Pound-force per square inch
rpm	Revolutions per minute
SEM	Scanning electron microscope
TEM	Transmission electron microscope
TGA	Thermalgravimetric analysis
v/v	Volume per volume
W	Watt
XRD	X-ray Diffraction
µm	Micrometer

Extraction of cellulose fiber from banana pseudo-stem using chemical pretreatment and ultrasonic

ABSTRACT

In this research, cellulose is extracted from banana pseudo stem by using chemical pretreatment followed by ultrasonic. Isolation of pure cellulose is difficult and different ultrasonic amplitudes and time affect the yield and properties of cellulose. The objectives of this study are to isolate cellulose from banana pseudo-stem by chemomechanical method and to determine the effect of ultrasonic amplitude and time on the yield of cellulose. The chemical used are sodium chlorite used as bleaching treatment to remove the lignin while potassium hydroxide acts as alkaline treatment to remove the hemicellulose and pectin. The chemically treated cellulose is proceed to ultrasonic for different ultrasonic time (15 minutes, 30 minutes and 45 minutes) and different amplitude (25 %, 50 % and 75 %). The cellulose composition of banana pseudo stem is 31.6 %. Chemical treated followed by ultrasonic cellulose has better properties than chemical treated cellulose. Thus, chemomechanical method is more effective in extracting the cellulose from banana pseudo stem. Ultrasonic time with 30 minutes and 25 % amplitude showed the best yield of cellulose (92.59 %). The morphology of cellulose are analyzed by SEM. The diameter of chemical treated cellulose is 15.690 µm. However, the chemical treated follow by ultrasonic with 30 minute and 75 % amplitude of cellulose show reduction in diameter with 1.418 µm. The ultrasonic time for 15 minutes and 75 % amplitude showed the highest crystallinity index (58.8 %). This is due to high amplitude can remove the amorphous region. The highest thermal stability of cellulose is obtained from ultrasonic with 30 minutes and 25 % amplitude showed that cellulose start to decompose at 280 °C. Result show that the removal of lignin, hemicellulose and pectin are confirmed by FTIR. Different properties of the cellulose can be applied in different application. Results from this work may be potentially applied in textile, paper, bioethanol, cellulose acetate, food packaging and thermoplastic polymer.

Keywords: Cellulose, Banana pseudo-stem, Chemical pretreatment, Ultrasonic, Characterization

Pengekstrakan serat selulosa dari pseudo-batang pisang dengan menggunakan pretreatment kimia dan ultrasonik

ABSTRAK

Dalam kajian ini, selulosa diekstrak daripada batang pseudo pisang dengan menggunakan pretreatment kimia diikuti oleh ultrasonik. Pengekstrakan selulosa amat sukar dan amplitud dan masa ultrasonik yang berbeza menjejaskan hasil dan sifat selulosa. Objektifnya ialah untuk mengekstrakan selulosa daripada batang pseudo pisang dengan chemomechanical dan menentukan kesan amplitud dan masa ultrasonik ke atas hasil selulosa. Natrium klorit digunakan untuk menghilangkan lignin manakala kalium hidroksida bertindak sebagai rawatan alkali untuk menghilangkan hemiselulosa dan pektin. Selulosa yang dirawat secara kimia diteruskan ke ultrasonik untuk masa ultrasonik yang berbeza (15 minit, 30 minit dan 45 minit) dan amplitud yang berbeza (25 %, 50 % dan 75 %). Objektifnya adalah untuk menentukan kesan amplitud ultrasonik dan masa ke atas hasil selulosa dan mencirikan sifat-sifat selulosa. Penyingkiran lignin, hemiselulosa dan pektin disahkan oleh FTIR dan SEM. Komposisi selulosa pokok pseudo pisang adalah 31.6%. Selulosa yang dirawat oleh kimia dan ultrasonik mempunyai sifat yang lebih baik daripada selulosa yang dirawat kimia. Oleh itu, kaedah chemomechanical lebih berkesan dalam mengekstrak selulosa daripada batang pseudo pisang. Masa ultrasonik dengan 30 minit dan amplitud 25 % menunjukkan hasil terbaik selulosa (92.59 %). Permukaan selulosa dianalisis oleh SEM. Diameter selulosa yang dirawat oleh kimia adalah 15.690 μm. Walau bagaimanapun, pengurangan diameter (1.418 μm) diwujudkan dalam selulosa yang dirawat oleh kimia diikuti ultrasonik dengan 15 minit dan 75% amplitud adalah. Masa ultrasonik selama 30 minit dan 75 % amplitud menunjukkan kristalografi tertinggi (58.8 %). Ini disebabkan oleh amplitud tinggi boleh mengeluarkan rantau amorf. Kestabilan haba selulosa tertinggi diperolehi daripada ultrasonik dengan 30 minit dan amplitud 25 % menunjukkan bahawa selulosa mula terurai pada 280 °C. Ciri-ciri selulosa yang berbeza boleh digunakan dalam aplikasi yang berbeza. Hasil daripada kerja ini mungkin berpotensi digunakan dalam tekstil, kertas, bioethanol, selulosa asetat, pembungkusan makanan dan polimer termoplastik.

Kata kunci: Selulosa, Pseudo-batang pisang, Prapreatment kimia, Ultrasonik, Pencirian

CHAPTER 1

INTRODUCTION

1.1 Background of study

Cellulose is a major structural polysaccharide in cell wall of plant. Cellulose is fibrous in structure and water-insoluble polysaccharide (Brigham, 2017). Cellulose exist as a crystalline form with intramolecular hydrogen bonds to provide stable, hydrophobic polymer with high tensile strength of megapascal pressure unit (MPa). Cellulose fiber has high thermochemical properties, high mechanical properties and high tensile strength of MPa. (Li *et al*., 2014).

Isolation of highly pure cellulose has been studied for many year due to the presence of lignin, hemicellulose, pectin and ash in cell wall. Different extraction methods and chemical composition of plant determine the successful of cellulose extraction. The conventional method usually involved chemical, mechanical and enzymatic hydrolysis. The conventional methods have limitation on extracting cellulose. However, the chemical method is not eco-friendly and high concentration of chemical cause degradation of cellulose (Zheng, 2014) while mechanical method required high

energy, expensive and high pressure homogenizer (HPH) causes clogging of homogenizer, damage the crystalline microfibril structure and decreases crystallinity of cellulose fiber. Mechanical method is insufficient to remove the lignin, hemicellulose, pectin and other non-cellulosic materials. HPH alone is insufficient to reduce the cellulose into nano-size. Enzymatic hydrolysis is expensive and required long time (Kalia *et al*., 2014). Lastly, modification of chemical method by sodium hydroxide pretreatment, oxygen delignification, sodium chlorite delignification and peracetic acid delignification produce high yield of cellulose nanofiber but a lot of chemical required.

Based on above mentioned methods, the researchers have developed the chemomechanical method which can decreases energy consumption, increase the defibrillation process, reduce the size of fiber and produce highly purified cellulose (Abdul Khalil *et al*., 2016). Chemomechanical method is using chemical as pretreatment followed by mechanical methods such as ultrasonic, microwave-assisted extraction and high pressure homogenizer. This method can decrease the high energy consumption and ease to scale up.

Cellulose can be isolated from algal, bacteria and plant. In plant cell wall, approximately 26 individual cellulose chain connect together through hydrogen bonds. The source of cellulose from plant are vegetable, wood and agriculture waste. Extraction of cellulose from agricultural waste has been highlighted due to its high surface area, good mechanical properties, biodegradability, inexpensive and non-toxic. Disposal of agricultural waste causes environmental problem. Agricultural waste such as corn stalk, rice straw, banana, coconut, sugarcane and pineapple consists of natural fiber (Dungani *et al*., 2016). Agricultural waste is a good source of fiber to produce value-added product such as fuel, paper, broad, textile (Albinante *et al*., 2014). However, banana has a great potential source of fiber because it has high cellulose, longer fiber length and mostly

abundant available in Malaysia. Banana fiber can produce textiles, paper, paper bags, board and rope (Zaida Ortega *et al*., 2016).

According to Food and Agriculture Organization Corporate Statistical Database (FOASTAT), banana is widely grow in Malaysia, about 309,508 tons in 2016. However, out of the weight of banana plant, banana fruit only represents about 12 % (Reddy and Yang, 2015). The banana stem are left over after the banana are harvesting because the banana plants cannot be used for next harvest. Historically, the banana stem waste acts as animal feed, left at soil that causes environmental pollution or managed by decomposition, burning, sanitary landfill and incineration. The banana stem, leaves, peels and peduncle are considerately agriculture waste. Bhathagar *et al*. (2015) studied that banana fiber contains 60-65% of cellulose, 6-19% of hemicellulose, 5-10% lignin. Cellulose in banana stem and peduncle has higher cellulose content than banana leaves and peels. Banana pseudo stem have higher specific strength modulus leaf and rachis fiber. (Bhatnagar *et al*., 2015).

Banana fiber can be used in wide range of application. Banana fiber is used to make ropes and cordage due to its resistance to sea water and buoyancy. In textile industries, banana fiber is used to produce fabrics, clothes, dress. In Japan, for example, banana fiber is being used for producing traditional dress like kimono due to its lightweight and comfortable to wear (Vigneswaran *et al*.,2015). Banana cellulose can be used to make paper, board, bioethanol, cellulose derivative such as cellulose acetate, methyl cellulose and ethyl cellulose.

1.2 Problem statement

Isolation of pure cellulose is difficult due to the presence of lignin, hemicellulose and pectin in cell wall. Different extraction methods affect the yield, morphology and properties of cellulose fiber. Conventional methods such as chemical, mechanical and enzymatic have limitation on extracting cellulose. Chemical method is not eco-friendly while mechanical methods require high energy, expensive and insufficient to remove lignin, hemicellulose and pectin. Enzymatic methods required long time and do not fully remove the lignin and hemicellulose.

However, chemomechanical method which is the combination of chemical and mechanical method helps in removal the amorphous region in plant to obtain pure cellulose. The parameter of ultrasonic like amplitude and time influence the yield and properties of cellulose. Different properties of the cellulose can be applied in wide range of application. There is no systematic study on the effect of ultrasonic on extracting chemically treated cellulose from banana pseudo-stem. Therefore, the different parameters of ultrasonic amplitude and time were conducted to determine the yield, morphology and properties of cellulose fibers from banana pseudo stem.

1.3 Objectives

1. To extract cellulose fiber from banana pseudo-stem using combination of chemical pretreatment and ultrasonic.
2. To determine the effect of ultrasonic amplitude and time on the yield of cellulose
3. To characterize the physical, chemical and thermal properties of chemical treated and chemical treated followed by ultrasonic of cellulose fiber.

1.4 Scope of study

The scope of study focus on the extraction of cellulose from banana pseudo stem by chemical pretreatment followed by ultrasonic. The effect of ultrasonic amplitude and time on yield of cellulose was investigated. The characterization of cellulose was done using FTIR, SEM, XRD and TGA. The function of FTIR is to study the chemical structure in the banana pseudo stem and cellulose. SEM aims to identify the morphology of cellulose and access any fiber damage. XRD is to determine the crystallinity of cellulose. TGA is to study the thermal stability of cellulose.

1.5 Significance of study

Utilization of banana waste is a way to sustain the natural resources. Cellulose fiber is extracted from chemical method followed by ultrasonic with different ultrasonic amplitude and time. The properties of cellulose from banana pseudo-stem is characterized to enhance the specific properties needed for different application. Chemomechanical is more cost effective.

CHAPTER 2

LITERATURE REVIEW

2.1 Cellulose

Cellulose is a structural component of the primary cell wall of plants. Cellulose is a glucose polymer linked by β-1,4 linkage allows the cellulose in linear configuration (Singanusong *et al*., 2014). The formula of cellulose is $C_6H_{10}O_5$ with repeating units contain hydroxyl groups. The OH groups form intermolecular and intra-molecular hydrogen bonding make the cellulose hydrophilic in nature (Ramamoorthy *et al*.,2015).

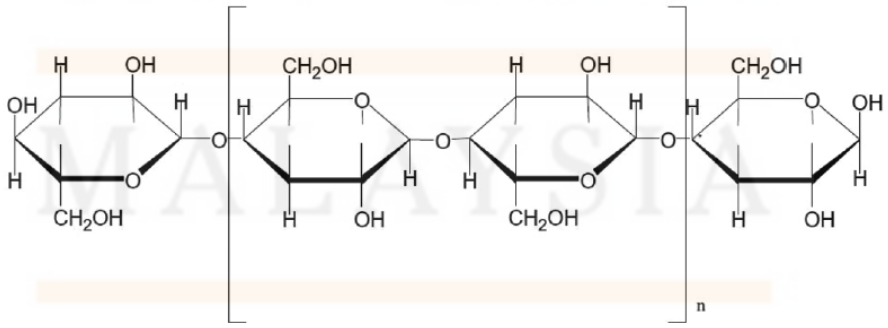


Figure 2.1: Chemical structure of cellulose

The source of cellulsoe are algal, tunicates, bacterial cellulose such as *Acetobacter, Sarcina, Agrobacterium, Pseudomonas, Rhizobium* and plant cell walls (Jonoobi *et al*., 2015). Approximately 36 individual cellulose molecular chain connect together through hydrogen bonds in plant cell wall (Sofla *et al*., 2016).

