STUDY OF ULTRASONIC EFFECT IN THE EXTRACTION PROCESS

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A project report submitted in partial fulfilment of the

requirements for the award of the degree of

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DECLARATION

I hereby declare that this project report is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for any other degree or award at UTAR or other institutions.

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APPROVAL FOR SUBMISSION

I certify that this project report entitled "STUDY OF ULTRASONIC EFFECT IN THE EXTRACTION PROCESS" was prepared by CHONG JUN TIAN has met the required standard for submission in partial fulfilment of the requirements for the award of Bachelor of Engineering (Honours) Petrochemical Engineering at Universiti Tunku Abdul Rahman.

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Date : _____

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STUDY OF ULTRASONIC EFFECT IN THE EXTRACTION PROCESS

ABSTRACT

Currently, the application of ultrasonic-assisted extraction (UAE) in cellulose extraction process is widely discussed. The abundance of biomass waste such as oil palm empty fruit bunch (OPEFB) has exacerbated environmental problems and the UAE is a promising environmentally friendly technology to extract the cellulose from the OPEFB. In this research, several important parameters were varied to investigate their influences towards the cellulose extraction efficiency from the OPEFB via ultrasonication. The UAE process conducted using sonication bath was manipulated using four different processing parameters, which are sonication duration at 120 minutes, 180 minutes and 240 minutes, temperature at 30 ^{o}C, 40 ^{o}C, 50 ^{o}C for ethanol, concentration of sodium hydroxide (NaOH) solution varied from 0.5 M, 0.75 M to 1.0 M as well as different types of solvent used such as ethanol, chloroform and NaOH solution. The characterization methods involving Fourier Transform Infrared Spectroscopy (FTIR), High Performance Liquid Chromatography (HPLC) and Differential Scanning Calorimetry (DSC) were carried out to determine the ultrasonic effect in the extraction process. After various ultrasonication parameters were conducted, it was found that the treatment using 1.0 M NaOH solution at 80 ^{o}C for 180 minutes yielded the highest cellulose content from the OPEFB, 78.12 wt%. The ultrasonic-assisted alkali extraction can remove the lignin from the raw OPEFB and increase the efficiency of cellulose extraction process. To further evaluated the strength of UAE, the raw OPEFB was treated using the obtained optimum condition: temperature at 80 ^{o}C, duration of 120 minutes, 1.0 M NaOH solution. The treated sample was further converted to carboxymethyl cellulose (CMC). The conversion of CMC was successful, and the CMC provided DS value of 0.5201, which is able to

form film for more applications. On the whole, the outcome is significant for the future development of UAE using OPEFB as feedstock to produce CMC at industry level.

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LIST OF SYMBOLS / ABBREVIATIONS

ATR	Attenuated Total Reflectance
CMC	Carboxymethyl Cellulose
DS	Degree of Substitution
DSC	Differential Scanning Calorimetry
EC	Ethyl Cellulose
EFB	Empty Fruit Bunch
FTIR	Fourier Transform Infrared Spectroscopy
HCl	Hydrochloric Acid
HPC	Hydroxy Propyl Cellulose
HPLC	High Performance Liquid Chromatography
HPMC	Hydroxy Propyl Methyl Cellulose
KOH	Potassium Hydroxide
MC	Methyl Cellulose
MEC	Methyl Ethyl Cellulose
NaOH	Sodium Hydroxide
OPEFB	Oil Palm Empty Fruit Bunch
PKS	Palm Kernel Shells
PPF	Palm Pressed Fibre
SEM	Scanning Electron Microscopy
SMCA	Sodium Monochloroacetate
H_2SO_4	Sulphuric Acid
UAE	Ultrasonic-Assisted Extraction
XRD	X-ray Diffraction

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

In Malaysia, palm oil has contributed a lot to the economy of the country. Nowadays, Malaysia is one of the world's leading producers and exporters for palm oil. Malaysia can produce around 15 million tonnes of EFB each year (Sultana Toma et al., 2021). During the extraction process of the palm oil from oil palm tree, the by-products such as empty fruit bunch (EFB), palm kernel shells (PKS) and palm pressed fibre (PPF) will be generated (Abdullah and Sulaim, 2013). The biomass waste will cause a vast amount of landfill space used up and the pollution of the environment. Therefore, the recycling of the biomass waste needs to be concerned so that all the resources can be fully used in a bid to minimize the environmental issue of palm oil production.

To remove the fruits from the stalks, the EFB is crushed and threshed in spinning steel drums (Muhamad Parid et al., 2017). In this process, the oil palm empty fruit bunch (OPEFB) is formed. The OPEFB which is the most prevalent waste product consists of 62.64% holocellulose, 21.64% lignin, and 1.29% extractives (Solikhin et al., 2021). The OPEFB can be a valuable biopolymer as it is biodegradable and renewable as well as it shows its potential to be a source of nanofibers. The OPEFB can act as the cheapest natural fibre with good qualities that is abundant in Malaysia (Padzil et al., 2020). Recently, the sustainable and non-toxic materials from biomass is a vital key in the future bioeconomy as the concerns about the safety and

environment issues of petroleum-based products have been raised (Tayeb et al., 2018); thus, the OPEFB is potential to be an alternative eco-friendly raw material.

The high cellulose content in the OPEFB makes it become a particularly attractive feedstock for nanocellulose extraction and the manufacture of diverse cellulose products (Padzil et al., 2020). Cellulose is one of the most common natural polysaccharides in the universe, with a complex structure and distinct capabilities. According to Bailey (2019), the plant cell walls are largely made of cellulose, which helps the plant stay robust and stiff, while the other component is made in conjunction with the cellulose. The formula of cellulose is $(C_6H_{10}O_5)_n$ and it is made up of hundreds to thousands of β(1,4) glucose units linked together to create a straight linear chain (Klemm et al., 2005). As the most ordinary organic polymer, cellulose is regarded as a nearly limitless supply of raw material for the growing need in ecologically friendly and biocompatible goods. The cellulose has distinctive characteristics such as low density, good mechanical capabilities, and biodegradability. Thus, the cellulose polymer has a wide range of applications, including veterinary feeds, wood and paper, textiles and clothing, cosmetic and pharmaceutical sectors as an excipient (Shokri and Adibki, 2013). For the purpose of widening the usage of cellulose, the non-water-soluble cellulose is developed by inserting a functional group to be the cellulose derivatives. For instance, the cellulose derivatives can be methyl ethyl cellulose (MEC), methylcellulose (MC), hydroxypropyl cellulose (HPC), hydroxypropyl methylcellulose (HPMC), sodium carboxymethylcellulose (CMC), and ethyl cellulose (EC) (Ergun, Guo and Huebner-Keese, 2016).

One of the cellulose derivatives is the carboxymethyl cellulose (CMC) and CMC is anionic and water soluble. CMC enables to hydrate quickly due to its very hygroscopic nature. When CMC powder is placed into water, rapid hydration will lead to agglomeration and the formation of a lump (Ergun, Guo and Huebner-Keese, 2016). Although CMC is seldom applied as biomaterials, it is widely utilized in paper, food, toothpaste, detergents, oil drilling mud, pharmaceuticals, coating, and others (Almlöf Ambjörnsson, Schenzel and Germgård, 2013). CMC is often used in drinks and beverage dry mixes to give rich mouthfeel owing to its excellent solubility and clarity

of its solutions. Currently, CMC is used to increase viscosity, manage rheology of a solution, prevent water separation from a suspension as well as improve surface or barrier qualities (Eliza et al., 2015).

To extract the cellulose from the OPEFB, the ultrasonic-assisted extraction (UAE) is one of the emerging technologies. UAE functions to employ kilohertz sonic vibrations that pass through the solution, causing cavitation bubbles (Al Jitan, Alkhoori and Yousef, 2018). These cavitation bubbles have the potential to break the hydrophobic wax coating on the surface of lignocellulosic biomass. UAE is an alternate approach that provides increased efficiency of the extracted yield in a short period of time compared to conventional solvent extraction (Natnoi and Pirak, 2019). Lately, the application of UAE has shown considerable promise in the study of plant extraction. UAE might improve heat and mass transfer by breaking matrix cell walls, allowing bioactive chemicals to be released (Dolatowski, Stadnik and Stasiak, 2007). Other than that, UAE can use mechanical effects to expedite the mass transfer and ease manipulation using less expensive equipment (Xu and Pan, 2013). UAE was used to extract a range of nutritious components, including phenolics, saponins, polysaccharides, and carotenoids.

In this project, the OPEFB was used to extract the cellulose through the UAE method. The cellulose was further processed to its cellulose derivative, CMC for further observations. The alkali cellulose was treated with the sodium salt of chloroacetic acid to produce CMC (BeMiller, 2019). After that, the determination of degree of substitution (DS) was conducted on the CMC to identify the number of the carboxymethyl groups in the molecular unit of the anhydrous glucose units. Through DS, the behaviour of CMC solutions can be discovered. CMC's salt tolerance, hygroscopicity, and alcohol tolerance rise as its DS increases, whereas its thixotropic nature diminishes (BeMiller, 2019).

1.2 Problem Statement

Although the oil palm empty fruit bunch (OPEFB) is classified as agriculture waste, it is a potential source to produce valuable products such as cellulose and CMC instead of discarding them. The OPEFB cellulosic fibres are densely packed with lignin, hemicelluloses, tiny wax depositions, and inorganic components. In order to extract cellulose from the OPEFB, the lignin, hemicelluloses, tiny wax depositions, and inorganic components need to be removed. The lignin, hemicelluloses, pectin, ash, and their spatial interlinks have formed as physical barriers to preserve cellulose from extraction (Zhao, Zhang and Liu, 2012). Lignin removal is a well-known pre-treatment technique for improving the efficiency of cellulose extraction. This technique can remove the physical barrier to cellulose accessibility as well as reduce the likelihood of non-productive adsorption of the cellulolytic enzyme onto the lignin matrix (Geng et al., 2018).

There are a lot of extraction methods introduced such as solvent extraction, distillation method, Soxhlet extraction, maceration and sublimation (Zhang, Lin and Ye, 2018). It is a step to get the natural product by separating it from the raw material. The extraction process of choosing is mostly determined by the running cost, simplicity of operation, amount of organic solvent required, and sample throughout. In the cellulose extraction process, chemical treatment is an ordinary method used to get a high purity of cellulose. Both acidic and alkali chemicals can be used in the chemical treatment (Izzaha et al., 2021). The acidic chemicals such as hydrochloric acid and sulphuric acid are often utilised in the acid hydrolysis of lignocellulosic biomass (Lenihan et al., 2010). When the acid used is too concentrated, it causes the corrosion of the equipment, whereas diluted acid may require a greater temperature to degrade hemicellulose, resulting in the creation of hazardous chemicals such as furfural and 5-hydroxymethyl furfural (HMF) (Sugiwati et al., 2021).

In addition, the alkali treatment procedure is used to dissolve lignin and hemicellulose by using sodium hydroxide. Alkaline treatment has been identified as

one of the most effective chemical treatments and modifications to natural fibre (Islam, Pickering and Foreman, 2010). This technique applying an alkali solution with a certain concentration and other conditions to natural fibre in order to remove lignin and a portion of hemicellulose while also increasing enzyme comprehensibility to cellulose (Latip et al., 2019). According to Sun and Cheng (2002), saponification of intermolecular ester linkages that cross-link carbohydrate and lignin in biomass is aided by an aqueous alkaline media.

In this research, ultrasonic is applied in the extraction process of cellulose from OPEFB. With ultrasonic-assisted extraction (UAE), it can boost the extraction yield and efficiency as well as minimise the amount of solvent use, resulting in time and energy savings with excellent repeatability (Xu and Pan, 2013). It is important to identify the best condition for the ultrasonic effect in the extraction of cellulose from OPEFB. Hence, the solvent used in the UAE was changed with different conditions. The effect of different factors in the UAE to isolate cellulose from OPEFB is a very important information for investigation. The success of this research would ease the extraction of cellulose from OPEFB. Also, this research would expose the extraction efficiency of cellulose from OPEFB and provide better understanding on the UAE performance.

1.3 Objectives

Based on the problem statement, the research objectives are:

i. To prepare and analyse powder form of oil palm empty fruit bunch (OPEFB).

ii. To study the strength of the ultrasonic under different types of solvent with various concentration of solvent, sonication duration and temperature.

iii. To evaluate the strength of the ultrasonic via characterization and carboxymethylation of the extracted products.

1.4 Scope of Study

The scopes of study are shown as below:

i. Study the preparation of oil palm empty fruit bunch (OPEFB) for cellulose production. The OPEFB is dried and cut into small pieces for cellulose extraction. The characteristics of OPEFB is analysed.

ii. Study the ultrasonic effect in cellulose extraction from OPEFB. The treatment is conducted by using various types of solvent, amount of solvent and temperature to evaluate the strength of the ultrasonic.

iii. Study the structure of OPEFB by conducting the characterization tests such as FTIR, DSC and HPLC to identify its properties.

iv. Study the yield of the extracted cellulose of OPEFB. The extracted cellulose is further processed to produce CMC.

CHAPTER 2

LITERATURE REVIEW

2.1 Cellulose Production Feedstock

The world's energy supplies are running out, thus a constant quest for alternate and renewable energy sources is vital nowadays. In order to find alternative energy sources, the exploration of cellulose from agricultural waste is significant due to its environmentally friendly and biocompatible properties. Based on energy content, cellulose is the most abundant renewable natural material, and it is a cost-effective energy source (Tiwari and Verma, 2019). About 10^{11}-10^{12} tons of cellulose is produced annually (Aswini, Gopal and Uthandi, 2020) and $7.5x10^{10}$ tons of cellulose can be consumed worldwide per year (Abdullah et al., 2016). Although some living species such as algae, bacteria, fungi, or some sea animals contain cellulose, plants which can be known as lignocellulosic sources, are still the main source of cellulose.

For the commercial source of cellulose, wood pulp and cotton fibres are the primary occurrence of cellulose. Large-scale industrial infrastructures for collecting, processing, and extracting cellulose are now accessible (George and S N, 2015). The paper and building products are commonly generated by wood pulp while cotton fibres are applied in numerous cellulose derivatives or chemical engineering use in the production of clothes, chromatography, paints, and explosives (Lavanya et al., 2011). Normally, deforestation is needed for large amounts of cellulose from the wood pulp and it will cause environmental concern. According to Hose et al. (2003), pesticides used in cotton production have also been shown to have a negative impact on river

ecosystems. For the purpose of reducing the environmental impact, the use of readily obtainable raw materials such as sugarcane bagasse, banana, and paddy straw and others, might serve as sustainable source for the synthesis of cellulose derivatives (Cheng, Catchmark and Demirci, 2009). Lately, the oil palm empty fruit bunch (OPEFB) which is a plantation waste, is investigated as an alternate feedstock for cellulose production.

As a result of crop production, large amounts of cellulose-rich waste biomass are created as plant leftovers such as empty fruit bunch, rice shells, sugarcane bagasse, maize straw, and others. The biomass can provide an outstanding amount of free raw material that has the potential to be transformed into important chemical feedstocks such as cellulose. In Malaysia, oil palm can define as the largest agricultural crop and 22 to 23 million tons of OPEFB can be produced annually (Padzil et al., 2020). The utilization of agriculture waste can contribute to reduce the undesirable environmental problem. There are three main polymers which are cellulose, hemicellulose, and lignin in lignocellulosic biomass, and they are chemically connected by non-covalent forces and covalent cross-linkages (Tiwari and Verma, 2019). The composition of the lignocellulosic agriculture waste is shown in Table 2.1.

Table 2.1: The Composition in Agriculture Waste.

Agriculture Waste	Cellulose %(w/w)	Hemicellulose %(w/w)	Lignin %(w/w)	References
Sugarcane Bagasse	30.2	56.7	13.4	(El-Tayeb, Abdelhafez, Ali and Ramadan, 2012)
Rice Straw	43.0	33.0	20.0	(Chen, Yu, Zhang and Lu, 2011)
Corn Stalks	35.0-45.0	25.0	17.0-21.0	(Qu et al., 2011)
Wheat Straw	29.0-35.0	26.0-32.0	16.0-21.0	(Tiwari and Verma, 2019)

Cotton Seed Hair	80.0-95.0	5.0-20.0	0.0	(Sharma et al., 2019)
Oil Palm Empty Fruit Bunch	37.1	39.9	18.6	(Megashah, Ariffin, Zakaria and Hassan, 2018)

Of late, the sugarcane bagasse, which is the by-product of crushed sugarcane, is a potential cellulose production feedstock as it can be found easily and has high cellulose content. Mahmud and Anannya (2021) stated that the extracted cellulose from sugarcane bagasse does not have exceptional mechanical properties, nevertheless their availability and versatility make them a useful material, particularly in composite industries. Other than that, the rice straw, corn stalks and wheat straw are abundant cellulosic by-product from the crop production. According to NL Agency (2013), the rice straw and wheat straw are low in nitrogen but abundant in ash. The high ash concentration brings on low degradability properties and they become less attractive compared to other cellulosic products. The commercial uses for those cellulosic by-products are still limited and needs to be developed more (Abdel-Halim, 2014). Also, each cotton seed may produce between 5000 and 20,000 solitary seed hairs and the cotton seed hair composes of high amount of cellulose. The cellulose extraction of the cotton seed hair is complicated, and it is not mature enough to be utilized commercially (Martins et al., 2011).

The high cellulose and low lignin content condition in the OPEFB opens up a wealth of potential resources for cellulose. Nazir et al. (2013) showed that lignocellulosic OPEFB fibres with a cellulose concentration of 444 g kg^{-1} can be converted into value-added biopolymers. At the same time, the OPEFB is cheap and plentiful to be the cellulose production feedstock. According to Rodríguez Couto (2008), the agricultural industries generate massive amounts of garbage each year, posing a severe disposal dilemma. As a result, the exploration of agriculture waste is vital so that the burden on environmental issues could be decreased. In order to obtain high purity of cellulose, the effective removal of lignin, hemicellulose, and other contaminants are the major study in the cellulose extraction process. Thus, the

investigation of the extraction methods and conditions can be important to isolate the cellulose.

2.2 Properties of Oil Palm Empty Fruit Bunch

The oil palm is a species of Elaeis guineensis in the Arecaceae family. This species originates in West African tropical forests (Britannica, 2021). Oil palm is grown as an agricultural crop in Malaysia which can produce a large number of biomass materials, primarily fibres from the trunk, frond, mesocarp, palm kernel shell, and empty fruit bunch (Abdullah and Sulaim, 2013). The oil palm empty fruit bunch (OPEFB) is produced when the fruits are extracted or prior to oil pressing. Based on Figure 2.1, the OPEFB is the major biomass from the oil palm industry as 23% to 25% of OPEFB can be generated from a ton of fresh fruit bunches. Every year, about 15.8 million tonnes of empty fruit bunch (EFB) are produced from the fresh oil palm fruit (Ying et al., 2014). According to Faizi et al. (2019), the secondary cycle for OPEFB is not mature and normally the OPEFB will be disposed of by burning, which is always a source of pollution. OPEFB is currently thought to be a possible feedstock to produce a few renewable and lucrative biofuels and bio-based chemicals such as cellulose, lignocellulose and sugar (Sunday Noah, 2022). Thus, the presence of vast amount of OPEFB is potentially to provide a sustainable resource for the cellulose extraction.

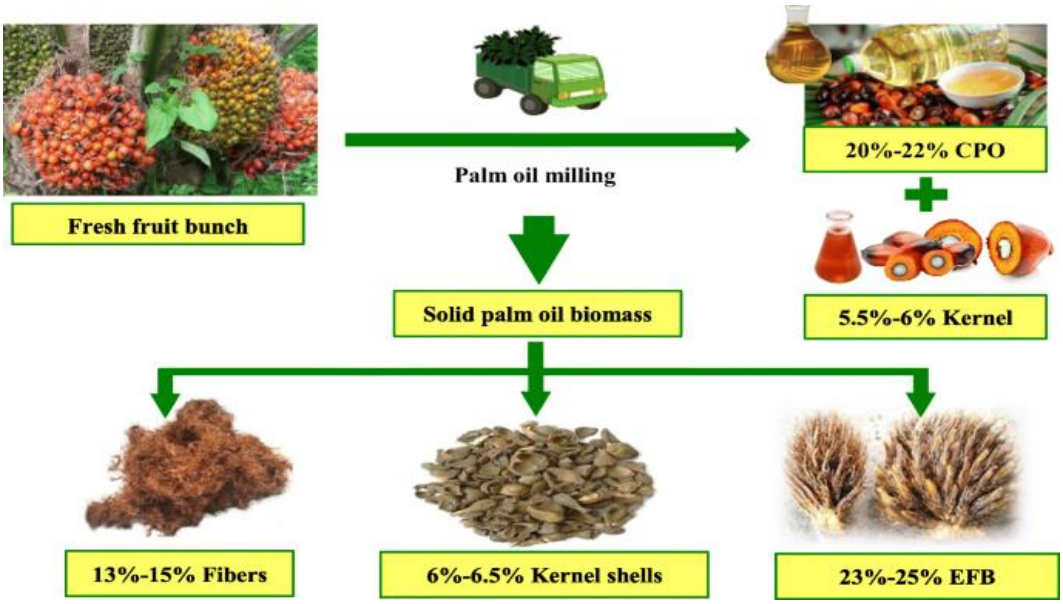


Figure 2.1: The Biomass Produced from the Oil Palm Industry. (Yan et al., 2019)

The OPEFB is a thick and voluminous brown bunch with an uneven form that weighs around 3.5 kg with a thickness of 130 mm and can range in length from 170 to 300 mm and width from 250 to 350 mm (Chang, 2014). Through different sources, the chemical composition of OPEFB can be varied. Chang (2014) states that OPEFB is made up of 20.6-33.5% hemicellulose, 23.7-65.0% cellulose, and 14.1-30.5% lignin while Megashah et al. (2018) revealed that 37% cellulose, 39.9% hemicellulose and 18.6% lignin in the OPEFB. Subsequently, extractives, pectin and pigments are present in lesser amounts in OPEFB (Ngadi and Lani, 2014). Collectively, these studies outline a critical role that there are high percentage of cellulose in the OPEFB. The chemical composition in the OPEFB may be varied depending on the age, size, and growth phase of the oil palm tree. The geographic location, soil quality, climatic influences, and testing methods employed also can result in different properties of the OPEFB.