Cellulose have gained interest due to biodegradable, low cost, high thermochemical properties, high mechanical properties and ability to form highly porous mesh. (Li *et al*., 2014). Cellulose have potential in paper production, biomedical, packaging material, nanocomposites, gas barrier films, and optically transparent materials.

2.2 Agricultural waste fiber

In the modern agricultural sector, there are plenty of waste generated that become threat to the environment. In 2016, cellulose represent about 1.5×10^{12} tons of total annual waste production (Khawas & Deka, 2016). Disposal of agricultural waste causes environmental problem. Agricultural waste problem must be resolved to conserve environment (Ogah & James, 2018). Agro waste consists of cellulose fiber that is a good alternative material to produce value-added product such as biodegradable film, writing papers, cement board. Use of agricultural waste provide sustainable, low cost materials and contribute to waste management.

Different type of plant and fiber extraction method affect the fiber quality in term of length of fiber, diameter of fiber, yield, crystallinity and thermal stability. Different agricultural waste fiber have different fiber length, width fiber, fibril angle that stated in Table 2.1. The table shows the fiber length of banana is longer than fiber length of oil palm, coconut, corn stalks and rice straw. Fiber length is important in fiber based composed because it indicate the strength properties (Dungani *et al*., 2016). Long fiber

has better mechanical properties and high degree of polymerization. The aspect ratio (fibril length to diameter ration) affect the mechanical strength of fiber. Smaller fibril angle provide high strength while large angle is responsible for higher ductility.

Table 2.1: Dimension of agricultural waste fiber.

Types of fiber	Fiber length (mm)	Width fiber (μm)	Fibril angle (degrees)	References
Oil palm	0.33-50.31	8.30-20.50	40-46	Abdul Khalil *et al.* (2008)
Sugarcane	1.22-1.59	19.35-20.96	10-40	Hemmasi *et al.* (2011) and Driemeier *et al.* (2012)
Banana	0.90-4.00	80.00-250.00	9-13	Alwani *et al.* (2015)
Coconut (coir)	0.30-1.00	92.00-314.00	39-49	Alwani *et al.* (2015) and Setyanto *et al.* (2013)
Corn stalks	0.50-1.50	10.00-20.00	33-39	Nguong *et al.* (2013)
Pineapple	3.00-9.00	20.00-80.00	10-15	Alwani *et al.* (2015)
Rice straw	0.40-3.40	4.00-16.00	31-35	Venkateshwaran *et al.* (2012)

(Source: Dungani *et al*., 2016)

Plant consists of cellulose, hemicellulose and lignin with different composition (Neto *et al*., 2013). These constituents are scattered through out primary and secondary cell wall. The main chemical composition in plant is cellulose which has higher than hemicellulose and lignin. Table 2.2 show the chemical composition of different agricultural waste. The table shows the cellulose composition in banana plant is higher than oil palm, sugarcane, coconut, corn stalks and rice straw. Among the agricultural wastes, banana has the potential source of cellulose fiber.

Table 2.2: Chemical composition of agricultural waste.

Types of fiber	Cellulose (%)	Hemicellulose (%)	Lignin (%)	References
Oil palm	44.20-49.60	18.30-33.54	17.30-26.51	Abdul-Khalil *et al.* (2007) and Lu *et al.* (2006)
Sugarcane	55.60-57.40	23.90-24.50	24.35-26.30	Wahlang *et al.* (2012) and Hemmasi *et al.* (2011)
Banana	60.25-65.21	48.20-59.2	5.55-10.35	Preethi and Murthy (2013)
Coconut (coir)	36.62-43.21	0.15-0.25	41.23-45.33	Satyanarayana *et al.* (2009)
Corn stalks	38.33-40.31	25.21-32.22	7.32-21.45	Reddy and Yang (2005)
Pineapple	70.55-82.31	18.73-21.90	5.35-12.33	Pardo *et al.* (2014)
Rice straw	28.42-48.33	23.22-28.45	12.65-16.72	Reddy and Yang (2005)

(Source: Dungani *et al*., 2016)

The better orientation of crystalline of cellulose causes better mechanical properties such as tensile strength of MPa and Young's modulus (Dungani *et al*., 2016). Higher degree of polymerization, longer fiber length, fiber with higher cellulose content pose better tensile strength of MPa and Young's modulus and. The physical and mechanical properties of different agricultural wastes are listed in Table 2.3.

Table 2.3: Physical and mechanical properties of agricultural waste.

Types of fiber	Density ($g\ m^{-3}$)	Tensile strength (MPa)	Young's modulus (GPa)	Elongation at break (%)	References
Oil Palm	0.7-1.55	227.5-278.4	2.7-3.2	2.13-5.00	Abdul Khalil *et al.* (2008) and Hemmasi *et al.* (2011)
Sugarcane	0.31-1.25	257.3-290.5	15-18	6.20-8.2	Driemeier *et al.* (2012)
Banana	0.65-1.36	51.6-55.2	3.00-3.78	1.21-3.55	Alwani *et al.* (2015), Sumaila *et al.* (2013) and Sakthive and Ramesh (2013)
Coconut (coir)	0.67-1.15	173.5-175.0	4.0-6.0	27.21-32.32	Alwani *et al.* (2015) and Sakthive and Ramesh (2013)
Corn stalks	0.21-0.38	33.40-34.80	4.10-4.50	1.90-2.30	Rodriguez *et al.* (2010)
Pineapple	1.25-1.60	166-175	5.51-6.76	2.78-3.34	Alwani *et al.* (2015)
Rice straw	0.86-0.87	435-450	24.67-26.33	2.11-2.25	Bouasker *et al.* (2014) and Reddy and Yang (2006)

(Source: Dungani *et al*., 2016)

Bhathagar *et al*. (2015), have studied the chemical composition of banana fiber. The pseudo-stem and peduncle are the major source of banana fiber. The cellulose in banana pseudo-stem is 59.22 % and the cellulose in peduncle is 60.41 % (Preethi and Balakrishna, 2013). Banana pseudo stem have higher specific strength modulus than leaf and rachis fiber. Banana fiber used as raw materials in industry for production of paper, tea bags, paper bag, board and rope. Banana fibers are used as natural sorbent, bioremediation agent for bacteria in water purifier. Banana fiber are cheap, renewable and environment friendly. Compared to conventional fiber like glass fiber, banana fiber has good specific strength properties, light weight, strong moisture adsorption, fire resistance quality, smaller elongation and biodegradability.

2.3 Extraction method on cellulose nanofiber

2.3.1 Conventional method

Extraction methods and chemical composition that vary from plant to plant affect the successful of extraction of cellulose and properties of cellulose. The conventional methods to extract cellulose usually involved are chemical, mechanical and enzymatic method.

(a) Chemical method

In the chemical method, alkaline hydrolysis, bleaching and ionic liquid are used to extract cellulose fiber (Rojas *et al*. 2015). Acidified sodium chlorite delignify wood materials for isolating cellulose. Alkali extraction to dissolve hemicellulose before or after delignification is a common method. Delignification significantly result high purity of cellulose. Sodium hydroxide is used to remove hemicellulose. To further purify the cellulose, acidified sodium chlorite and potassium hydroxide are used to treat cellulose fiber. Ionic liquids break the intermolecular hydrogen bonds and dissolve the cellulose.

The yield of cellulose obtained from acid hydrolysis is lower than 50 % (Li *et al*., 2014). Isroi *et al*., (2017) stated that purification of cellulose increase from 36.67 % to 97 % in oil plam empty fruit bunch by sodium hydroxide methods and bleached using sodium hypochlorite. The cellulose from oil palm are used as fuel. Kopania *et al*., (2012) studied that hemp straw has 85.5 % cellulose by using sodium hydroxide pretreatment, oxygen delignification, sodium chlorite delignification and peracetic acid delignification.

Reddy *et al*., (2016) studied the pretreated and untreated cellulose from ficus leaf fibers by chemical method including 5 % sodium hydroxide, dilute acetic acid, toluene-

ethanol, 0.7 % sodium chlorite and 2 % sodium bisulphite. The yield of cellulose from chemical treated ficus leaf is 55 %.

Advantage of chemical method is required less energy and low cost compared to mechanical method. The crystallinity of chemically cellulose of banana pseudo-stem is 61.2 % which is more crystalline than mechanically (56.6 %) and enzymatic extracted cellulose (60.2 %) (Xu *et al*., 2015). This is because the removal of amorphous region by chemical. The higher the crystallinity of cellulose indicate the higher thermal stability and better mechanical properties. The chemically extracted cellulose is softer than mechanically and enzyme-extracted fiber due to the lignin and pectin are remove effectively than mechanical and enzymatic method (Xu *et al*., 2015).

The disadvantage of chemical method is high concentration of chemical cause the degradation of cellulose (Zheng, 2014). The yield of cellulose is low. Chemical is not eco-friendly as improper disposal of chemical cause environmental problem.

(b) Mechanical methods

Mechanical methods including grinding, high pressure homogenization (HPH), microfluidization, steam explosion, cryocrushing and high intensity ultrasonication are used to extract cellulose (Jonoobi *et al*., 2015). Extraction by mechanical is environmental friendly that reduce chemical use and chemical hazard discharge into environment.

By mechanical method, the cellulose yield from wheat straw is more than 50 % but the crystallinity of cellulose were damage. The cellulose content from wheat straw increased from 44.81 % to 94.23 % by combined microfludization, steam explosion and microwave-assisted hydrolysis (Liu *et al*., 2017). Li *et al*. (2014), isolate cellulose from depectinated sugar beet pulp by high pressure homogenizer (HPH). The mechanism of the HPH involves bombardment of fluid steam within a reaction chamber (Saelee *et al*.,

2016). Rapid change in pressure causes high pressure, high velocity, high shear, turbulence and cavitation. The homogenized sample generate shear rates, reducing size of cellulose fiber.

Saelee *et al*. (2016) isolated cellulose from sugarcane baggasses by xylanase-assisted pretreatment, steam explosion and HPH. The xylanase-assisted pretreatment dry cellulose fiber is homogenized at 15,000 psi for 30 passes. The diameter of cellulose have the range from 5 to 10 nm. Sugarcane fiber can be used to make cement board. HPH causes thermal stability decrease and the crystallinity damage.

Xu et al., (2015) isolated the cellulose fiber from banana pseudo stem by fiber-extracting machine called mechanical decorticator. The result showed that non-cellulosic components were present on the surface of fiber and many short fibers were observed due to the fibers were damaged by machine. The tensile strength of mechanically extracted cellulose is lower (210 MPa) than chemically extracted (333 MPa). This is because complete removal of lignin and hemicellulose by chemical.

Advantage of mechanical method is can extract the cellulose from cell wall without degrading cellulose severely. Hydrogen bonding between the cellulose are broken. High intensity ultrasonic and microfluidization cause cleavage along the longitudinal axis of cellulose by high shear gradients. Thus, by reducing the degree of crystallinity and molar mass, the microfibril structure is damaged (Rojas *et al*., 2015).

However, the disadvantages of mechanical method are high cost and required high energy. Mechanical method is insufficient to remove the amorphous region resulting less pure cellulose. HPH causes clogging of homogenizer, damage the crystalline microfibril structure. The increasing of number of passes in HPH cause the crystallinity decreases.

(c) Enzymatic method

Enzyme such as ligninases, xylanases are used to degrade the lignin and hemicellulose but not cellulose. Cellulase including endoglucanase, exoglucanase, bglucosidases (bGLs) that hydrolyse 1-4 bonds to produce cellulose. Endoglucanase shows highest reduction rate with increase in dose (Karim *et al*., 2017). Cellobiohydrolases produce enzyme that attack the crystalline portion of cellulose and attack the disordered structure of cellulose (Anderson *et al*., 2014). However, enzyme hydrolysis that converts cellulose to cellulose nanofiber is complex due to complexity of cell wall (Karim *et al*., 2017). The hydrolysis rate depends on the crystallinity of cellulose. Higher crystallinity of cellulose shows low rate of hydrolysis.

Tibolla *et al*., (2014) isolated cellulose nanofiber from banana peel by using xylanses. Xylanases can modify the amorphous components in the plant fiber and initiate the hydrolysis of β-1,4 non reducing terminal regions in the glycosidic linkages (Tibolla *et al*., 2014). However, the results showed xylanase did not solubilize the hemicellulose and penetrate the cellulose chain result in cellulose hydrolysis difficulty. The TEM image showed the cellulose is less pure as the non-cellulosic components are presents. Since xylanase has specific action, it works mainly on removing xylan fraction. Thus, enzyme treatment did not fully remove the hemicellulose.

Enzyme is specific and effective. Only low concentration of enzyme is required to use for degrading lignin and hemicellulose. The molecular weight and fiber length of cellulose are preserved. *A.xylinum* and *Trichoderma reesei* produce enzyme that can reduce the size of microcrystalline cellulose (Rojas *et al*., 2015). Enzyme treatment is environmental friendly as it does not involved chemical reagent. Enzyme method on isolating cellulose can achieve high yield and pure cellulose (Karim *et al*., 2017).

However, enzyme is expensive and required long time for hydrolysis process (Kalia *et al*., 2014). Enzyme did not completely remove the hemicellulose region. Since xylanase has specific action, it works mainly on removing xylan fraction.

2.3.2 Chemomechanical method

Combination of chemical and mechanical treatments is dissolution of lignin, hemicellulose and non-cellulosic components. Chen *et al* (2015) successfully isolated nanocellulose from lotus leaf stalks by chemical pretreatment and high intensity ultrasonication with 75 % crystallinity and width of 20 nm. Deepa *et al*, (2011) stated that the banana cellulose increased from 64 % to 95 % by alkaline and acid treatment followed by steam explosion. Banana fiber is used as polymer composites (Albinante *et al*., 2014). Xu *et al*., (2015) stated that more than 95 % of cellulose is isolated from coconut palm using chemical pretreatment and high pressure homogenizer. Coconut fiber is used as heat insulator.

Tibolla *et al*., (2018) isolated cellulose nanofiber with average diameter of 3.72 nm from banana peel by bleaching, alkaline treatment, sulphuric acid hydrolysis and followed by high pressure homogenizer. The crystallinity ranged from 63.1 % to 66.4 % that suitable to use for good reinforcing agents.

Gopinathan *et al*., (2107) studied the cellulose from “Grand Naine” and “Poovan” banana pseudo-stem by using bleaching agent, nitric acid and acetic acid hydrolysis coupled with ultrasonication. The results show that cellulose of “Grand Naine” has peak reading of thermal stability at 385 °C and 349 °C for “Poovan” banana pseudo-stem which are lower in raw fiber that have peaks at 443 °C and 458 °C respectively. Raw fiber has higher thermal stability due to the association of lignin decomposition. The higher

thermal stability is a good properties in exploiting cellulose as reinforcement materials and in processing thermoplastic polymers.

Xie *et al*., (2016) isolated cellulose nanofiber from bamboo by using microwave liquefaction with chemical treatment and ultrasonication with frequency of 25 kHz, 750 W power for 30 minute. The results show that 70 % cellulose nanofiber with the diameter within 2-10 nm, 22 % cellulose have 12-20 nm and 9 % has diameter more than 20 nm. The TEM images revealed that large number are elemental fibril, some are fibril bundles and small amount of aggregated bundles.

Chemomechanical method decreases energy consumption (Chirayil *et al*., 2014) and reduce the concentration of chemical used. Chemomechanical facilitate the disintegration of cellulose that increase the defibrillation process. This method reduce the size of fiber and produce highly purified cellulose (Abdul Khalil *et al.*, 2016).

2.3.3 Extraction of cellulose by ultrasonic technique

Ultrasonic uses solvents and ultrasonic energy to extract target compounds by ultrasound pressure waves and resulting cavitation (Khawas & Deka, 2016). Cavitation is formation, growth, and violent collapse of cavities in water. The energy transferred is within the hydrogen bond energy scale. The ultrasonic energy break the cellulose fiber with higher surface area. Cavitation breaks the cell walls of plant and releases the cellulose into the medium by collapse bubbles and highly localized temperature. Ultrasonic provides penetration of solvent into the material to improve mass transfer and break the vegetative tissue to facilitate the release of cellulose.

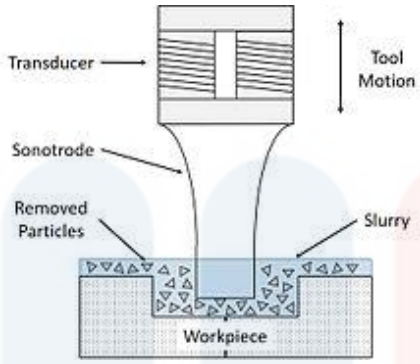


Figure 2.4: Ultrasonic principle

The crystallinity increases gradually with increase in ultrasonic power. Khawas and Deka (2016) extracted cellulose from banana peel by chemical treatment and high intensity ultrasonication showed that the crystallinity of cellulose are 30.50 %, 44.14 %, 50.74 % and 63.64 % for 0 W, 400 W, 800 W and 1000 W respectively. This is due to the part of amorphous region are removed. However, Xie *et al*., (2016) studied that the crystallinity of cellulose from ultrasonication (67.4 %) is lower than alkali treated cellulose (74.2 %) due to breakdown of hydrogen bonds. Ultrasonication damage the crystalline region of cellulose causes the crystallinity index decreases (Lu *et al*., 2015).

Longer sonication time result in high yield of cellulose. The yield of cellulose increases from 42.3 % to 56.5 % for 30 minute sonication time. However, 50 minute sonication time only results in increased 2.3 % in yield. Thus, 30 minute of sonication time is found to the best extraction time to isolate cellulose (Li *et al*., 2014).

Liao *et al*., (2016) stated that the higher the ultrasonic energy, the higher the yield of quercetin and rutin but the yield decreases when 400 W ultrasonic power is applied. This is due to suitable ultrasound power disrupt the cell wall and dissolving the target compounds into liquid medium. However, higher ultrasonic power causes cavitation bubbles grow too big to collapse that lead to weaken the cavitation effect (Liao *et al*., 2016). Excessive cavitation bubbles production hinder the mass transfer and lead to

ultrasound waves to scatter. Thus, from the journal, the optimum ultrasound power to extract quercetin and rutin from *Euonymus alatus* is 200 W.

The higher the cellulose concentration, the lower the yield of cellulose. At 0.5 % of cellulose concentration, the yield of extracted cellulose is 85.4 %. At 2.5 % of cellulose concentration, the yield of cellulose is 35.5 %. The solution is viscous and difficult to isolate the cellulose if the cellulose concentration is high because initiation of cavitation process required more energy in viscous system (Li *et al*.,2014).

2.4 Characterization of cellulose

2.4.1 Functional group determination

The functional group of plant such as lignin, hemicellulose and cellulose were analyzed by FTIR. The reduction in intensity peaks or disappearance of certain peak prove that the functional groups are changed at different treatment. Untreated fibers have peak at 3443 cm^{-1} due to the presence of strong absorption for intermolecular bonded hydroxyl groups. Peak at 1509 cm^{-1} attributed to aromatic asymmetric stretching of lignin and peaks at 1435 cm^{-1} attributed to C-O-C linkages in lignin (Aprilia *et al*., 2015). Disappearance of peak at 1435 cm^{-1} and 1509 cm^{-1} confirmed the removal of lignin (Saurabh *et al*., 2016). The absence of peak at 1732 cm^{-1} confirmed the removal of hemicellulose.

The absorption peak at 1025 cm^{-1} attributed to C-O stretching vibration in cellulose showed that the higher cellulose content while the peak at 828 cm^{-1} refer to the characteristics of β-glycosidic bond (Saurabh *et al*., 2016). The peak near 2913 cm^{-1} is the aliphatic saturated C-H stretching vibration in cellulose. The bands between 1370-1390 cm^{-1} attributed to the C-H deformation of cellulose (Pelissari *et al*.,2014).

Peak at 3000-3650 cm^{-1} region that attributed to –OH groups which reflected the hydrophilicity of banana fiber. The wavelength between 1635-1640 cm^{-1} attributed to the O-H bond of water absorbed from cellulose.

2.4.2 Morphology analysis

Morphological is investigated by SEM. The clustered nature of fiber is due to formation of interfibrillar hydrogen bonds or Van der Waals forces between nanoparticle (Lani *et al*., 2014). From the SEM result, the surface of the cellulose can be determined. The surface of untreated banana pseudo is rough and irregular due to the deposit composed of lignin, hemicellulose, pectin and waxes (Li *et al*.,2015). In the delignification, the lignin, hemicellulose and waxes are removed resulting in clean and smooth surface with large wrinkles (Xu *et al.*, 2015). With the chemical treatment, the fibers are divided into individual microfibril.

Mechanical method crack the surface of the sample show that ultrasonication break up the cellulose wall (Li et al., 2014). Many short fibers were observed in SEM image due to the fibers were damaged by machine (Xu *et al*.,2015). Ultrasonic result in more separate network to show large amount of filament

SEM has limitation on the size of sample. The image resolution of SEM is less than TEM. The image resolution for SEM is 20 nm to 10^6 nm. The specimen must be conductive which coating such as platinum and gold coating is required. Another disadvantage is images produced by SEM are in black and white.

2.4.3 Crystallinity

XRD is used to determine the crystallinity of cellulose. XRD can measure the diffraction of the beam from different section of the compound to show the composition of compound on atomic level because all compounds diffract the beam differently (Yao *et al*., 2014). XRD can be used to measure the sample purity.

The crystallinity of cellulose affect the thermal and mechanical properties of cellulose. The better orientation of crystalline of cellulose has better mechanical properties (Dungani *et al*.,2014). Both intramolecular and intermolecular hydrogen bond in cellulose via hydroxyl groups results in different ordered crystalline arrangements. The realignment of cellulose molecule is due to the removal of hemicellulose (Li *et al*.,2015).

The crystallinity index (CrI) is the large number of secondary molecular bonds in the crystalline regions and the level of compaction in crystalline region (Andrade-Mahecha *et al*., 2015). The higher the crystallinity index, the higher the crystallinity. The degree of crystallinity affect the compactability and absorption of water (Gopinathan et al., 2017).

By XRD, treated sample is more crystalline than untreated sample due to amorphous region is break. Cellulose does not have peak at 18° because the lignin and hemicellulose are removed (Gopinathan *et al*., 2017). The peak at 2θ of 15° and 16° is the structure of crystalline cellulose.

The disadvantage of XRD is the sample must be grinded into powder. There will be overlap of sample that cannot be detected and worsens for high angle “reflections” (Andrei *et al*., 2015).

2.4.4 Thermal stability

The thermal stability of cellulose is investigated using thermogravimetric analysis (TGA). The thermal stability of cellulose nanofiber is lower than micron cellulose because of the smaller fiber dimension that leads to higher surface areas exposed to heat (Jiang and Hsieh, 2013).

Banana cellulose undergoes three weight loss region. First region is in 50-100 °C due to moisture evaporation (Chen *et al*., 2015). Region from 220-300 °C is due to cleavage of cellulose linkage and thermal depolymerization of hemicellulose. The region from 200 to 500°C is due to lignin components (Chirayil *et al*., 2014). When heated to 500 °C, smaller amount of carbonized residue was left because non-cellulosic constituents are removed (Neto *et al*., 2013). Cellulose has better thermal properties than lignin, hemicellulose and pectin (Rojas *et al*, 2015). Ultasonication reduce crystallinity of cellulose due to damage in crystal region between cellulose cause thermal stability decreases.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Reagents

The reagents were toluene, ethanol, sodium chlorite, acetic acid solution, potassium hydroxide, distilled water.

3.2 Methods

3.2.1 Raw material preparation

The harvested banana pseudo stem were collected at Agropark, UMK Jeli. The banana stem was washed and the outer bark was separated from the pseudo-stem. The sample was cut into small pieces and dried for 8 hours in oven. After that, the sample was ground using blender machine. The final weight of 300 g of dried sample were obtained.

3.2.2 Chemical pretreatment

Dried banana stem weight 60 g was placed in filter paper and placed in the extraction sleeve of a Soxhlet extractor and covered with a little glass wool. The sample was extracted with toluene-ethanol (2:1,v/v) for 6 hours at 110 °C to remove the wax. The sample was then filtered by vacuum pump and washed with distilled water. The residues was treated with 20 % potassium hydroxide in a water bath at 22 °C for 2 hours to remove hemicellulose and pectin followed by washing with distilled water. After filtering, the residue was treated with 6% sodium chlorite in acetic acid solution with pH 3.6-3.8 at 75 °C for 2 hours to remove lignin until the sample become white powder. The delignify sample was filtered by vacuum pump. After filtering, the residue was treated again with 6 % sodium chlorite solution at 75 °C for 1 hour, then follow by 8 % potassium hydroxide at 90 °C for 2 hours to obtain purified cellulose. Next, the residues was washed with 95 % ethanol followed by distilled water and 95% ethanol. The purified cellulose was dried in oven at 60 °C for 12 hours.

3.2.3 Ultrasound technique

Chemical treated cellulose weight 3g was soaked in 300 ml distilled water with 1% concentration. The ultrasonic process was carried out in ice bath. The sample was sonicated at 20-25 kHz for 15 minute, 30 minute, 45 minute with amplitude 25 %, 50 % and 75 % respectively. The sonicated suspension was centrifuged for 10000 rpm and 15 minute to collect cellulose from supernatant. Three samples was replicated under the same condition to obtain the average values.

3.2.4 Yield of cellulose nanofiber

After extracting of cellulose, the dried cellulose was weighted and the yield was calculated using Equation 3.1.