Abdullah et al. (2016) have stated that the lignocellulose has a parallel rod-like structure, cellulose as its backbone, and lignin as well as hemicellulose have been deposited on top. The OPEFB consists of naturally occurring composites which are hard, crystalline cellulose microfibrils embedded in a soft, amorphous hemicellulose and lignin matrix as shown in Figure 2.2. The cellulose is a highly crystalline linear

glucose polymer while the hemicellulose is a branching polymer composed of pentose and hexose sugars that undergo moderate acid hydrolysis to liberate their sugar components. For the lignin, it is thermally stable and prevents bio-composite materials from further deterioration. The lignin gives the matrix a structural function in which cellulose and hemicellulose are incorporated (Harmsen et al., 2010). When the lignin is degraded, the interior content becomes more prone to breakdown, and the fibres physically begins to lose its surface properties (Hassan et al., 2010).

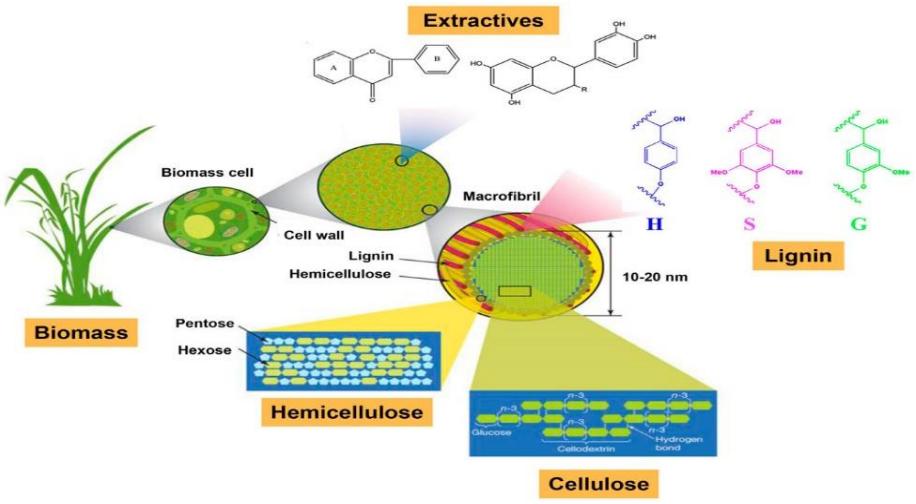


Figure 2.2: The Important Components in a Biomass. (Huang et al., 2022)

Because of their complicated structures, the lignocellulose components of OPEFB fibres have rendered them resistant to various physico- and biochemical conversion processes. The hydrogen connections formed between the distinct layers of cellulose chains, together with the crosslinking of lignin with both cellulose and hemicellulose, have resulted in a complex web of linkages that not only offer structural strength to OPEFB fibres, but also provide a degrading problem (Chang, 2014). To get these compounds with high purity, an isolation strategy should be used. In this research, the isolation method for cellulose was investigated so that the oil palm biomass waste can be reduced effectively.

2.3 Cellulose Extraction Process

Cellulose is the most abundant, renewable polymer material accessible today on a global scale. When lignocellulose is untreated, it is unsuitable for industrial usage because of its recalcitrance and diverse composition (Shrotri, Kobayashi and Fukuoka, 2017). For successfully extracting different types of materials, different solvents and procedures will be used and developed. In the cellulose extraction process, the removal of the amorphous materials such as hemicellulose and lignin are compulsory to produce the high purity of cellulose from the biomass. Due to the less closely linked microfibril in the main cell wall than wood, the nonwooden biomass sources such as oil palm empty fruit bunch (OPEFB) contain less lignin and need fewer processing steps and energy usage (Rojas, Bedoya and Ciro, 2015).

Figure 2.3 shows the general processing steps of cellulose extraction. Firstly, the dried biomass is grinded into small pieces. Through the grinding process, the cell wall structure is torn down by shear stresses, but this process may cause mechanical damage of fibre for some biomass (Rojas, Bedoya and Ciro, 2015). After that, the cellulose can be isolated through chemical treatment assisted mechanical disintegration. The mechanical treatment can break the primary and secondary cell wall without significantly damaging cellulose. However, the production cost for mechanical treatment is higher compared to the chemical treatment. The most often used chemicals for isolation are sodium hydroxide, acetic acid, aqueous ammonia, and hydrogen peroxides as they can promote delignification and bleaching (Mohaiyiddin et al., 2016). According to Abdullah et al. (2016), the chlorite technique for delignification and cellulose extraction from wood products employs the use of acidified sodium chlorite. Other than that, the chemical treatment through sodium hydroxide is a popular extraction method but it will cause the harmful side effects from alkaline or alkaline earth hydroxides and takes a huge amount of water to clean fibres after extraction procedure treatment (Boonterm et al., 2016). After the removal of the amorphous materials, the treated biomass will undergo purification by washing and drying. Then, the bleaching process is necessary to obtain high purity of cellulose from the biomass as well as enhance age resistance preventing brittleness and yellowing.

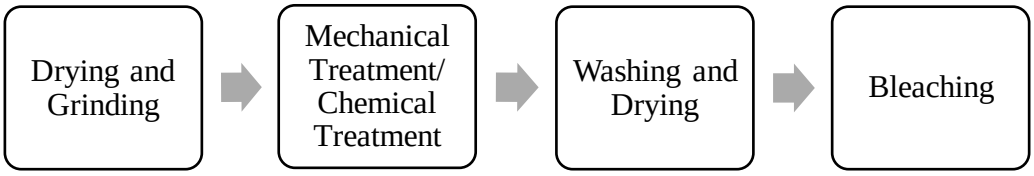


Figure 2.3: Process Flow Diagram of Cellulose Extraction.

A lot of studies have showed that the removal of unwanted materials such as lignin, hemicellulose and extractives is important in the cellulose extraction process. Although the chemical treatments as well as the mechanical treatments are varied in different research, the purpose of the treatments is same. The treatments are implemented to obtain high purity of cellulose. The extraction yield is determined by treatment conditions such as chemical type, concentration utilised, time, and temperature (Ching and Ng, 2014). In this research, the alternative method to extract the cellulose is determined.

2.4 Pre-treatment Process in Cellulose Extraction

The presence of lignin and hemicelluloses, as well as cellulose's high degree of crystallinity contribute to the complexity of lignocellulosic biomass such as OPEFB. Pre-treatment is required for lignocellulosic biomass because of the complicated structure of cellulosic biomass in order to make it more accessible for chemicals to extract cellulose as shown in Figure 2.4. The optimal pre-treatment attempts to remove lignin, remove hemicellulose, change lignin structure, and increase lignocellulosic material surface area and porosity (Zulkiple, Maskat and Hassan, 2016). According to Harmsen et al. (2010), the pre-treatment may be broadly categorised into four types: physical, biological, chemical, and combination of pre-treatment.

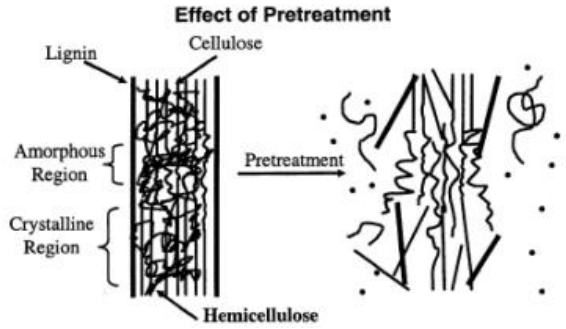


Figure 2.4: The Effect of Pre-Treatment Process. (Hsu et al, 1980)

2.4.1 Autoclaving

Autoclaving is an ordinary laboratory work as it is normally used to kill hazardous bacteria, viruses, fungi, and spores. Autoclaves exist in a variety of sizes, temperatures, and pressure capabilities. However, it is typically featuring a cylindrical form to handle the internal gas pressure while providing the most usable inside space for parts and tools of varied geometries (Fernlund, Mobuchon and Zobeiry, 2018). The autoclaving method takes use of the phenomena that elevate the boiling point of water under high pressure. With the increasing heat content in the steam, it can penetrate through the object and kill the bacteria. The autoclaving machine, it uses high pressure and a recommended temperature of 121°C for 15-20 minutes to disinfect the materials (Microlit, 2021). In the cellulose extraction process, the high temperature steam can break the chemical bonding such as the aromatic -OH, C-C, R-O-R of the lignin and the H-bond in RCOOR and R-O-R of the hemicelluloses or celluloses (Abdullah et al. 2016).

Because of its ability in creating large fibre volume fractions, low porosity levels, and compatibility with highly toughened matrix systems, autoclave processing has generally been the conventional treatment method for high-performance composites (Fernlund, Mobuchon and Zobeiry, 2018). From the study area, Abdullah

et al. (2016) applied the autoclave and ultrasonication as pre-treatment for cellulose extraction of OPEFB. The OPEFB sample was autoclaved at 121°C and 1.5 bar for an hour. Through the autoclave pre-treatment, the high energy steam is generated, and it dislocates fibrillar arrangements along with aids in the penetration of sodium hydroxide and hydrogen peroxide for partial oxidation (Nazir et al., 2013). Also, the autoclave treatment is used with combination of alkaline pre-treatment in coconut husk for 40 minutes at 121℃ and 1 atm so that high efficiency of lignin removal and hemicellulose dissolution can be achieved (Cabral et al., 2016). In 1996, Oosterveld et al. (1996) published a paper to extract polysaccharides from sugar beet pulp through autoclave treatment at 121℃ for 40 minutes.

The studies clearly indicate that the autoclaving pre-treatment can function effectively in cellulose extraction. Although the autoclaving process is not a new developed technology, it is still popular to be used in the pre-treatment for cellulose extraction due to its economic cost and consistent pressure provided. Nevertheless, an autoclave cannot ensure uniform heat transmission, and selecting the thermal system for a particular production load remains difficult (Fernlund, Mobuchon and Zobeiry, 2018).