Analysis of cellulose yield:

$$\frac{\text{weight of extracted cellulose (g)}}{\text{Weight of the plant part used for extraction (g)}} \times 100 \qquad (3.1)$$

3.3 Sample preparation for characterization

3.3.1 Functional group analysis

The chemical structure of untreated, chemical treated and chemical treated followed by ultrasonic of cellulose fiber were analyzed by FTIR in the range of 400-4000 cm^{-1} with the resolution of 4 cm^{-1}.

3.3.2 Morphology analysis

The morphology of raw fibers, chemical treated fiber and chemical treated followed by ultrasonic of cellulose fiber were analyzed by SEM. The fiber was put on a tape on aluminum for analysis.

3.3.3 Crystallinity Determination

Crystallinity of samples were determined by X-ray Diffraction (XRD) diffractometer. The scattered radiations was detected in the range 2θ range of 10° to 90° at speed of 0.04°/min (Gopinathan et al., 2017). The degree of crystallinity were determined by crystallinity index (CrI) using Equation 3.2.

$$CrI = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \qquad 3.2$$

where I_{002} is the maximum intensity for the crystalline portion in samples (i.e., cellulose) at about $2\theta = 22$ º and Iam is the intensity attributed to the amorphous portion of samples (i.e., hemicellulose and lignin) at $2\theta = 18$ º.

3.3.4 Thermal analysis

Thermal stability of celluloses were determined by Thermalgravimetric analysis (TGA) measurements. Thermal analysis was carried out for raw fibers, chemical treated fiber and chemical treated followed by ultrasonic of cellulose fiber. Approximately 7 mg of sample was transferred into the alumina crucible with a pinhole and followed by heating from room temperature to 550 ºC at a heating rate of 10 K min^{-1}. All measurements were performed under a nitrogen atmosphere with a gas flow rate of 40 mL/min.

The research flow chart below shows the methodology that have been carried out during this study.

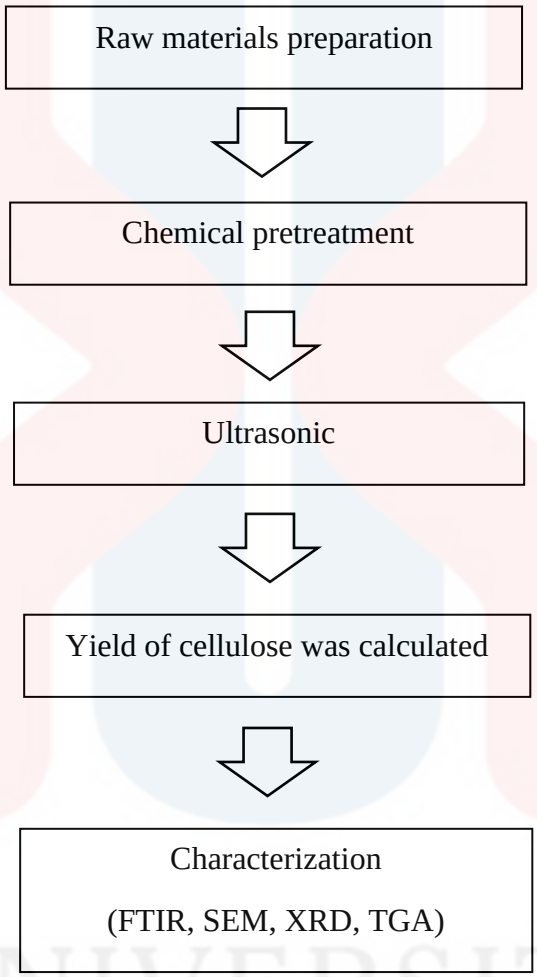


Figure 3.1: The research flow chart of extraction of cellulose from banana pseudo-stem.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Physical appearance of cellulose

The initial colour of banana pseudo stem is dark brown after extraction with ethanol-toluene. It changed to light brown as a result of alkali treatment by using 20 % potassium hydroxide in Figure 4.1. Potassium hydroxide is used to hydrolyse and solubilize hemicellulose, starch and pectin (Khawas & Deka, 2016).

Figure 4.1: The banana pseudo stem changes colour from dark brown to light brown as a result of alkali treatment.

The bleaching treatment of 6 % sodium chlorite cause the gradual discoloration from light brown to white after the second bleaching treatment as shown Figure 4.2 (Khawas & Deka, 2016). The changing in colour from brown to white is due to leaching out of lignin that is brown colour of banana pseudo stem. The chromogen groups like double bonds, conjugated carbonyls and their combination underline the brown colour of lignin were removed (Tibolla *et al*., 2018). As chlorite and chlorine oxidize lignin, it produce hydroxyl, carbonyl and carboxyl groups which make lignin soluble in alkali medium in order to obtain pure cellulose (Pelissari *et al*., 2014).

Figure 4.2: The colour transition from brown and gradual discoloration to white due to bleaching treatment.

4.2 Chemical composition of banana pseudo-stem

The chemical treatment was using 6 % sodium chlorite in acetic acid solution is to remove the lignin. However, the function of 20 % potassium hydroxide was used to remove hemicellulose. The chemical composition of banana pseudo-stem was recorded in the Table 4.1. Plant consists of lignin, hemicellulose and cellulose. The cellulose of banana pseudo stem is 31.6 % which is higher than lignin composition (24.57 %) but lower than hemicellulose (43.83 %).

Table 4.1: The average chemical composition of banana pseudo-stem

.

Sample	Chemical composition		
	Lignin (%)	Hemicellulose (%)	Cellulose (%)
Replicate 1	32.56	33.71	33.73
Replicate 2	23.17	48.13	28.70
Replicate 3	30.17	38.78	31.05
Replicate 4	12.39	54.69	32.92
Average	**24.57**	**43.83**	**31.6**

Banana fibers are used to make rope and cordage due to its resistance to the sea water and natural buoyancy. Banana fiber is lightweight and comfortable to wear, thus it is suitable to make clothes. Banana fiber is used as bioremediation for purify water and sorbent in absorbing spilled oils in refineries (Vigneswaran et al.,2015). Development of packaging like paper box, paper bag, paper cup from banana fiber help to eliminate environment problem because banana fibers biodegradable.

4.3 Yield of extraction of cellulose fiber from banana pseudo-stem by ultrasonic

Different ultrasonic amplitude and time result in variation of fiber quality in term of fiber yield, crystallinity and thermal stability (Liao *et al.*, 2016). Ultrasonic causes dispersion of size of cellulose fiber. High intensity of ultrasound causes the cavitation bubbles develop and grow. Energy is released from the collapse of the bubbles create mechanical shock wave and cause disruption of particle agglomerates (Li *et al*., 2014).

Table 4.2: The average yield of extraction of cellulose fiber from banana pseudo-stem by ultrasonic.

Time (minute)	**Amplitude (%)**		
	25	50	75
15	88.16%	88.99%	91.22%
30	92.59%	91.57%	90.86%
45	91.17%	90.33%	89.86%

The yield increases with the longer sonication time. The yield increases from 15 minutes to 30 minutes but as the time prolong to 45 minutes, the yield decreases. From Table 4.2, at amplitude 50 %, the yield for 15 minutes is 88.99 %. When the time increases to 30 minutes, the yield increases to 91.57 % but decreases to 90.33 % at 45 minutes. This is because there is larger surface area between sample and solvent, thus the ultrasonic wave can disrupt the cell wall (Liao *et al.*, 2016). When the time is prolonged to 45 minutes, the contact area decreases on the cell due to the increasing distance and the inner cell is exposed to the cavitation causes the crystal structure of cellulose break down to form amorphous phase. Therefore, the sonication time of 30 minute is found to be the best of cellulose extraction since it has higher yield of product.

The effect of ultrasonic amplitude on the yield is examined with amplitude 25 %, 50 %, 75 %. Table 4.2 shows for 15 minutes, the yield at 25 % amplitude is 88.16 %. The yield increases to 88.99 % with the increasing of amplitude at 50 %. The yield increases to 91.22 % at amplitude of 75 %. At low sonication amplitude (25 %), only weak bonds are broken. High sonication amplitude (50 % and 75 %) can break the molecular bonds to produce higher yield of cellulose. This is why the yield increases when the sonication amplitude increases (Li *et al.*, 2014). The suitable ultrasound wave can facilitate the cell walls to disrupt, allow the dissolving of target compound in the liquid medium (Liao *et al.*, 2016). However, at 45 minute, the yield decreases with increasing of amplitude. This

is because high amplitude can weaken the cavitation effect because the cavitation bubbles grow too big to collapse (Carail *et al*.,2015). Moreover, excessive cavitation bubbles production can hinder the mass transfer and lead the ultrasound waves to scatter, which weaken the effect of ultrasonic power (Liao *et al*., 2016).

For the high yield of cellulose obtained from the ultrasonic, it can be turned into bioethanol by fermentation with the help of microbes. The cellulose is break down into simple glucose by enzyme. The microbes like *Zymomonas mobilis* and yeast ferment the sugar into ethanol. The higher the yield of cellulose, the higher the amount of the bioethanol produced. Besides, the high yield of cellulose act as eco-friendly substitute in textile industry like fabrics and textiles woven (Vigneswaran et al.,2015). Higher surface area can also increases the strength of paper.

4.4 Characterization of cellulose fiber from banana pseudo-stem

4.4.1 Functional group analysis of untreated, chemical treated and chemical treated followed by ultrasonic cellulose

The prepared sample were analyzed by FTIR at wavenumber region of 400-4000 cm^{-1} to determine the chemical structure. The aim of using FTIR in this study is to determine the lignin and hemicellulose are removed by chemical in order to obtain cellulose. Untreated, chemical treated fiber and ultrasonic extracted fiber present vibration bands of components mainly corresponding to lignin, hemicellulose and cellulose. The vibration is summarized in Table 4.3.

For all sample, a broad absorption of band at 3000-3650cm^{-1} region that attributed to –OH groups which reflected the hydrophilicity of banana fiber (Obi Reddy *et al*., 2012).

The peak at 3329 cm^{-1}, 3331 cm^{-1} and 3332 cm^{-1} were observed in Figure 4.3 for raw material, chemical treated fiber and chemical treated with ultrasonic respectively.

Table 4.3: The vibration bands of components of lignin, hemicellulose and cellulose.

Group frequency wavenumber, cm^{-1}	Origin	Assignment
800-950	C-H	C-H deformation vibration in cellulose
1000-1100	C-O	C-O stretching vibration in cellulose
1100-1200	C-O-C	C-O-C asymmetrical stretching in cellulose
1250-1290	C-O	C-O stretching of acetyl in hemicellulose
1370-1390	C-H	C-H symmetric deformation cellulose
1420-1440	CH_2	CH_2 asymmetric stretching in lignin
1500-1700	C=C	Aromatic C=C bonding in lignin
1635-1640	O-H	O-H of water absorbed from cellulose
1718	C=O	C=O stretching of carboxylic acid
1744	C=O	C=O stretching of carboxyl ester
2850-2950	C-H	C-H stretching in cellulose-rich materials
3000-3650	-OH	Free and hydrogen bonded OH stretching

(Sources: Obi Reddy *et al*., 2012)

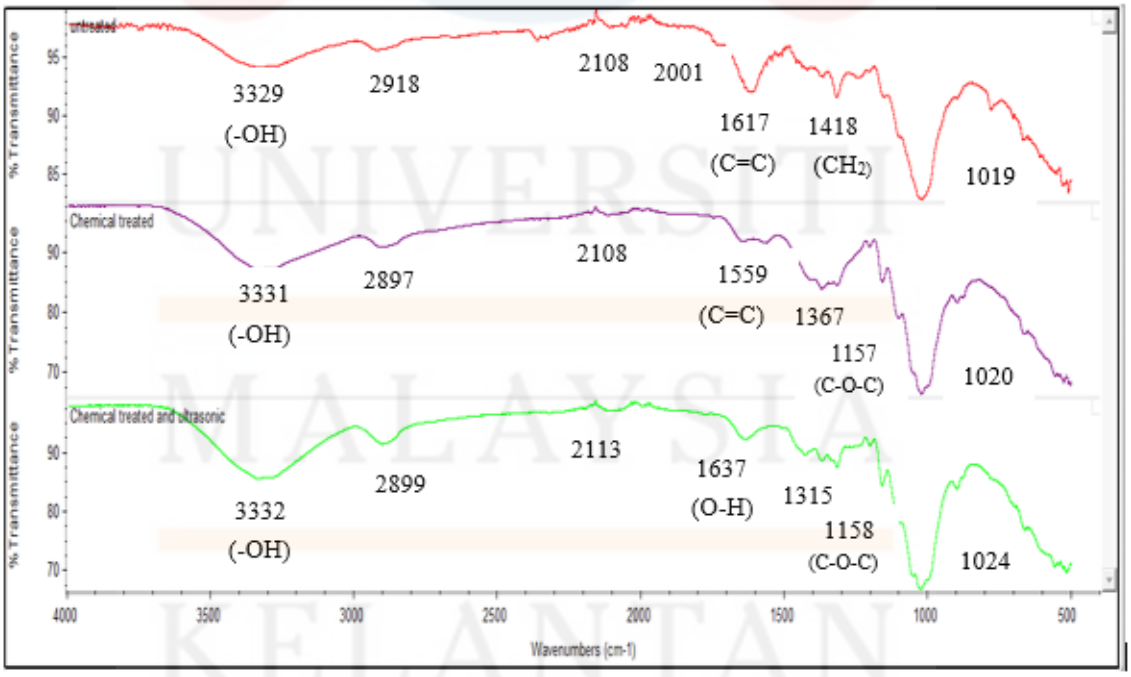


Figure 4.3: FTIR spectra of untreated, chemical treated, chemical treated followed by ultrasonic of cellulose fiber.