2.4.2 Microwave Treatment

Microwaves are electromagnetic waves with frequency ranging from 300 MHz to 300 GHz and wavelengths ranging from 1 m to 1 mm (Banik, Bandyopadhyay and Ganguly, 2003). The microwave technology has been extensively employed for a variety of reasons in the food production and consumption sectors. In contrast to conduction and convection heating, which are dependent on surface heat transfer, microwave heating creates heat through direct interaction between a heated item and an applied electromagnetic field (Hu and Wen, 2008). The microwave treatment is conventional, and it can ease the cellulose extraction effectively through its electromagnetic radiation

to the material. The microwave treatment of lignocelluloses can selectively warm the more polar region and generates a "hot spot" with the inhomogeneous materials (Hu and Wen, 2008). It can evaporate the water in the cell and put significant pressure on the cell wall, causing the cells to break apart.

The microwave treatment is normally combined with the chemical treatment. According to Ndruru et al. (2019), microwave irradiation properly boosts heating to alkaline, bleaching, and hydrolysis treatment. The cellulose from Theobroma cacao L. husk can be extracted through the microwave-assisted extraction in 30 minutes with power rated 100W (Ndruru et al., 2019). The microwave-assisted extraction can increase efficiency of the cellulose isolation in the alkali treatment. Other than that, the microwave treatment can be utilized in the synthesis of carboxymethyl cellulose from cellulose isolated of brewer's spent grain as the microwave irradiation is a viable way for modifying cellulose's physical-chemical characteristics (Santos et al., 2015). The study of Nomanbhay, Hussain and Palanisamy (2013) also stated that well controlled microwave pre-treatment considerably enhances the enzymatic saccharification of the OPEFB by removing more lignin and hemicellulose.

The microwave exposure might alter the ultrastructure of cellulose, destroy lignin and hemicellulose in lignocellulosic materials, and make lignocellulosic materials more susceptible to enzymatic degradation (Binod et al., 2012). The microwave treatment can be seen to be popular among the cellulose extraction treatments as it can easily cope with the other chemical treatments and increase the chemical reaction rate. Though there are certain limitations to microwave heating, such as non-uniform heat distribution and expensive equipment prices, the microwave treatment can be a promising method to accelerate the breakdown of crystal structures in cellulose extraction (Banik, Bandyopadhyay and Ganguly, 2003).

2.4.3 Organosolv Treatment

To extract cellulose from the lignocellulosic biomass, an organic solvent termed organosolv can be used in the treatment process. The organosolv treatment can remove lignin with an organic solvent or combination of organic solvents and water. In the organosolv treatment, ethanol, methanol, acetone, and ethylene glycol are typical solvents used and the choice of solvents is vital to increase the treatment efficiency (Harmsen et al., 2010). The solvent's principal function is to promote tissue impregnation and solubilization of the generated lignin fragments (Sannigrahi, Ragauskas and Miller, 2010). The temperature of the treatment can reach 200 ^{o}C but a lower temperature is acceptable in the presence of a catalyst. The organosolv treatment is a promising process by reasons of the organic solvents are always simple to distillate and reuse as well as the chemical recovery can separate the lignin as a solid material (Zhao, Cheng and Liu, 2009). Nevertheless, the organosolv treatment is expensive to be used because extra equipment is required in recycling the solvent.

In the 1970s, the organic solvent was already utilized in the production of pulp and high-quality lignin. Through the organosolv treatment, it fractionates and solubilizes lignin and hemicellulose using a combination of organic and aqueous solvents (Shrotri, Kobayashi and Fukuoka, 2017). Lower alcohols, particularly ethanol, are the most suitable solvents due to their low cost and ease of recovery by distillation. The lignocellulose can be fractionated effectively without considerable loss of components or monomeric sugars owing to degradation. As a result, the approach is advantageous for catalytic lignocellulose conversion since all three components may be handled individually following pre-treatment (Shrotri, Kobayashi and Fukuoka, 2017).

According to Macfarlane, Mai and Kadla (2014), the organosolv treatment is more effective for hardwoods rather than softwoods such as OPEFB as their lignin chemical structures are varied. The organosolv treatment is more applied in the lignin extraction. It is because after the organosolv treatment solid crystalline cellulose is left

behind with dissolved lignin and hemicellulose. Subsequently, the solvent is separated to produce aqueous solutions of hemicellulose fractions and precipitates of lignin (Shrotri, Kobayashi and Fukuoka, 2017). The lignin produced through the organosolv treatment is normally with great purity, high quality, low molecular weight, and without any sulphur (Bai et al., 2013). The study of Huijgen et al. (2014) in producing high purity wheat straw lignin using organosolv treatment supports the evidence of organosolv treatment in production of high purity lignin. Thus, more studies on lignin extraction using organosolv treatment are done compared to cellulose extraction.

Despite that, the organosolv treatment in ethylene glycol with sodium hydroxide as an alkaline catalyst can produce 90 wt% cellulose from the OPEFB within 45 minutes at 80 ^{o}C (Chin et al., 2021). Furthermore, organosolv treatment can couple with ultrasound treatment to increase the cellulose selectivity from the oil palm fronds (Ofori-Boateng and Lee, 2014). The study found that the pre-treatment temperature and time can be reduced by combining organosolv treatment with ultrasound treatment. This indicates that the organosolv treatment can be utilized in the cellulose extraction of lignocellulosic biomass. Although considerable body of research is done, much less is known about the cellulose extraction through organosolv treatment from the OPEFB.

2.4.4 Alkali Hydrolysis Treatment

The alkali hydrolysis treatment is a common chemical pre-treatment method in the cellulose extraction process. Normally the alkaline solutions such as sodium hydroxide (NaOH), lime ($Ca(OH)_2$), or ammonia (NH_3) are used in the treatment to eliminate lignin and a portion of the hemicellulose as well as improve the enzyme accessibility to the cellulose (Duangwang and Sangwichien, 2012). The process of alkaline hydrolysis depends on saponification of intermolecular ester linkages connecting xylan hemicelluloses and other components such as lignin. The alkaline solution

breaks down the ester and glycosidic linkages, causing the changing of lignin structure and dissolving of hemicellulose. Among the alkaline solutions, the sodium hydroxide (NaOH) is widely used as it is beneficial in enhancing the digestion of low-lignin hardwood and agricultural leftovers (Bali et al., 2014).

According to Duangwang and Sangwichien (2012), the alkali pre-treatment can be done at mild temperatures in the interest of impeding lignin condensation and increasing lignin solubility. At the same time, it takes a longer time and a higher concentration of the alkali to complete the hydrolysis process. A standard treatment is immersing fibres in a 5% sodium hydroxide solution for 48 hours at 30°C (Rojas, Bedoya and Ciro, 2015). Controlling of the alkaline solution is vital to prevent the degradation of cellulose. In the literature, a cellulose yield of 68.8% can be extracted from the OPEFB in the condition of 15% NaOH concentration at 130^{o}C for 40 minutes (Duangwang and Sangwichien, 2012). Besides, Mustikaningrum, Cahyono and Yuliansyah (2021) reported that a total of 55.6% lignin and hemicellulose is removed from oil palm trunk using 4 %(w/v) NaOH. By promoting hydroxyl group ionisation to alkoxides, it can have a direct impact on cellulose fibrils, polymerization degree, as well as lignin and hemicellulose content. Other than that, the crystallinity index of cellulose increases from 38% to 63% after the OPEFB undergoes the alkali hydrolysis treatment (Ngadi and Lani, 2014).

Through the studies, the NaOH has considerable promise in alkaline hydrolysis treatment since it acts at low temperatures and has a remarkable delignification capability in comparison to its harshness. Nevertheless, the price of NaOH is high and it is very corrosive. To increase the efficiency of the pre-treatment process, the alkali hydrolysis treatment can work with other mechanical actions. The alkaline hydrolysis treatment can combine with ultrasonication. The ultrasonic-assisted alkali extraction can extract 77.14 wt% of cellulose from the OPEFB using 0.75 M potassium hydroxide (KOH) (Wong et al., 2021). The study proved that the alkali hydrolysis treatment can cope well with the mechanical treatment to enhance the efficiency of removing the impurities such as hemicellulose and lignin.

2.4.5 Acid Hydrolysis Treatment

The acid hydrolysis treatment is a favourable method in cellulose extraction process because it is a direct hydrolysis process in biomass conversion and the process is simple. Sulphuric acid (H_2SO_4) and hydrochloric acid (HCl) are the most widely employed acid in the acid hydrolysis treatment. To extract cellulose, the acid hydrolysis procedure can be conducted in either concentrated or diluted form. The acid hydrolysis treatment can be a cost-effective approach because it employs economical mineral acid and a moderate reaction condition (Shrotri, Kobayashi and Fukuoka, 2017). In the acid hydrolysis treatment, the influence of acid concentration and temperature is significant.

In the dilute acid hydrolysis treatment, lignocellulose is sprayed with 0.2-2.5 wt% sulphuric acid and then stored at a high temperature which is between 127^{o}C-210 ^{o}C for a few minutes or a few hours depending on the temperature set (Shrotri, Kobayashi and Fukuoka, 2017). Through the reaction, the hemicellulose is hydrolysed, and the cellulose and lignin are left behind. With the removal of hemicellulose, it can enhance porosity as well as improve the enzymatic digestibility, with total hemicellulose removal generally resulting in maximal enzymatic digestibility (Chen et al., 2007). Based on Fatriasari et al. (2018), the reducing sugar yield of Jabonkraft pulp contain 0% lignin using the microwave-assisted acid hydrolysis at 190^{o}C. The study also indicates that the rising lignin removal is accompanied by the increment of hemicellulose loss, and it causes the increase of cellulose in the pulp. The dilute acid treatment is successfully dissolving the hemicellulose of biomass. Nazir et al. (2013) also proved that the dilute formic acid with hydrogen peroxide can extract 64% w/w of cellulose from the OPEFB.

Despite its low cost, the dilute acid hydrolysis of cellulose has some drawbacks. It generates by-products such as hydroxymethylfurfural (Harmsen et al., 2010). At the same time, the acid is caustic and neutralisation are needed for the solid waste produced. On the other hand, the concentrated acid hydrolysis treatment can normally

operate at mild temperature and without any enzymes to achieve high yields. Nevertheless, the concentrated acid will corrode the equipment and more affords are required to neutralize it. Thus, the cost operation for concentrated acid hydrolysis treatment is more expensive compared to the dilute acid hydrolysis treatment. The acid hydrolysis treatment is limited to be applied as it does not work well in removing lignin.

2.4.6 Ultrasonic-assisted Treatment

The ultrasonic-assisted treatment is a mechanical technique that isolates cellulose from lignocellulosic biomass utilising the hydrodynamic forces of ultrasound. It is an established technology for treating wastewater treatment plant sludge and it is getting attention to be used to extract the cellulose from the biomass (Harmsen et al., 2010). Acoustic cavitation is the primary mechanism responsible for ultrasonic-assisted treatment's high extraction efficiency (Xu and Pan, 2013). The ultrasonic-assisted treatment employs high frequency sonic vibrations that pass through the solution, causing cavitation bubbles. When cavitation bubbles burst on the surface of the plant sample matrix, shockwave-induced cell wall destruction cleaves the complicated network structure of the lignocellulosic biomass and promotes the removal of components such as lignin and hemicellulose (Al Jitan, Alkhoori and Yousef, 2018). The ultrasonic-assisted treatment is normally conducted with other chemical treatments such as acid hydrolysis, alkali hydrolysis and organsolv treatment. With the ultrasonic-assisted treatment coped with chemical treatment, it can significantly increase solvent penetration into the matrix and expedite the chemical reactions of targeted chemicals (Xu and Pan, 2013).