From the Figure 4.3, sodium chlorite is proved to remove the lignin in the sample as there are no peak at 1420-1440 cm^{-1} that attributed to CH_2 asymmetric stretching in lignin and waxes. The aromatic rings and carboxyl groups in the polyphenolic structure of lignin are removed (Tibolla *et al*., 2014). However, the peak at 1418 cm^{-1} present in the untreated raw material.

Chemical treated cellulose is not considered as pure cellulose because it has peak at 1559 cm^{-1} that attributed aromatic C=C bonding in lignin. However, the chemical treated followed by ultrasonic cellulose does not have any peak in region of 1500-1700 cm^{-1} that indicate the cellulose obtained is pure. Besides, the sample does not contain hemicellulose because there is no peak present at 1250-1290 cm^{-1} region. The result show that hemicellulose are removed by 20% potassium hydroxide.

The peak near 2913 cm^{-1} is the aliphatic saturated C-H stretching vibration in cellulose. The bands between 1370-1390 cm^{-1} attributed to the C-H deformation of cellulose (Pelissari *et al*.,2014). The peak at 1157 cm^{-1} of chemical treated cellulose and 1158 cm^{-1} of chemical treated followed by ultrasonic cellulose indicate that C-O-C asymmetrical stretching in cellulose.

The peak at 1025 cm^{-1} corresponding to C-O-C pyranose ring skeletal vibration in cellulose (Tibolla *et al*., 2014). The fiber from chemical treated followed by ultrasonic has band at 1024 cm^{-1} that shows higher cellulose content than chemical treated fiber (1020 cm^{-1}) . Besides, the chemical treated followed by ultrasonic cellulose has peak at 1637 cm^{-1} that attributed O-H of water absorbed from cellulose. This band is absent in the chemical treated cellulose. This is because ultrasonic break the chain or bonds to produce higher content of cellulose than only chemical treated fiber. Thus, chemical treated followed by ultrasonic cellulose show higher content cellulose and more pure than chemical treated fiber alone.

4.4.2 Morphology of cellulose fiber

The morphology of chemical treated and chemical treated followed by ultrasonic were observed in SEM image in Figure 4.4. In general, cellulose have a web-like network structure with long filaments. With the chemical treatment, the fibers are divided into individual microfibril shown in Figure 4.4 (a).

Ultrasonic associated with fibers' individualization result in more separate network to show large amount of filament in Figure 4.4 (b) and Figure 4.4(c) (Tibolla *et al*., 2014). The plant fibers are multilayered because of the interfibrillar hydrogen bonds and aggeregated with wide distribution. From the SEM result in Figure 4.4 (e), the surface of the sample show that ultrasonication break up the cellulose wall (Li *et al*., 2014).

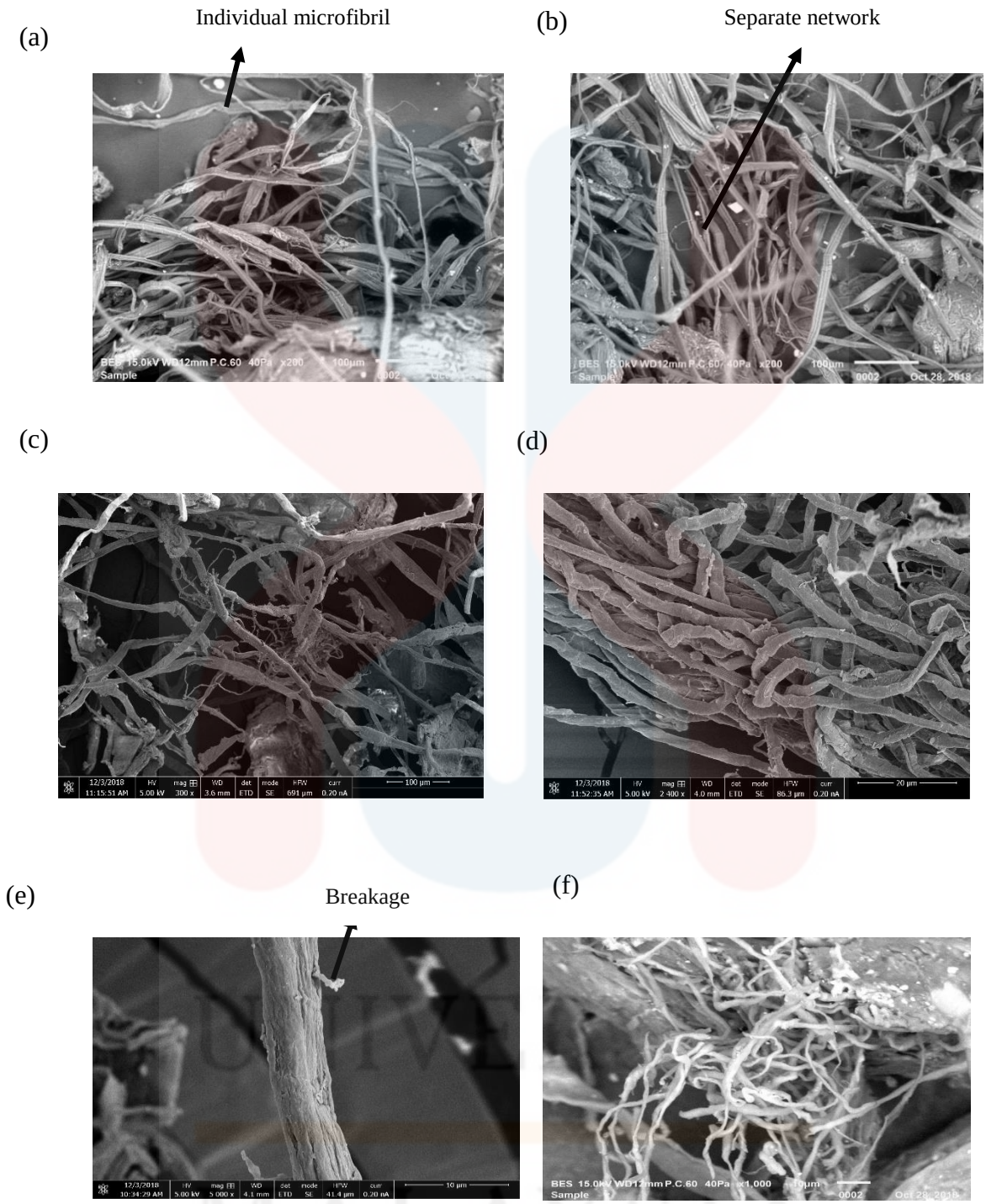


Figure 4.4: SEM image of (a) chemical treated at 200x magnification, (b) chemical treated followed by ultrasonic with 30 minute and amplitude 25 % at 200x magnification, (c) chemical treated followed by ultrasonic with 30 minute and amplitude 75 % at 300x magnification, (d) chemical treated followed by ultrasonic with 30 minute and amplitude 75 % at 2400x magnification, (e) chemical treated followed by ultrasonic with 30 minute and amplitude 75 % at 5000x magnification, (f) chemical treated followed by ultrasonic with 45 minute and amplitude 50 % at 1000x magnification.

Table 4.4: Average diameter of cellulose.

Sample			Diameter (µm)
	Time (Min)	Amplitude (%)	
Chemical treated	-	-	15.690
chemical treated followed by ultrasonic	15	75	7.843
	30	25	13.379
	30	75	1.4182

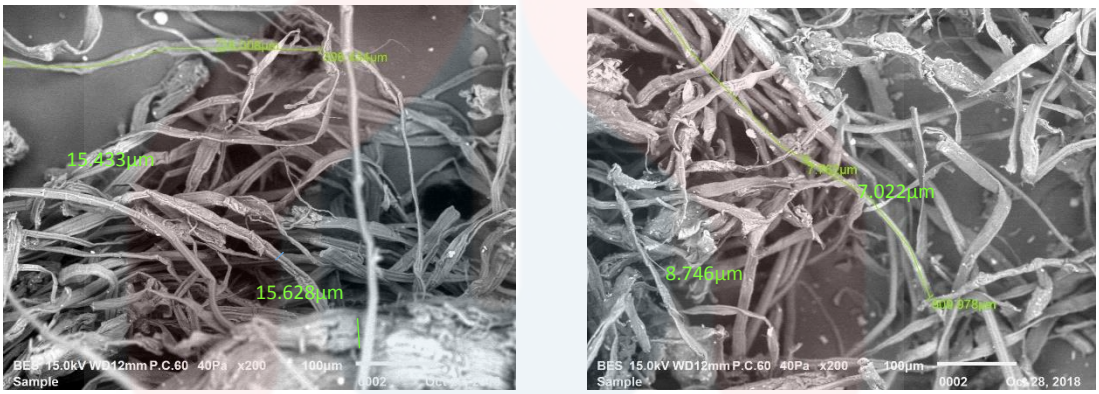
The average diameter of chemical treated cellulose is 15.690 µm. The diameter of cellulose is reduced to 7.843 µm after ultrasonic of 15 minute and 75 % amplitude. This is because ultrasonic break the cellulose cell wall resulting in reducing the size of cellulose (Chen *et al*., 2014). Ultrasonic treatment increases the dispersion of cellulose. Smaller diameter of cellulose has higher surface area.

The higher the ultrasonic amplitude resulting in decreased in size of cellulose (Khawas & Deka,, 2016). At 30 minute and 25 % amplitude of ultrasonic, the diameter is cellulose is 13.379 µm. The diameter decreases to 1.4182 µm when the amplitude increases to 75 %. This is because the higher the amplitude, the stronger the mechanical oscillating power. The molecules absorb ultrasonic energy leads to formation, expansion and implosion of gas bubbles. Within the cavitation bubbles, a strong shock waves are produced to break down the cellulose (Khawas & Deka,, 2016).

The longer the ultrasonic time, the shorter the diameter of cellulose. At 15 minute ultrasonic time and 75 % amplitude, the diameter of cellulose is 7.843 µm. When the ultrasonic time increases to 30 minute, the diameter of cellulose decreases to 1.4182 µm. The longer ultrasonic time result in reduction of size of cellulose is due to stronger mechanical oscillating power of ultrasonic are produced in longer time. The cavitation produces strong waves to break the cell wall of cellulose.

For smaller size of cellulose, it has higher surface area that suitable for bioremediation and sorbent as higher surface area can absorb more contaminants. The larger surface can bind a lot of the surrounding materials resulting in strong adhesion between the fibers and surrounding matrix which is beneficial in composite materials to provide higher strength properties (Kalia *et al*., 2014). Food packages are the example of fiber composite. Besides, cellulose with higher surface area can act as carrier for enzyme and protein to deliver the drug (Mishra *et al*., 2018 .

(a) (b)



(c) (d)

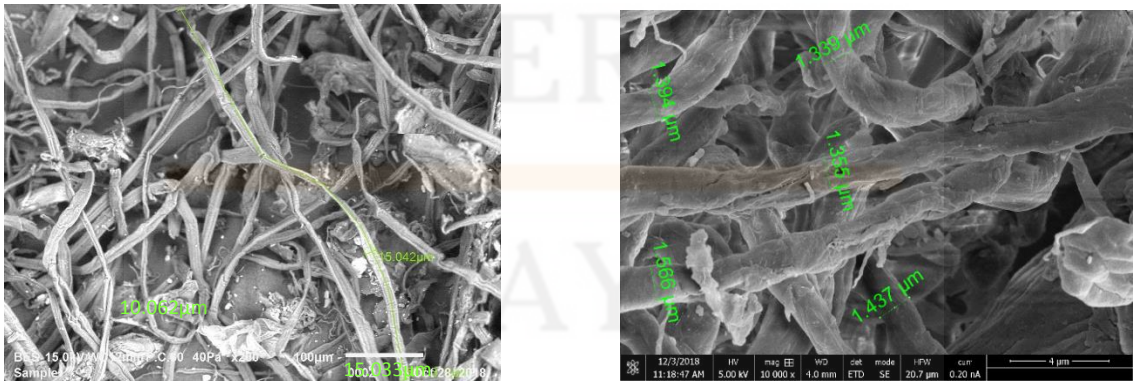


Figure 4.5: SEM image of diameter of (a) chemical treated cellulose at 200x maagnification, (b) chemical treated followed by ultrasonic with 15 minute and amplitude 75 % at 200x magnification, (c) chemical treated followed by ultrasonic with 30 minute and amplitude 25 % at 200x magnification, (d) chemical treated and ultrasonic with 30 minute and amplitude 75 % at 10,000x magnification.