Abdullah et al. (2016) revealed that the cellulose can be isolated from OPEFB through the autoclave-based and ultrasonication pre-treatments. In the research, OPEFB was suspended with sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2). Then, the solution with OPEFB was sonicated at 40 kHz for few hours at room

temperature. After that, the treated OPEFB was dried in the oven at 100°C for half day before it was bleached from yellow to white fibre. From the result, it found that the ultrasonic treatment with H_2O_2 yielded 49% cellulose successfully.

Other than that, Wong et al. (2021) also carried out an ultrasonic-assisted alkali treatment to extract the cellulose from OPEFB. The treated OPEFB was mixed with the potassium hydroxide (KOH) and heated till 80°C. Next, the ultrasonication was carried out at ultrasonic amplitude of 30% and temperature of 80 °C, for 30 minutes. The treatment using 0.75M KOH is able to extract highest cellulose content of 77.14 wt%. Overall, both studies give evidence that the ultrasonic-assisted treatment can be conducted to extract the cellulose from OPEFB successfully with different solvents used. The chemical such as alkali with ultrasonic treatment can damage the cell wall of OPEFB as well as solubilise hemicellulose that was present on the outer surface of OPEFB, resulting in an increase in cellulose quantity.

The ultrasonic-assisted treatment has the ability to aid in the disruption of different lignocellulosic materials. It can accelerate the cellulose extraction process effectively and improve the extraction yield in a short period. At the same time, it is easy to use, requires less solvent than other procedures, and may be used with other extraction techniques (Louie et al., 2020). Since this treatment can be conducted at room temperature, it can help to avoid oxidation and degradation of natural goods. Consequently, the ultrasonic-assisted treatment can be performed with other chemical treatments to extract the cellulose from OPEFB.

2.5 Affecting Factors on the Ultrasonic-assisted Treatment

The ultrasonic-assisted treatment has successfully used in the cellulose extraction process. It can ease the cellulose extraction process with higher productivity, lesser

solvent consumed as well as higher purity of product (Chemat et al., 2017). Yet, the process is energy demanding, and extensive research are required to improve the process parameters for large-scale applications. There are some major factors such as ultrasonic strength, extraction temperature, extraction time and the solvent composition affecting the ultrasonic-assisted treatment.

2.5.1 Ultrasonic Strength

The ultrasonic-assisted treatment can be implemented using different ultrasonic strength. Ultrasonic-assisted extraction (UAE) can be accomplished using a probe-type ultrasonic homogenizer or an ultrasonic bath. Despite the fact that both methods use ultrasonic to treat the sample, there are substantial differences in their efficacy, efficiency, and process capacities. When it comes to ultrasound intensity, amplitude, homogeneous processing, and repeatability, probe-type ultrasonicators much outperform ultrasonic baths (Hielscher Ultrasonics, n.d.). Besides, the ultrasonic strength can be varied due to the ultrasonic power used. The most typical frequencies utilised in UAE procedures are between 20 and 100 kHz.

The different equipment used in the ultrasonic-assisted treatment may bring out different effects on the lignocellulose biomass. In general, the ultrasonic bath device may only deliver 20-40 W/L of ultrasonication with a relatively non-uniform distribution, but the ultrasonic probe device can provide 20,000 W/L into the fluid (Hielscher Ultrasonics, n.d.). Nevertheless, ultrasonic bath can work in a larger volume than the ultrasonic probe. An ultrasonic bath is better suited for low-power applications that require diffuse rather than concentrated energy. Nascentes et al. (2001) reported that the ultrasonic bath's characterisation is critical for sonochemical processes, and the strength of cavitation increased linearly with sonication time. Also, Jambrak et al. (2008) showed the ultrasonic effect on pH, electric conductivity and surface of the biomass using ultrasonic bath and ultrasonic probe. Since the 20kHz probe is more

powerful than the 40 kHz bath, the pH reduction was greatest with the ultrasonic treatment of probe for 10 minutes.

During the ultrasonic-assisted treatment, the electrical energy is converted into ultrasonic energy by ultrasound generators. which is a type of mechanical energy. Ultrasound frequencies ranging from 10 to 100 kHz are beneficial for aiding in the decomposition and breaking of lignocellulosic biomass (Ur Rehman et al., 2013). According to Ni, Li and Fan (2021), cellulose nanoparticles were created by treating cellulose microparticles from ginkgo seed shells with ultrasonic treatments at output powers around 150W to 600W and durations from 10 minutes to 60 minutes. The increase of ultrasonic power can decrease the reaction time as well as provide higher efficiency to alter the morphology and properties of cellulose nanoparticles.

Although the academic literature of the ultrasonic strength on the efficiency of ultrasonic-assisted extraction is less, the ultrasonic-assisted treatment is successful to be applied in cellulose extraction in various ultrasonic strength. In the study, the pH of the solvent is checked from time to time in order to determine the ultrasonic strength of the ultrasonic bath as the sonication can affect the pH of the solution.

2.5.2 Extraction Temperature

In the ultrasonic-assisted treatment, the extraction temperature is one of the significant impacts on the extraction efficiency. Temperature is a critical operating parameter that influences viscosity, mass transfer rate, diffusivity, solubility, and cavitation (Moradi et al., 2017). When the temperature increases, it causes a decrease in viscosity and surface tension, as well as a rise in vapour pressure. Capelo-Martínez (2009) stated that the increasing vapor pressure results in more solvent vapours will reach the bubble cavity, resulting in a greater number of cavitation bubbles that will collapse less

forcefully and lessen sonication effects. Thus, the ultrasonic-assisted treatment is more preferred to be conducted in lower and controllable temperature to avoid the reducing of collapse of cavitation bubbles due to high temperature.

Prior to the work of Yi et al. (2019), the role of temperature in ultrasonic-assisted alkaline extraction is significant to improve the yield of cellulose. The yield of cellulose from treated sugarcane bagasse (SCB) increased when the temperature had risen from 60 ^{o}C to 70 ^{o}C. It is because more kinetic energy is gained in the solution when the temperature increases, and the alkaline solution can interact more with the biomass to remove the other components. Nevertheless, the cellulose content did not increase at 80^{o}C since the hemicellulose, lignin as well as the cellulose were degraded by the alkaline solution.

The temperature contributes much in the efficiency of the ultrasonic-assisted extraction. Increasing of temperature can cause the enhancement of cavitation bubbles and a better collision between the solid-solvent contact area. This impact, however, is diminished when the temperature is near the boiling point of the solvent (Chemat et al., 2017). Hence, it essential to select an extraction temperature based on the target component of extraction. In this study, the optimum temperature of the solvent for the ultrasonic-assisted extraction is investigated to get the highest yield of cellulose without any degradation.

2.5.3 Extraction Time

The ultrasonic-assisted treatment can reduce the extraction time compared to conventional extraction method. The extraction duration for the ultrasonic-assisted treatment is varied depends on the ultrasonic equipment and its strength. Generally,

the increased time without exceeding the limit aids in the delignification of lignocellulosic biomass, thus improving cellulose extraction.

When the ultrasonic-assisted extraction time increases, more time is given for the solvent to penetrate through the surface of plant matrix. Nonetheless, Mojerlou and Elhamirad (2018) demonstrated that the high extraction time could form the sound chemical components which generates free radicals and have oxidative effect on phenolic compounds. According to Methrath Liyakathali et al. (2016), the enzymatic digestibility of sugarcane bagasse rises with increasing of sonication duration and temperature. The higher cellulose digestibility at longer time is owing to the greater exposure to the microjetting and microstreaming processes, which change the biomass structure. However, ultrasonication over an extended length of time may have an undesirable effect due to particle collision and aggregation (Baruah et al., 2018). Dinh Vu et al. (2017) also increased the ultrasonic-assisted extraction time from 10 minutes to 30 minutes in alkaline medium and the lignin separation yields of rice straw increases from 72.8% to 84.7%.

It is clearly indicating that the cavitation along the liquid-solid interface and asymmetrically collapsing bubbles generate micro jets, which can assist the extraction process by allowing more solvent penetration into the plant cell, breakage of plant cell walls, and the release of cellular components with the increasing of the extraction time (Moradi et al., 2017). In spite of that, the yield of cellulose may remain similar or have negative effect with the increasing of extraction time since the time allowed is adequate for the disruption to occur, eliminating the majority of the lignin and hemicellulose. In fact, the extraction time in the ultrasonic-assisted treatment needs to be investigated to optimize the best parameter for the ultrasonic-assisted treatment in the cellulose extraction from OPEFB.

2.5.4 Solvent

The choice of solvents is an important issue in the ultrasonic-assisted treatment for cellulose extraction. A solvent can change the mass transport process and subsequent efficiency of the extraction. Besides, the solvent used in the ultrasonic-assisted treatment will affect the acoustic cavitation phenomenon and cavitation threshold. Thus, the choice of solvents (dilute aqueous solutions of inorganic acids or alkalis, organic solvents or ionic liquids) is essential in selecting the best conditions for ultrasonication pre-treatment (Ko et al., 2015). Despite of the solvent selection, the solvent concentration and the solvent/material ratio are potential factor in affecting the ultrasonic-assisted extraction efficiency.

Zhang, Xie and Che (2020) studied the effect of ultrasound with different solvents such as hexane, ethanol and aqueous enzymatic medium on the bioactive compound in kenaf seed oil. The using of different solvents gives different yield of kenaf seed oil extracted. The combination of ethanol solvent and ultrasonic extraction gives the highest yield as more cavitation bubble is produced in ethanol due to its low vapor pressure.

For cellulose extraction from OPEFB, Solikhin et al. (2021) utilized different solvents such as hot water, ethanol and mixture of ethanol and benzene to remove the extractives in OPEFB for the reason that the extractive compound will inhibit the cellulose purification and cellulosic nanofibers (CNFs) isolation. The ethanol/benzene mixture is the most effective solvent to remove the extractives with the number of CNFs increases by 69.52% using the ethanol/benzene extraction. In the research, it is suggested that ultrasonication can be used in order to avoid aggregation and promote surface modification.

It can see that the research on the various solvents applied in the ultrasonic-assisted treatment is limited. Thus, this study aims to find out the efficiency of different types of solvents such as ethanol, chloroform, and sodium hydroxide solution in

ultrasonic-assisted treatment to extract cellulose from OPEFB. At the same time, the solvents used have different characteristic as the ethanol is polar with moderate pH value, the chloroform is non-polar with low pH value while the sodium hydroxide solution is ionic and high pH value. From the study, it can indicate the suitable solvent in the cellulose extraction process.

2.6 Carboxymethyl Cellulose (CMC)

The carboxymethyl cellulose (CMC) is a white to yellowish powder-like material without any odour and taste. CMC is a water-soluble anionic cellulose derivative and CMC's solubility is influenced by the degree of substitution and polymerization as well as the homogeneity of the substitution distribution (Ergun, Guo and Huebner-Keese, 2016). Lately, CMC is the most widely utilized cellulose ether with various applications such as detergent, food exploration, paper, textile, pharmaceutical and paint industries (Pushpamalar et al., 2006). CMC is often used as an additive in food items due to its harmless properties to ingest in the pure form. For instance, it can used as stabilizer and functional ingredient in ice cream (Parid et al., 2021). The present extraction of CMC from wood has put the wood industry in competition. The cellulose will undergo carboxymethylation in the CMC production. In Figure 2.5, it shows the process flow diagram of the CMC production.

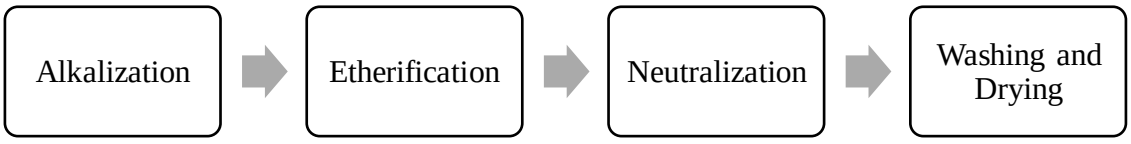
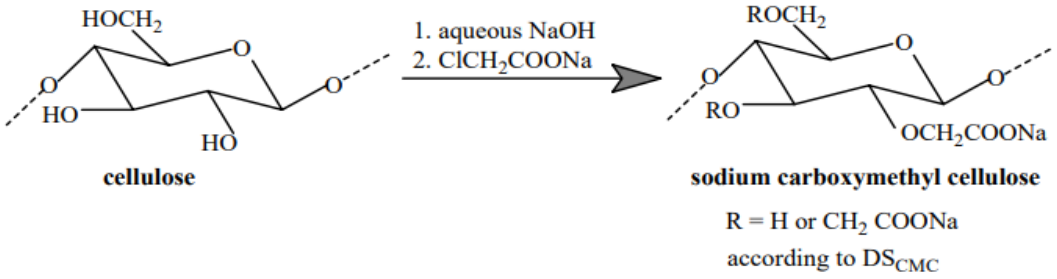


Figure 2.5: CMC Process Flow Diagram.

In the CMC production process, it involves two reaction stages which are alkalization and etherification as shown in Figure 2.6. In the first stage, the cellulose powder is mixed with the sodium hydroxide (NaOH) with the presence of isopropanol as solvent at 20 ^{o}C to 30^{o}C. Klunklin et al. (2020) also stated that the concentration of NaOH can affect the CMC yield and 30% concentration of NaOH gives the highest yield of CMC. It is important to use low polarity solvent such as isopropanol or ethanol to increase the efficiency of dissolution process (Eliza et al., 2015). The solvent functions to expand and disintegrate the cellulose pulp, increasing the reactive surface and allowing NaOH to penetrate (Almlöf Ambjörnsson, Schenzel and Germgård, 2013). When NaOH is combined with the exposed hydroxyl groups, the swollen and dissolved cellulose is converted into alkali cellulose (or Na-cellulose) which is highly reactive towards monochloroacetic acid (MCA) (Almlöf Ambjörnsson, Schenzel and Germgård, 2013).

In the second stage, the etherification reaction is started with the adding of monochloroacetic acid (MCA) at 50 ^{o}C to 70 ^{o}C (Almlöf Ambjörnsson, Schenzel and Germgård, 2013). In this reaction, carboxymethylation reaction occurs as a hydroxyl group of cellulose is substituted with a carboxymethyl group at the molecular level. In the carboxymethylation, the negatively charged surface promotes the creation of a stable suspension and accelerates the breakdown of lignocellulosic fibres (Rojas, Bedoya and Ciro, 2015). According to Pushpamalar et al. (2006), the increasing in the concentration of the MCA results in the high yield of CMC. The rise is most likely because of the increased availability of acetate ions at greater amount in the presence of cellulose molecules. After that, the slurry formed is soaked in the organic solvent for overnight before it is neutralized with acetic acid in the next day. The neutralized CMC is washed for several times to remove the impurities, and it is dried in the oven at 60 ^{o}C (Bono et al., 2009).



cellulose **sodium carboxymethyl cellulose**

R = H or CH_2 COONa

according to DS_{CMC}

Figure 2.6: Reaction in Synthesis CMC. (Pushpamalar et al., 2006)

CHAPTER 3

METHODOLOGY

3.1 Research Flow Chart

Figure 3.1 presents the research flow chart to achieve the research objectives. The research activities started by preparation of OPEFB in powder form and followed with characteristics analysis to identify the nature of powder. After that, the powders were undergoing sonication treatment under various types of solvent and condition to extract cellulose. The best condition and solvent were then identify based on the characteristic analysis on treated products for subsequent carboxymethylation reaction to synthesis CMC.

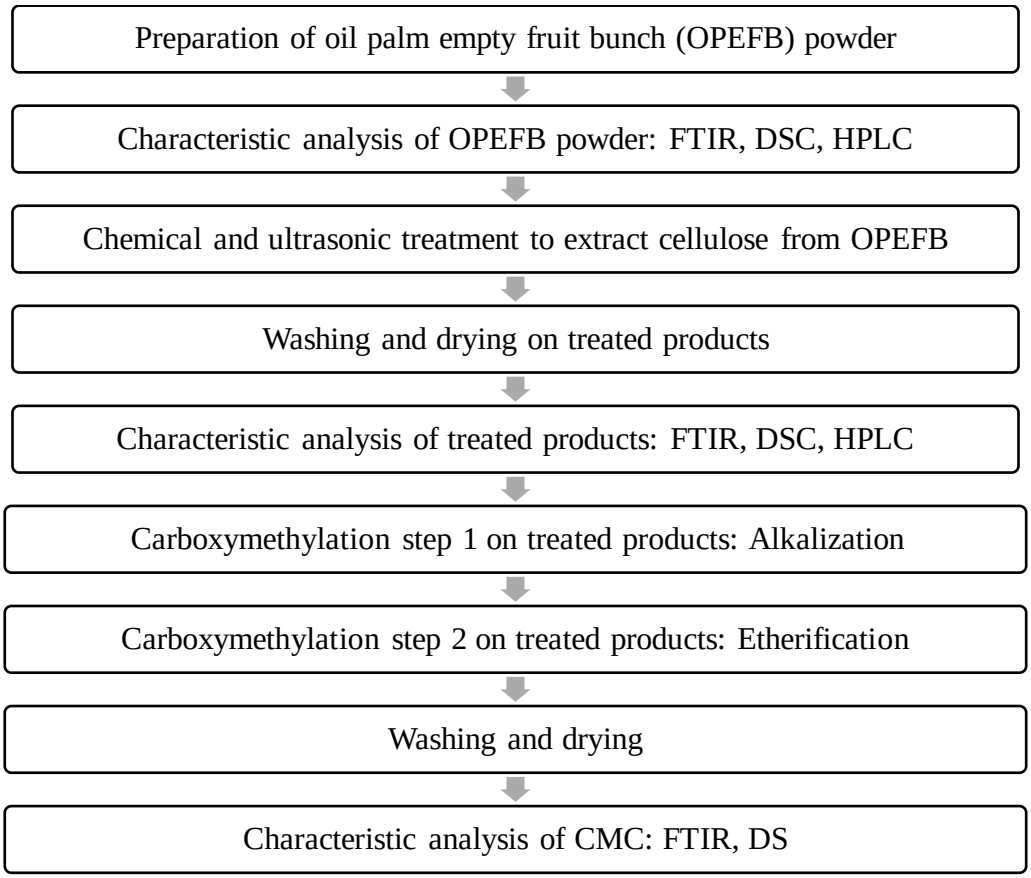


Figure 3.1: Research Flow Chart.

3.2 Materials and Chemicals

Table 3.1: Materials and Chemicals Used in this Research.

Material & Chemical	Source	Purity
Carboxymethylcellulose sodium	Alfa Aesar	-
Chloroform	Merck, Germany	95-98%
Ethanol	HmbG Chemicals, UK	95%
Dextrose Standard	HmbG Chemicals, UK	-
Isopropanol	HmbG Chemicals, UK	-

Raw OPEFB	Tian Siang Oil Mills Sdn Bhd, Malaysia	-
Nitric Acid	R&M Chemicals, UK	68%
Sodium monochloroacetate	Sigma Aldrich, Germany	98%
Sodium hydroxide pellet	R&M Chemicals, UK	-
Sulphuric acid	R&M Chemicals, UK	95-98%

3.3 Sample Preparation

The oil palm empty fruit bunch (OPEFB) was cleaned and grinded into powder form. The powder was dried in the oven at a constant temperature, 55 ℃ for 24 hours until constant weight was obtained. The powders were sieved through.

3.4 Cellulose Extraction Process

There were 14 sets of experiments conducted to determine the ultrasonic effect with manipulated variables on the extraction process. All parameters are kept constant by varying one parameter at each time in this experiment as shown in Table 3.2, Table 3.3, Table 3.4, Table 3.5, and Table 3.6. Three different types of solvents were used for extraction which are ethanol, chloroform, and sodium hydroxide. The extraction process will be operated in the presence of ultrasonic water bath as shown in Figure 3.2.

Figure 3.2: Set-Up of Treatment in an Ultrasonic Water Bath.

3.4.1 Extraction with Solvent – Ethanol

For the first investigated parameter, 5 g of dried OPEFB samples was added to 150 ml of ethanol into a Scott bottle. The Scott bottle was located in the ultrasonic bath and the pH of solution was monitored along the extraction process. The solution was stirred at constant speed of 600 rpm with motor stirrer. The temperature of the ultrasonic treatment was fixed at 40 ℃. After 120 minutes, the solution was filtered via vacuum filtration and washed with distilled water for 3 times to remove the impurities. The precipitate was dried in oven set at 60 ℃ until constant weight and kept for subsequent analysis. The experiment was repeated using different sonication duration 180 minutes and 240 minutes as tabulated in Table 3.2 while the temperature was varied to 30 ℃ and 50 ℃ at constant time as shown in Table 3.3. The changing of the temperature was controlled under boiling point of ethanol, 78 ℃ to meet the requirement that the ideal temperature for usage in the ultrasonic water bath which is roughly 65% of the solvent's boiling point (Zenith Ultrasonics, 2020).

Table 3.2: Treatment at Various Duration Using Ethanol as Solvent.

Experiment No.	Mass of OPEFB (g)	Temperature (°C)	Volume of ethanol (ml)	Duration (minutes)
1	5	40	150	120
2	5	40	150	180
3	5	40	150	240

Table 3.3: Treatment at Various Temperature Using Ethanol as Solvent.