(a) (b)

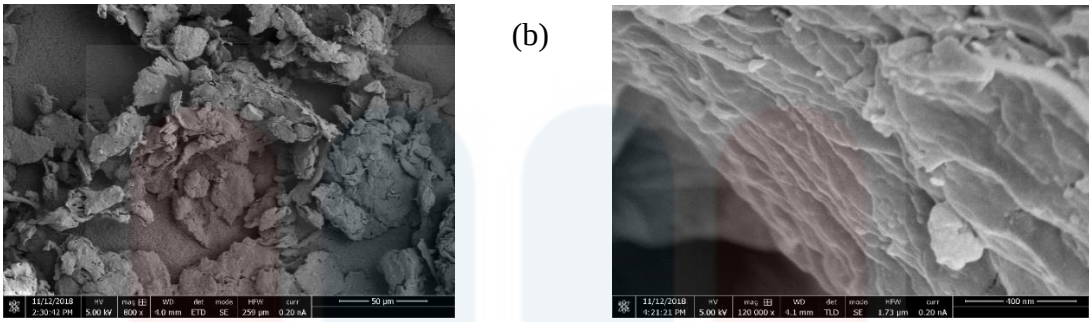


Figure 4.6: SEM image of (a) cellulose after ball milling at 800x magnification, (b) cellulose after ball milling at 120,000x magnification.

After ball milling of the cellulose, the microfibrils bundles get closely packed in Figure 4.6. The cellulose become compact due to the formation of new hydrogen bonds between the chain of cellulose microfibrils bundles. The microfibrils bundles rearrange themselves in a more compact manner resulting in closer packing (Li *et al*.,2015).

Thus, chemomechanical prove that reduction of diameter of cellulose to produce high surface area cellulose. The longer the time and the higher the ultrasonic amplitude result in reduction of diameter. After ball milling, the cellulose become compact that does not show the filaments in SEM image.

4.4.2 X-ray Diffraction

The crystallinity of cellulose are analysed by XRD. The XRD graph in Figure 4.7 reveal sharp peak at 2θ =22° represent the crystalline structure of cellulose (Khawas & Deka, 2016). All the samples do not have peak at 18° to show that lignin and hemicellulose are removed (Gopinathan *et al*.,2017).

The crystallinity index of treated fiber (53.9 %) is higher than untreated fiber (44.1 %) because the amorphous region are removed in the chemical treated cellulose.

High value of crystalline index indicate order compact structure while lower value of crystallinity index refer to disordered structure, resulting in more amorphous (Han *et al.*, 2013). Thus, the banana fiber are converted into more crystalline during chemical treatment.

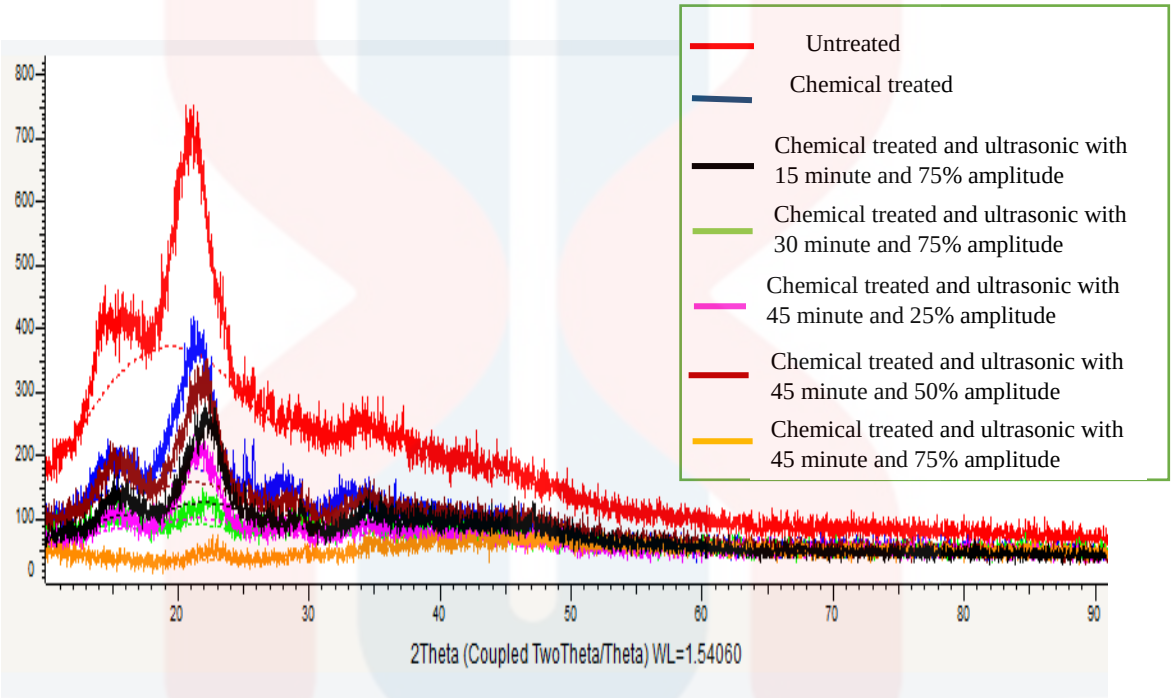


Figure 4.7: XRD diffraction pattern of cellulose from banana pseudo stem.

Pelissari *et al*. (2014) stated that mechanical method affects the degree of crystallinity of cellulose because mechanical treatment can remove a fraction of amorphous portion of cellulose fibers. From Table 4.5, the crystallinity index of chemomechanical cellulose is generally higher than chemical treated cellulose alone. The crystallinity index of chemical treated cellulose is 53.9 %. However, the crystallinity index of chemical treated followed by ultrasonic with 15 minutes and 75 % amplitude is higher (58.8 %) than chemical treated alone.

Table 4.5: Crystallinity and amorphous of cellulose with different parameter.

Sample			Crystallinity index (%)
	Time (Min)	Amplitude (%)	
Untreated	-	-	44.1
Chemical treated	-	-	53.9
chemical treated followed by ultrasonic	15	25	55.9
	15	50	56.9
	15	75	58.8
	30	25	44.2
	30	50	43.4
	30	75	54.6
	45	25	48.5
	45	50	48.1
	45	75	40.3

The crystallinity index increases with the increasing with the ultrasonic amplitude. From Table 4.5 for 15 minute, the crystallinity index of cellulose increases from 55.9 % at 25 % amplitude to 56.9 % at 50 % amplitude. The crystallinity index continue increases to 58.8 % at 75 % amplitude. This is because higher amplitude of ultrasonic produce stronger shock wave to remove the amorphous region. However, for 45 minutes ultrasonic time, the crystallinity index of cellulose is 48.5 % at 25 % amplitude. When the amplitude increases to 50 %, the crystallinity decreases to 48.1 %. At 75 % amplitude, the crystallinity decreases to 40.3 %. This is because the crystal is damaged by the high amplitude of ultrasonic for 45 minute.

The longer the ultrasonic time, the lower the crystallinity index due to the crystal was damage. With the control of amplitude 75 %, when the time is 15 minute, the crystallinity index of cellulose is 58.8 %. The crystallinity index decreases to 54.6 % at 30 minute and continuous decreases to 40.3 % at 45 minutes. This is due to longer time with high power result in strong interaction of cellulose and complex compound particle which cleave the cellulose chain and damage the crystal structure forming an amorphous phase (Li *et al*., 2014). Therefore, chemical treated followed by ultrasonic with 15 minute and 75 % amplitude show the highest crystallinity index that indicate the highest crystallinity and lowest amorphous region.

For high crystallinity of cellulose, the cellulose can act as barrier material as the high crystallinity cellulose has the ability to form dense network held together by strong inter-fibrillar bonds. In paper and paperboard industries, cellulose with high crystallinity has a strong reinforcement effect on paper materials due to the fiber-fiber bond strength. Cellulose can be used as a barrier in grease-proof type papers and as wet-end additive to enhance retention, dry and wet strength in paper and paperboards. High crystallinity indicate the high mechanical properties of cellulose that can be reinfored plastics. Film made from high crystalline cellulose have high strength over 200 MPa and high stiffness around 20 GPa (Khalil *et al*., 2014)

4.4.3 Thermal stability analysis

The thermal stability of the untreated, chemical treated and chemical treated followed by ultrasonic are analysed by using thermogravimetric analysis from 30 °C to 550 °C. The left limit refers to temperature which weight loss starts. Right limit refers to final temperature of weight loss. Inflection point: A point of curve which change in a direction.

The curve show initial weight loss in the region of 25 °C to 150 °C is due to the evaporation of moisture and initial thermal decomposition. There is water retention in the cellulose due to the cellulose hydrophicity. Hemicellulose and lignin are degraded at 200 °C. Thermal depolymerization of hemicellulose and cleavage of glycosidic linkages of cellulose occur in the range of 268-300 °C (Li et al., 2015). The broad peak 270 °C to 400 °C is attributed to cellulose degradation (Khawas & Deka, 2016).

Treated cellulose has higher thermal stability than untreated due to the removal of less thermally stable substances like hemicellulose and lignin in treated cellulose (Ortega *et al*., 2016). The chemical treated followed by ultrasonic of cellulose has higher thermal stability than chemical treated cellulose due to fully removal of hemicellulose and lignin.

Table 4.6: Thermal stability of untreated and treated cellulose.

Sample condition			**Left limit (°C)**	**Right limit (°C)**	**Inflection point (°C)**	**Weight loss (%)**
	Time (min)	Amplitude (%)				
Untreated	-	-	40.81	385.73	225.12	-63.7329
Chemical treated	-	-	41.03	542.69	243.44	-69.0839
Chemical treated followed by ultrasonic	15	25	40.24	544.91	271.93	-65.3995
	15	50	40.33	539.98	264.74	-67.3600
	15	75	40.82	542.07	270.44	-72.2033
	30	25	38.58	543.55	280.00	-77.1748
	30	50	37.24	543.33	273.32	-80.1870
	30	75	40.85	542.25	269.85	-75.4600
	45	25	40.73	541.68	269.60	-75.3200
	45	50	40.77	541.74	271.35	-76.4779
	45	75	40.75	540.83	276.85	-72.6548

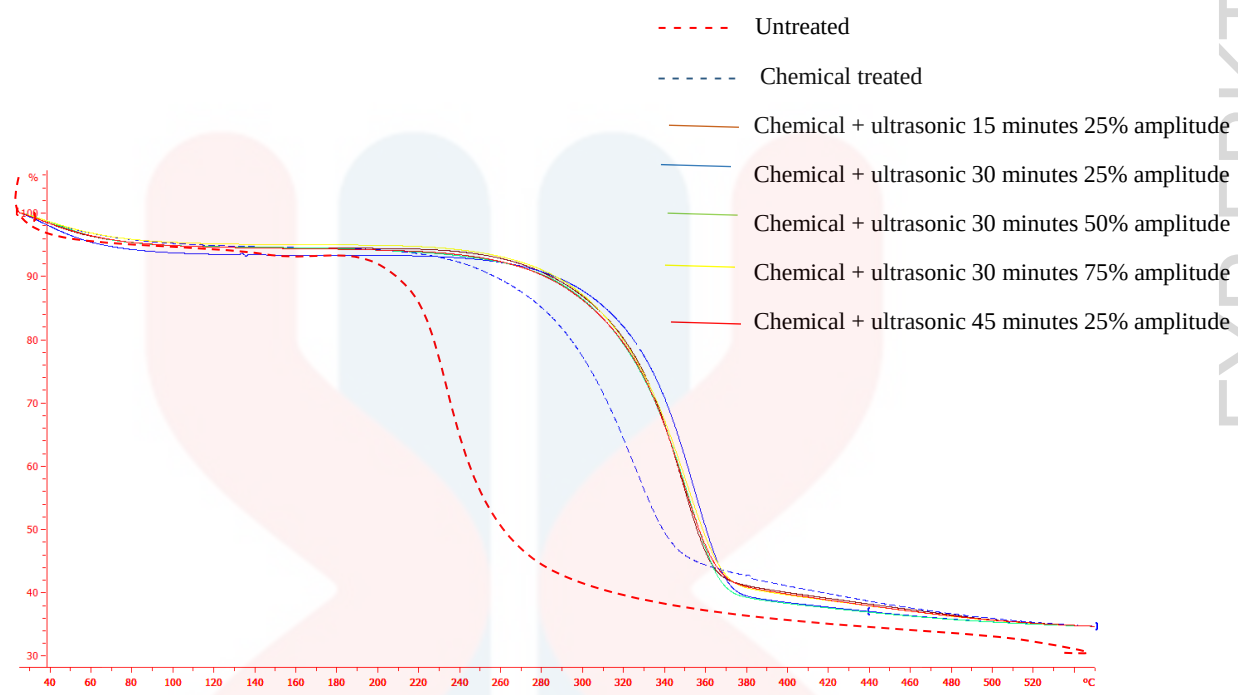


Figure 4.8: TGA curve of untreated, chemical treated and chemical treated with ultrasonic banana pseudo stem.