Experiment No.	Mass of OPEFB (g)	Temperature (°C)	Volume of ethanol (ml)	Duration (minutes)
4	5	30	150	240
5	5	50	150	240

3.4.2 Extraction with Solvent – Chloroform

For the second investigated parameter, 5 g of dried OPEFB samples was added to 150 ml of chloroform into a Scott bottle. The Scott bottle was located in the ultrasonic bath and the pH of solution was monitored along the extraction process. The solution was stirred at constant speed of 600 rpm with motor stirrer. The temperature of the ultrasonic treatment was fixed at 40 °C. After 120 minutes, the solution was filtered via vacuum filtration and washed with distilled water for 3 times to remove the impurities. The precipitate was dried in oven set at 60 °C until constant weight and kept for subsequent analysis. The experiment was manipulated using different sonication duration 180 minutes and 240 minutes as tabulated in Table 3.4. The temperature was set as 40 °C for the chloroform treated samples which was around 65% of the solvent's boiling point at 61 °C.

Table 3.4: Treatment at Various Duration Using Chloroform as Solvent.

Experiment No.	Mass of OPEFB (g)	Temperature (°C)	Volume of chloroform (ml)	Duration (minutes)
6	5	40	150	120
7	5	40	150	180
8	5	40	150	240

3.4.3 Extraction with Solvent – Sodium Hydroxide

For the third investigated parameter, 5 g of dried OPEFB samples was added to 150 ml of 0.75 M sodium hydroxide (NaOH) solution into a Scott bottle. The Scott bottle was located in the ultrasonic bath and the pH of solution was monitored along the extraction process. The solution was stirred at constant speed of 600 rpm with motor stirrer. The temperature of the ultrasonic treatment was fixed at 80 ℃. After 120 minutes, the solution was filtered via vacuum filtration and washed with distilled water for 3 times to remove the impurities. The precipitate was dried in oven set at 60 °C until constant weight and kept for subsequent analysis. The experiment was repeated using different sonication duration 180 minutes and 240 minutes using 0.75 M NaOH solution. as tabulated in Table 3.5 while the concentration of NaOH solution was manipulated from 0.5 M to 1.0 M by keeping other parameters constant in Table 3.6. The temperature was set as 80 °C as the boiling point of NaOH solution is high and 80 °C is the highest temperature that can be set for the ultrasonic water bath (Wong et al., 2021).

Table 3.5: Treatment at Various Duration Using NaOH as Solvent.

Experiment No.	Mass of OPEFB (g)	NaOH (M)	Volume of NaOH (ml)	Duration (minutes)
9	5	0.75	150	120
10	5	0.75	150	180
11	5	0.75	150	240

Table 3.6: Treatment at Various Concentration Using NaOH as Solvent.

Experiment No.	Mass of OPEFB (g)	NaOH (M)	Volume of NaOH (ml)	Duration (minutes)
12	5	0.50	150	180
13	5	0.75	150	180
14	5	1.00	150	180

After all the treatment, the treated, dried OPEFB was stored in a polyethylene bag. The characterization tests including FTIR, HPLC and DSC were carried out to study the properties of treated OPEFB and the effect of ultrasonic under different conditions. All the samples were named according to their experiment number that set in Table 3.2, Table 3.3, Table 3.4, Table 3.5 and Table 3.6.

3.5 Synthesis of CMC from Cellulose

3 g of dried cellulose was prepared and added with 60 mL of ethanol/isopropanol with ratio 0.7 as the solvent into a Scott bottle. In order to undergo alkalization process, 6 mL of 17.5 w/v % aqueous NaOH was added drop by drop into the mixture was stirred

using motor stirrer at 700 rpm for 1 hours at 30 ℃ in the water bath. Then, 3.6 g of sodium monochloroacetate (SMCA) was added into the mixture and it was stirred for about 2 hours in water bath by maintaining at 57 ^{o}C. This step is the carboxymethylation process. After 2 hours, the mixture was filtered and purified with ethanol for three times to remove the impurities. The product was dried in the oven at 55 ^{o}C until constant weight was obtained. Then, the CMC was further characterized.

3.6 Characterization of raw OPEFB, treated OPEFB and CMC

3.6.1 Fourier Transform Infrared Spectroscopy (FTIR)

The Fourier Transform Infrared Spectroscopy (FTIR) is used to identify functional groups of the materials such as raw OPEFB, treated OPEFB and CMC in the experiment. FTIR can create an infrared absorption spectrum to identify chemical bonds in a molecule. In the FTIR test, the OPEFB samples used were grinded into powders. Attenuated total reflectance (ATR) is applied in the sampling procedure of the FTIR. The powder form samples were placed onto ATR crystal pressed down using the swivel press to make sure the optimal contact between sample and crystal (Agilent, n.d.). FTIR spectra analysis was determined within the wavelength range of 400 to 4000 cm^{-1} (Sindhu, Binod and Pandey, 2015).

3.6.2 Differential Scanning Calorimetry (DSC)

The differential scanning calorimetry (DSC) detects the energy transferred to or from a sample that is experiencing a physical or chemical change (Usmani et al., 2017). The DSC concept assumes that the change in heat in every transition is comparable to the

change in enthalpy under constant pressure circumstances. With this analysis, it can detect the melting point, glass transition point and crystallization temperature of lignocellulosic biomass. In DSC test, 3 to 9 mg of OPEFB samples was placed in an empty aluminium crucible which was covered with lid. The test was operating at pure nitrogen flow rate of 20 ml/min and the samples were heated from 25 to 250 ^{o}C at a rate of 10 °C/min. The DSC result reveals the information on the relationship of wood components and the change in chemical composition caused by heat treatment. The raw OPEFB as well as the treated OPEFB from each solvent were analysed by DSC.

3.6.3 High Performance Liquid Chromatography (HPLC) for Carbohydrate Content Analysis

The high performance liquid chromatography (HPLC) functions to determine the cellulose content in the sample by converting the cellulose to simple sugar unit. HPLC can isolate and detect chemicals in any material that can be dissolved in a liquid at trace quantities as low as parts per trillion. A refractive index detector in the HPLC machine is employed to determine the sugar content.

After the treatment process, 0.3 gram of OPEFB samples was mixed with 3ml of 72% sulphuric acid in a boiling tube. The boiling tube with mixture was placed in a water bath at 30 ^{o}C for 24 hours. On the next day, it was transferred into a Scott bottle and diluted with 84 ml distilled water. Then, it was dispersed using sonicate water bath. After that, the mixture was autoclaved at 121 ^{o}C for 1.5 hours. Then, the mixture was neutralized by adding 3 ml of 2 M sodium hydroxide. 1 ml of neutralized mixture was mixed with 9 ml of distilled water in a centrifuge tube for dilution. The syringe filter was used to filter the mixture into a glass vial before it was analysed through HPLC. In the HPLC analysis, the 0.025 M H_2SO_4 was used as mobile phase solution in Agilent Hi-Plex column with a pump flow rate of 0.6 ml/min. To determine the sample composition, calibration curves were developed using basic sugar standards, arabinose,

xylose, and dextrose. The calibration line where cellulose breaks down into simple sugars was used to assess cellulose quantity.

3.6.4 Determination of Degree of Substitution (DS) of CMC

The degree of substitution (DS) can be used to represent the number of hydrogens in the hydroxyl group of a glucose unit that have been replaced by carboxymethyl group. The DS can affect the solubility, thickening property, stability as well as acid resistance of the CMC. To calculate DS of CMC, 4 g of CMC was prepared and agitated with 75 ml of 95% (v/v) ethanol in a 250 ml Scott bottle for 3 minutes. After that, 5 ml of 2 M nitric acid was added drop by drop. The mixture was stirred and heated with hot plate until it boiled. After the mixture was boiled, it was removed from the hotplate and stirred for 10 minutes. Then, the solution was filtered and washed with ethanol for 3 times. 50 ml of methanol was further used to wash the precipitate. The washed product was dried in the oven at 105ºC for 3 hours and cooled for overnight. Next, 1 g of dried CMC was added in 100 ml of distilled water and 25 ml of 0.3 N NaOH. The mixture was heated and stirred for 15 minutes. Finally, the mixture underwent titration using 0.3 N HCl. 2 drops of phenolphthalein were used as the indicator to observe the neutralization of the mixture by changing from pink to colourless (Bono et al., 2009). The Equation 3.1 and 3.2 are used in the calculation of DS for CMC.

$$DS = \frac{0.162 \times A}{1 - 0.058 \times A} \qquad (3.1)$$

$$A = \frac{BC - DE}{W} \qquad (3.2)$$

Where B = Volume of NaOH added, ml

C = Concentration in normality of sodium hydroxide added

D = Volume of HCl used ml

E = Concentration in normality of HCl used

W = Weight of CMC, g

3.6.5 CMC Film Preparation

In order to produce the CMC film, 1 g of CMC sample is mixed with 30 ml of distilled water at 50 ^{o}C in a Scott bottle. The solution is agitated continuously till it is fully solubilized. After that, the solution is homogenized with 0.5 ml of glycerol which acts as a plasticizer for 10 minutes. Then, the film solution is cooled down to room temperature before it is cast on a petri dish. The film solution is dried at ambient temperature for 48 hours before it is peeled off as a film (Łopusiewicz et al., 2021).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 OPEFB Characterization

The raw OPEFB is the main material used for the cellulose extraction process using ultrasonic method. In this experiment, the OPEFB was collected to determine its characterization through different characterization methods such as fourier-transform infrared (FTIR) spectrophotometer, high performance liquid chromatography (HPLC) and differential scanning calorimetry (DSC). The results of the characterization are used to make some comparisons with the treated OPEFB to identify the changes.

Based on Figure 4.1, it shows the FTIR spectrum of the raw OPEFB and the functional group of the sample can be determined. From the diagram, there is a broad absorption band at 3333 cm^{-1} which indicates the hydroxyl (O-H) stretching vibration in the cellulose structure (Suraya Rosli et al., 2017). The absorption at 2921 cm^{-1} ascribable to the presence of the C-H_2 asymmetric stretching vibration from the methyl and methylene group in the lignocellulose (Faizi et al., 2019). The presence of C=O acetyl group of hemicelluloses or carbonyl group of lignin causes the infrared spectroscopy at 1734 cm^{-1} (Zulkiple et al., 2016). Meanwhile, the band at 1634 cm^{-1} relates to deformation of absorbed water by cellulose or hemicellulose (Suraya Rosli et al., 2017). According to Sayakulu & Soloi (2022) and Al-Samarrai et al. (2018), the peaks at 1601 $cm^{-1}$and 1512 cm^{-1} represents to the C=C stretching from aromatic ring of lignin while 1244 cm^{-1} is the C-O-C of aryl alkyl ether in lignin. Other than that, the

absorbance at 1423 cm^{-1}, 1369 cm^{-1} and 1321 cm^{-1} are linked to the bending vibrations of -CH_2, C-H, and C-O respectively (Abdullah et al., 2016). Besides, the sharp peak at 1034cm^{-1} is in connection with aliphatic C–O stretching (Md Salim et al., 2021). The existence of β-glycosidic linkage between glucose units in cellulose can be seen clearly at 896 cm^{-1} (Haji Badri and Syakilla Hassan, 2016). Lastly, the weak peaks occur in between 800 cm^{-1}- 400 cm^{-1} can be the C-O-H bending or the aromatic –CH stretch vibration (Md Salim et al., 2021). The absorption bands of raw OPEFB are demonstrated in Table 4.1 with their sources. In short, the OPEFB is a lignocellulose component with varied fraction of cellulose, hemicellulose, and lignin.