Untreated sample showed the least weight loss (-63.7329 %) among all the sample. The higher amount of residue left from the untreated sample is due to the presence of lignin, hemicellulose and noncellulosic materials (Saurabh et al., 2016).

When the ultrasonic amplitude increases, the thermal stability of chemomechanical cellulose decreases. The result is shown in Table 4.6, for 30 minutes ultrasonic time, at 25 % amplitude, the cellulose start to degrade at 280 °C. At 50 % amplitude, the sample start to decompose at 273.32 °C. This is due to partial dissolution process of reduction in crystallinity (Ghaderi et al., 2014). When the amplitude increases, the crystallinity decreases leads to decreased in thermal stability. The esterification of hydroxyl group lows the activation energy for cellulose degradation that reduce the pyrolysis resistance. The sample is decomposed at lower temperature (269.85 °C) when the amplitude is 75 %. This is because the smaller size of cellulose lead to higher surface area are exposed to heat.

When the ultrasonic time increases, the thermal stability of chemomechanical cellulose also increases. However, prolong time result in damage crystallinity causes thermal stability decreases. At 25 % amplitude, the cellulose for 15 minutes ultrasonic time start to decompose at 271.93 °C. The thermal stability increases to 280 °C for 30 minutes ultrasonic time. This is because more densely packed cellulose chain lead to higher hydrogen bond intensity between neighboring cellulose chain to hinder heat transfer by diffusion through cellulose chain (Saurabh *et al*., 2016). When the time is prolonged to 45 minutes, the cellulose start to degrade at 269.6 °C. The size of cellulose is smaller and crystallinity of cellulose is damaged and causes thermal stability decreases.

For high thermal stability cellulose, the cellulose are used to produce cellulose acetate. Cellulose acetate is easily bonded with heat, pressure and plasticizers. Cellulose acetate is widely used in textile industries such as blouses, dresses and draperies. Cellulose acetate is also used to make high absorbency products like surgical product and diapers. In the nanocomposite field, the processing temperature for thermoplastic polymer rises above 200 °C (Khawas & Deka, 2016). Thus, the high thermal stability of cellulose from banana pseudo-stem serve as reinforcing material in food packaging.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In conclusion, cellulose was successfully extracted from banana pseudo-stem by chemical pretreatment and ultrasonic with different ultrasonic amplitude (25 %, 50 % and 75 %) and different time (15 minute, 30 minute, 45 minute). Sodium chlorite and potassium hydroxide shows successful removal of lignin and hemicellulose respectively with 31.6% of cellulose obtained from banana pseudo stem.

Ultrasonic amplitude and time do affect the yield of cellulose fiber. Ultrasonic time with 30 minutes and amplitude of 25 % show the best parameter to extract high yield of cellulose. The yield increases with the longer sonication time. However, prolong time (45 minute) causes the yield decreases because of crystal damage. The yield increases with the increasing with ultrasonic amplitude. However, at high ultrasonic amplitude (75 %), the yield decreases because high amplitude can weaken the cavitation effect because the cavitation bubbles grow too big to collapse.

The presence of cellulose were characterized with FTIR and SEM. SEM image show the ultrasonic reduce in diameter of cellulose. The crystallinity of cellulose were analyzed by XRD. The highest crystallinity (58.8 %) obtained in ultrasonic with 15 minutes and 75 %. The thermal stability of cellulose were characterized by TGA. The sonication time of 30 minute and 25 % amplitude is found to have highest thermal stability (280 °C) of cellulose fiber.

5.2 Recommendations

Theoretically, mechanical extraction method reduce the cellulose size from micron to nanometer. To confirm the presence of nanocellulsoe, it is suggested to characterize by transmission electron microscope (TEM).

Atomic force microscopy (AFM) can be used to achieve quantitative surface potential measurement with nanoscale resolution. AFM provide detailed results about the surface in order to expand the horizon of cellulose application (Tibolla et al., 2018). To determine the diameter of cellulose, software like ImageJ can used to measure the diameter and at least 100 counting should be included.

To determine the cellulose content, degree of polymerization test can be carried out. The higher the cellulose content, the higher degree of polymerization and lower mircofibrillar angle. It gives better mechanical properties. To determine the mechanical properties of cellulose, hardness test like tensile strength or modulus can be used to expand the cellulose application. Besides, the moisture content test can be carried out as moisture content in fibers affect the degree of crystallinity and tensile strength. The ability of fiber to absorb or desorb moisture should be considered in paper, textiles or composites application.

Different extraction method affect the cellulose properties such as yield, crystallinity, structure, morphology and thermal stability. Thus, different extraction method such as high pressure homogenizer, microwave assisted extraction, enzyme assisted method or pulsed electric field extraction can be carried out to study the properties of cellulose.

REFERENCES

Abdul Khalil, H. P. S., Davoudpour, Y., Saurabh, C. K., Hossain, M. S., Adnan, A. S., Dungani, R., Haafiz, M. K. M. (2016). A review on nanocellulosic fibres as new material for sustainable packaging: Process and applications. *Renewable and Sustainable Energy Reviews*, 64, 823-836.

Albinante SR, Pacheco EBAV, Visconte LLY, Platenik G, Batista LN.(2014). Modification of Brazilian natural fibers from banana's tree to apply as fillers into polymer composites, *Chemical Engineering Transactions*.;37: 715-720.

Anderson, S. R., Esposito, D., Gillette, W., Zhu, J. Y., Baxa, U., & Mcneil, S. E. (2014). Enzymatic preparation of nanocrystalline and microcrystalline cellulose. *TAPPI Journal*, *13*(5), 35-42.

Andrade-Mahecha, M. M., Pelissari, F. M., Tapia-Bl_acido, D. R., & Menegalli, F. C. (2015). Achira as a source of biodegradable materials: Isolation and characterization of nanofibers. *Carbohydrate Polymers*, 123, 406e415.

Andrei A. Bunaciu, Elena gabriela Udriştioiu & Hassan Y. Aboul-Enein (2015) X-Ray Diffraction: Instrumentation and Applications, *Critical Reviews in Analytical Chemistry*, 45:4, 289-299

Aprilia, N. S., Hossain, M. S., Mustapha, A., Suhaily, S. S., Norulaini, N. N., Peng, L. C.,& Khalil, H. A. (2015). Optimizing the isolation of microfibrillated bamboo in high pressure enzymatic hydrolysis. *BioResources*, *10*(3), 5305-5316.

Bhatnagar, R., Gupta, G., & , & S, Y. (2015). A Review on Composition and Properties of Banana Fibers. *Cellulose*, 60-65.

Brigham, C. (2017). Biopolymers: Biodegradable alternatives to traditional plastics. In *Green chemistry* (pp. 753-770).

Carail, M., Fabiano-Tixier, A. S., Meullemiestre, A., Chemat, F., & Caris-Veyrat, C. (2015). Effects of high power ultrasound on all-E-β-carotene, newly formed compounds analysis by ultra-high-performance liquid chromatography–tandem mass spectrometry. *Ultrasonics sonochemistry*, *26*, 200-209.

Chen, Y., Wu, Q., Huang, B., Huang, M., & Ai, X. (2015). Isolation and characteristics of cellulose and nanocellulose from lotus leaf stalk agro-wastes. *BioResources*, 10(1), 684-696.

Chirayil, C. J., Mathew, L., & Thomas, S. (2014). Review of recent research in nanocellulose preparation from different lignocellulosic fibers. *Reviews on advanced materials science*, 37.

Deepa, B., Abraham, E., Cherian, B. M., Bismarck, A., Blaker, J. J., Pothan, L. A., Kottaisamy, M. (2011). Structure, morphology and thermal characteristics of banana nano fibers obtained by steam explosion. *Bioresource technology*, 102(2), 1988-1997. doi: 10.1016/j.biortech.2010.09.030

Dungani, R., Karina, M., Subyakto, Sulaeman, A., Hermawan, D., & Hadiyane, A. (2016). Agricultural Waste Fibers Towards Sustainability and Advanced Utilization: A Review. *Asian Journal of Plant Sciences, 15*(1), 42-55.

FOASTAT, (2016). Malayisa: Bananas, production quantity (tons). Retrieved from http://www.factfish.com/statisticcountry/malaysia/bananas,+production+quantity

Ghaderi, M., Mousavi, M., Yousefi, H., & Labbafi, M. (2014). All-cellulose nanocomposite film made from bagasse cellulose nanofibers for food packaging application. *Carbohydrate polymers, 104*, 59-65.

Gopinathan, P., Subramanian, K. S., Paliyath, G., & Subramanian, J. (2017). Genotypic Variations in Characteristics of Nano-Fibrillated Cellulose Derived from Banana Pseudostem. *BioResources*, 12(4), 6984-7001.

Han, J., Zhou, C., French, A. D., Han, G., & Wu, Q. (2013). Characterization of cellulose II nanoparticles regenerated from 1-butyl-3-methylimidazolium chloride. *Carbohydrate polymers, 94*(2), 773-781.

Hu, R., Zhang, M., Adhikari, B., & Liu, Y. (2015). Effect of homogenization and ultrasonication on the physical properties of insoluble wheat bran fibres. *International Agrophysics*, 29(4), 423-432.

Isroi, Cifriadi, A., Panji, T., Wibowo, N. A., & Syamsu, K. (2017). Bioplastic production from cellulose of oil palm empty fruit bunch. *IOP Conference Series: Earth and Environmental Science, 65*, 012011

Jiang, F., & Hsieh, Y. L. (2013). Chemically and mechanically isolated nanocellulose and their self-assembled structures. *Carbohydrate Polymers, 95*(1), 32-40.

Jonoobi, M., Oladi, R., Davoudpour, Y., Oksman, K., Dufresne, A., Hamzeh, Y., & Davoodi, R. (2015). Different preparation methods and properties of nanostructured cellulose from various natural resources and residues: a review. *Cellulose, 22*(2), 935-969.

Kalia S, Boufi S, Celli A, Kango S. (2014). Nanofibrillated cellulose: surface modification and potential applications. *Colloid Polymer Science*.;292(1):5–31.

Karim, Z., Afrin, S., Husain, Q., & Danish, R. (2017). Necessity of enzymatic hydrolysis for production and functionalization of nanocelluloses. *Critical Review Biotechnology, 37*(3), 355-370. doi: 10.3109/07388551.2016.1163322

Khawas, P., & Deka, S. C. (2016). Isolation and characterization of cellulose nanofibers from culinary banana peel using high-intensity ultrasonication combined with chemical treatment. *Carbohydrate Polymer, 137*, 608-616. doi: 10.1016/j.carbpol.2015.11.020

Khalil, H. A., Davoudpour, Y., Islam, M. N., Mustapha, A., Sudesh, K., Dungani, R., & Jawaid, M. (2014). Production and modification of nanofibrillated cellulose using various mechanical processes: a review. *Carbohydrate polymers, 99*, 649-665.

Kopania, E., Wietecha, J., & Ciechańska, D. (2012). Studies on isolation of cellulose fibres from waste plant biomass. *Fibres & Textiles in Eastern Europe*.

Lani, N. S., Ngadi, N., Johari, A., & Jusoh, M. (2014). Isolation, characterization, and application of nanocellulose from oil palm empty fruit bunch fiber as nanocomposites. *Journal of Nanomaterials*, *2014*, 13.

Li, M., Wang, L.-j., Li, D., Cheng, Y.-L., Adhikari, B., (2014). Preparation and characterization of cellulose nanofibers from de-pectinated sugar beet pulp. *Carbohydrate Polymer*. 102, 136–143.

Li, W., Zhang, Y., Li, J., Zhou, Y., Li, R., & Zhou, W. (2015). Characterization of cellulose from banana pseudo-stem by heterogeneous liquefaction. *Carbohydrate Polymer*, *132*, 513-519. doi: 10.1016/j.carbpol.2015.06.066

Liao, J., Qu, B., & Zheng, N. (2016). Effects of Process Parameters on the Extraction of Quercetin and Rutin from the Stalks of Euonymus Alatus (Thumb.) Sieb and Predictive Model Based on Least Squares Support Vector Machine Optimized by an Improved Fruit Fly Optimization Algorithm. *Applied Sciences*, *6*(11), 340. doi: 10.3390/app6110340

Liu, Q., Lu, Y., Aguedo, M., Jacquet, N., Ouyang, C., He, W.,Richel, A. (2017). Isolation of High-Purity Cellulose Nanofibers from Wheat Straw through the Combined Environmentally Friendly Methods of Steam Explosion, Microwave-Assisted Hydrolysis, and Microfluidization. *ACS Sustainable Chemistry & Engineering*, 5(7), 6183-6191.

Lu, Q., Lin, W., Tang, L., Wang, S., Chen, X., & Huang, B. (2015). A mechanochemical approach to manufacturing bamboo cellulose nanocrystals. *Journal of materials science*, *50*(2), 611-619.

Mishra, R. K., Sabu, A., & Tiwari, S. K. (2018). Materials chemistry and the futurist eco-friendly applications of nanocellulose: Status and prospect. *Journal of Saudi Chemical Society*. doi: https://doi.org/10.1016/j.jscs.2018.02.005

Neto, W.P.F., Silverio, H.A., Dantas, N.O., and Pasquini, D. (2013). Extraction and characterization of cellulose nanocrystals from agro-industrial residue – Soy hulls, *Industrial Crops and Products* 42, 480-488.

Obi Reddy, K., Shukla, M., Uma Maheswari, C., & Varada Rajulu, A. (2012). Mechanical and physical characterization of sodium hydroxide treated Borassus fruit fibers. *Journal of Forestry Research*, *23*(4), 667-674. doi: 10.1007/s11676-012-0308-7

Ogah, A., & James, T. (2018). Mechanical Behavior of Agricultural Waste Fibers Reinforced Vinyl Ester Bio-composites. *Asian Journal of Physical and Chemical Sciences, 5(1), 1-10.*

Ortega, Z., Moron, M., Monzon, M. D., Badallo, P., & Paz, R. (2016). Production of Banana Fiber Yarns for Technical Textile Reinforced Composites. *Materials (Basel)*, *9*(5). doi: 10.3390/ma9050370

Pelissari, F. M., do Amaral Sobral, P. J., & Menegalli, F. C. (2014). Isolation and characterization of cellulose nanofibers from banana peels. *Cellulose*, *21*(1), 417-432.

Preethi, P., & Balakrishna Murthy, G. (2013). Physical and chemical properties of banana fibre extracted from commercial banana cultivars grown in Tamilnadu State. *Agrotechno S11*, 8(2).

Ramamoorthy, S. K., Skrifvars, M., & Persson, A. (2015). A review of natural fibers used in biocomposites: plant, animal and regenerated cellulose fibers. *Polymer reviews*, *55*(1), 107-162.

Reddy N., Yang Y. (2015) Fibers from Banana Pseudo-Stems. In: Innovative Biofibers from Renewable Resources. Springer, Berlin, Heidelberg, p: 25-27

Reddy, K. O., Uma Maheswari, C., Muzenda, E., Shukla, M., & Rajulu, A. V. (2016). Extraction and characterization of cellulose from pretreated ficus (peepal tree) leaf fibers. *Journal of Natural Fibers*, *13*(1), 54-64.

Rojas, J., Bedoya, M., & Ciro, Y. (2015). Current trends in the production of cellulose nanoparticles and nanocomposites for biomedical applications. *In Cellulose-Fundamental Aspects and Current Trends*. InTech

Saelee, K., Yingkamhaeng, N., Nimchua, T., & Sukyai, P. (2016). An environmentally friendly xylanase-assisted pretreatment for cellulose nanofibrils isolation from sugarcane bagasse by high-pressure homogenization. *Industrial Crops and Products*, 82, 149-160.

Satyamurthy, P., & Vigneshwaran, N. (2013). A novel process for synthesis of spherical nanocellulose by controlled hydrolysis of microcrystalline cellulose using anaerobic microbial consortium. *Enzyme and Microbial Technology*, 52(1), 20-25.

Saurabh, C. K., Mustapha, A., Masri, M. M., Owolabi, A. F., Syakir, M. I., Dungani, R., Abdul Khalil, H. P. S. (2016). Isolation and Characterization of Cellulose Nanofibers fromGigantochloa scortechiniias a Reinforcement Material. *Journal of Nanomaterials*, 1-8.

Sofla, M. R. K., Brown, R. J., Tsuzuki, T., & Rainey, T. J. (2016). A comparison of cellulose nanocrystals and cellulose nanofibres extracted from bagasse using acid and ball milling methods. Advances in Natural Sciences: *Nanoscience and Nanotechnology*, 7(3), 035004.

Singanusong, R., Tochampa, W., Kongbangkerd, T., & Sodchit, C. (2014). Extraction and properties of cellulose from banana peels. *Suranaree Journal Science Technology*, *21*, 1-19.

Thakur, V. K., Thakur, M. K., & Gupta, R. K. (2014). raw natural fiber–based polymer composites. *International Journal of Polymer Analysis and Characterization*, *19*(3), 256-271.

Tibolla, H., Pelissari, F. M., & Menegalli, F. C. (2014). Cellulose nanofibers produced from banana peel by chemical and enzymatic treatment. *LWT - Food Science and Technology*, *59*(2), 1311-1318. doi: 10.1016/j.lwt.2014.04.011

Tibolla, H., Pelissari, F. M., Martins, J. T., Vicente, A. A., & Menegalli, F. C. (2018). Cellulose nanofibers produced from banana peel by chemical and mechanical treatments: Characterization and cytotoxicity assessment. *Food Hydrocolloids*, *75*, 192-201. doi: 10.1016/j.foodhyd.2017.08.027

Vigneswaran, C., Pavithra, V., Gayathri, V., & Mythili, K. (2015). Banana fiber: scope and value added product development. *Journal of Textile and Apparel, Technology and Management*, *9*(2).

Xie, J., Hse, C. Y., Cornelis, F., Hu, T., Qi, J., & Shupe, T. F. (2016). Isolation and characterization of cellulose nanofibers from bamboo using microwave liquefaction combined with chemical treatment and ultrasonication. *Carbohydrate polymers*, *151*, 725-734.

Xu C, Zhu S, Xing C, Li D, Zhu N, Zhou H (2015) Isolation and Properties of Cellulose Nanofibrils from Coconut Palm Petioles by Different Mechanical Process. *PLoS ONE* 10(4): e0122123.

Xu, S., Xiong, C., Tan, W., & Zhang, Y. (2015). Microstructural, thermal, and tensile characterization of banana pseudo-stem fibers obtained with mechanical, chemical, and enzyme extraction. *BioResources*, *10*(2), 3724-3735.

Yao, Z.; Yang, Y.; Ying, H.; Dong, Y.(2014). Mineral Characteristics and Their Geological Significance of Black Shales in Southeastern Ordos Basin by X-ray Diffraction Analysis. *Chin. J. Geochem*; 33, 119–124

Zaida Ortega, Moisés Morón , Mario D. Monzón, Pere Badalló and Rubén Paz (2016). Production of Banana Fiber Yarns for Technical Textile Reinforced Composites, *Materials*, 9, 370.

Zheng H. Production of fibrillated cellulose materials—effects of pretreatments and refining strategy on pulp properties. Aalto University; 2014. p. 74

APPENDIX A

The yield of chemical treated cellulose extraction by ultrasonic were analyzed by equation below:

Analysis of cellulose yield:

$$\frac{\text{weight of extracted cellulose (g)}}{\text{Weight of the plant part used for extraction (g)}} \times 100$$

$$\text{Yield of cellulose for 15 minute and 25 \% amplitude} = \frac{2.63\ \text{g}}{3.09\ \text{g}} \times 100$$

$$= 85.11\ \%$$

Table 1: Yield of extraction of chemical treated cellulose fiber from banana pseudo-stem by using ultrasonic for 15 minutes sonication with different amplitude.

Time	Amplitude	Yield 1	Yield 2	Yield 3	Average
15 minutes	25 %	85.11 %	91.69 %	87.67 %	88.16 %
	50 %	86.24 %	92.41 %	88.33 %	88.99 %
	75 %	87.00 %	95.67 %	91.00 %	91.22 %

Table 2: Yield of extraction of chemical treated cellulose fiber from banana pseudo-stem by using ultrasonic for 30 minutes sonication with different amplitude.

Time	Amplitude	Yield 1	Yield 2	Yield 3	Average
30 minutes	25 %	90.11 %	95.34 %	92.33 %	92.59 %
	50 %	88.33 %	95.34 %	91.03 %	91.57 %
	75 %	85.57 %	95.00 %	92.00 %	90.86 %

Table 3: Yield of extraction of chemical treated cellulose fiber from banana pseudo-stem by using ultrasonic for 45 minutes sonication with different amplitude.

Time	Amplitude	Yield 1	Yield 2	Yield 3	Average
45 minutes	25 %	87.12 %	95.05 %	91.33 %	91.17 %
	50 %	86.33 %	95.00 %	89.67 %	90.33 %
	75 %	85.24 %	94.00 %	90.33 %	89.86 %

APPENDIX B

The average diameter of cellulose fiber.

The average diameter of chemical treated cellulose

$$= \frac{16.008\ \mu m + 15.628\ \mu m + 15.433\ \mu m}{3} = 15.690\ \mu m$$

The average diameter of chemical treated followed by ultrasonic with 15 minute and 25 % amplitude of cellulose

$$= \frac{7.760\ \mu m + 8.746\ \mu m + 7.022\ \mu m}{3} = 7.843\ \mu m$$

The average diameter of chemical treated followed by ultrasonic with 30 minute and 25 % amplitude of cellulose

$$= \frac{10.062\ \mu m + 15.042\ \mu m + 15.033\ \mu m}{3} = 13.379\ \mu m$$

The average diameter of chemical treated followed by ultrasonic with 30 minute and 75 % amplitude of cellulose

$$= \frac{1.339\ \mu m + 1.394\ \mu m + 1.355\ \mu m + 1.437\ \mu m + 1.566\ \mu m}{5} = 1.418\ \mu m$$

APPENDIX C

The degree of crystallinity were determined by Crystallinity Index (CrI) using equation below:

$$CrI = \frac{I_{002} - I_{am}}{I_{002}} \times 100$$

where I_{002} is the maximum intensity for the crystalline portion in samples (i.e., cellulose) at about 2θ= 22 º and Iam is the intensity attributed to the amorphous portion of samples (i.e., hemicellulose and lignin) at 2θ= 18 º.

CrI of untreated sample

$$= \frac{746-417}{746} \times 100$$

= 44.1 %

APPENDIX D

Raw materials preparation

Figure 1: Dried banana pseudo stem

Figure 2: The dried banana pseudo stem is ground into powder

Figure 3: Banana pseudo stem powder

APPENDIX E

Apparatus used in this research

Figure 4: Soxhlet apparatus

Figure 5: Ultrasonic machine

APPENDIX F

Chemical treated and ultrasonic of cellulose

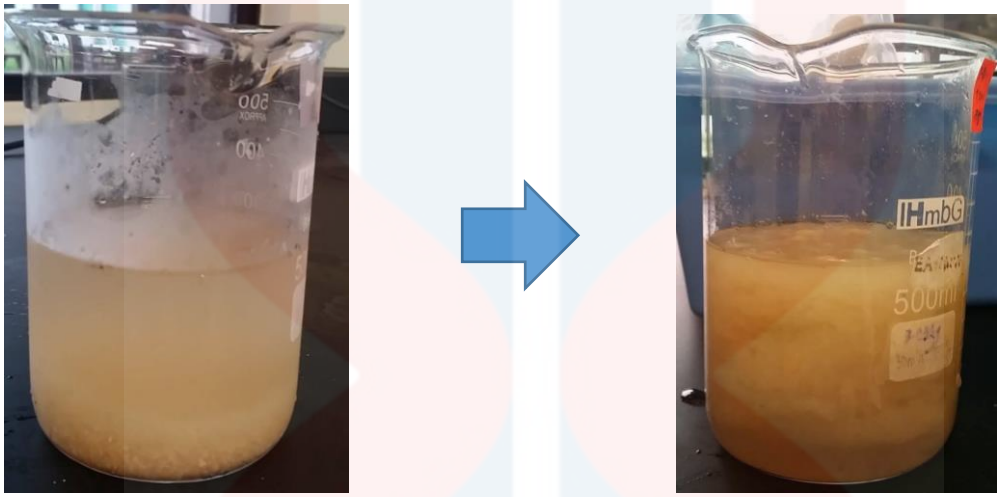


Figure 6: Extraction of cellulose after ultrasonic

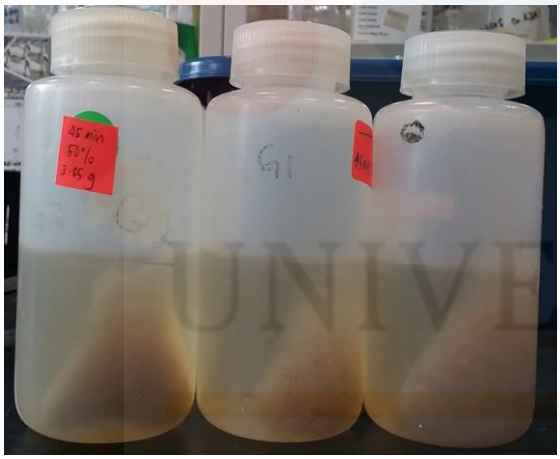


Figure 7: Extraction of cellulose after centrifuge

Figure 8: Removal of supernatant

(a)

(b)

(c)

Figure 9: Dried cellulose after ultrasonic with (a) 15 minute (b) 30 minute and (c) 45 minute