Table 4.1: Absorption Band of Raw OPEFB.

ABSORPTION BANDS (WAVENUMBER, CM^{-1})	ASSIGNMENT	SOURCE
3333	O-H stretching	Cellulose
2921	C-H_2 asymmetric stretching	Cellulose
1734	C=O stretching	Hemicellulose, lignin
1634	OH bending	Cellulose
1601	C=C stretching with aromatic ring	Lignin
1512	C=C stretching with aromatic ring	Lignin
1423	-CH_2 bending	Lignin
1369	C-H bending	Cellulose, hemicellulose
1321	C-O bending	Cellulose, hemicellulose
1244	C-O-C stretching	Lignin
1034	C–O symmetric stretching	Cellulose, hemicellulose
896	β -Glycosidic linkage	Cellulose
598	C-O-H bending	-
427	aromatic –CH stretching	-

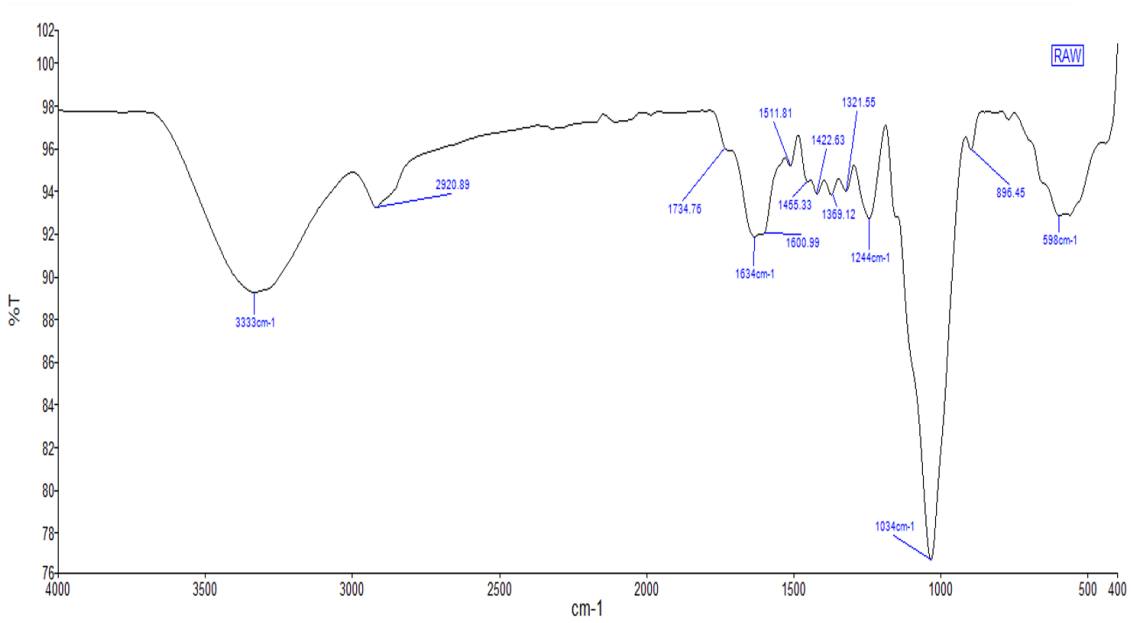


Figure 4.1: FTIR Result of Raw OPEFB.

From the HPLC graph, the cellulose content in the biomass can be calculated by breaking down the polysaccharides into the simple sugar molecules through the acid hydrolysis treatment. The HPLC is a significant method to conduct the qualitative and quantitative evaluations. In the HPLC analysis, the basic sugar standards such as arabinose, xylose, and dextrose are prepared at various concentration to make the HPLC calibration curve in Appendix A. According to the plotted HPLC calibration curve, the calculation for the cellulose content in the samples can be done. As depicted in Figure 4.2, there is a sharp peak in the beginning due to the appearance of water molecules while the peak shown behind is the sugar content from the raw OPEFB. The calculation step of the cellulose content in the raw OPEFB is shown in Appendix B. From the calculation, the cellulose content in the raw OPEFB is 33.77 wt%. The low content of cellulose in raw OPEFB is due to presence of significant amount of lignin and hemicellulose.

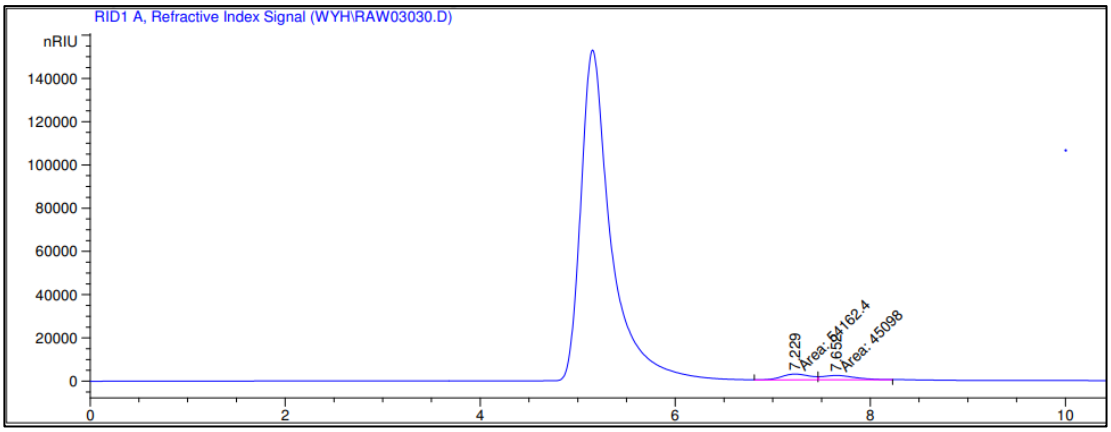


Figure 4.2: HPLC Result of Raw OPEFB.

Throughout Figure 4.3 which demonstrates the DSC result of raw OPEFB, the thermal degradation of the lignocellulosic materials such as cellulose, hemicellulose and lignin can be discovered. In the DSC result, a broad endothermic peak is spotted within the temperature of 34℃ to 144℃. The appearance of this peak denotes the elimination of water and low-temperature degradation of hemicelluloses from the raw OPEFB (Solikhin et al., 2016). As illustrated in Figure 4.3, the specific enthalpy of the raw OPEFB is -23.89 W/g and it starts dehydrated at an onset temperature of 43℃ and peaked at 81.17℃. Even though the temperature set for DSC is limited till 250℃, it can view that a peak is going to form at 246℃ and it could be the occurrence of cellulose degradation (Solikhin et al., 2016).

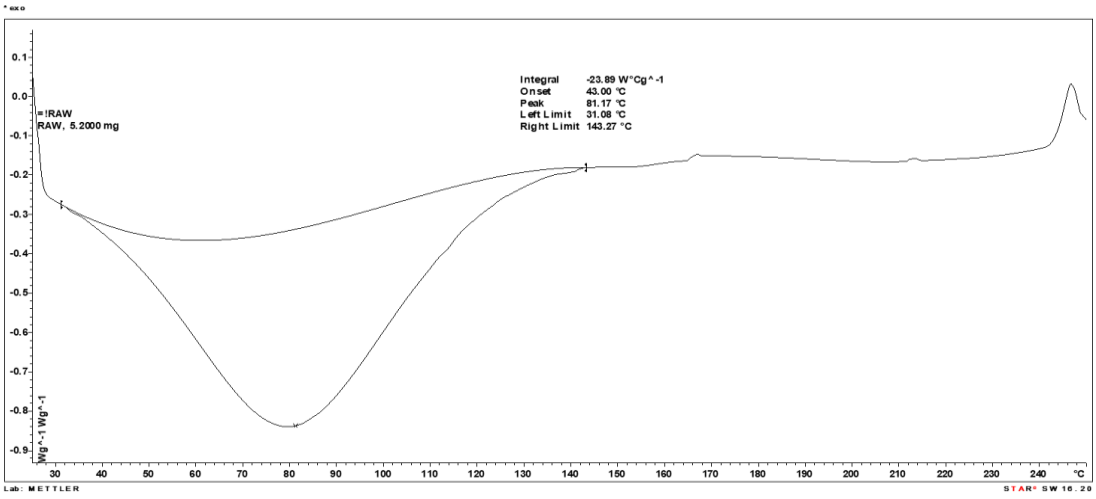


Figure 4.3: DSC Result of Raw OPEFB.

4.2 Effect of Treatment Conditions on Cellulose Content

4.2.1 Effect of Sonication Duration on Cellulose Content

The OPEFB is treated with an ultrasonic bath using different types of solvent. In the process, the sonication duration was manipulated to 120 minutes, 180 minutes, and 240 minutes for each solvent. The cellulose contents that are calculated through the HPLC analysis were revealed in Table 4.2. With the cellulose content result in Table 4.2, it can clearly observe that the increasing of sonication duration can increase the cellulose content gradually for samples using ethanol and NaOH. For sample using chloroform as solvent, the cellulose content is slightly decreases when the sonication duration increases from 120 minutes to 240 minutes. The reason of the reduction of cellulose content in Sample 8 at 240 minutes may be caused by the degradation of cellulose for high sonication duration (Shojaeiarani et al., 2020). This situation can be called as ultrasonic degradation which is the adverse effect of long-term ultrasonic duration (Lianfu and Zelong, 2008). Apart from that, long sonication duration can result the secondary oxidation-reduction reactions of the organic solvent such as chloroform by generating free radicals (Chukwumah et al., 2009). Consequently, the optimal duration of the cellulose extraction using ultrasonication could be varied depending on the solvent used in the treatment.

Table 4.2: Cellulose Content of Samples with Various Sonication Duration.

Sample	Type of Solvent	Sonication Duration (min)	Temperature (ºC)	Concentration of Solution (M)	Cellulose Content (%)
1	Ethanol	120	40	-	52.50
2	Ethanol	180	40	-	51.09
3	Ethanol	240	40	-	57.51
6	Chloroform	120	40	-	52.50
7	Chloroform	180	40	-	41.89
8	Chloroform	240	40	-	51.78
9	NaOH	120	80	0.75	68.46

10	NaOH	180	80	0.75	68.08
11	NaOH	240	80	0.75	70.25

During the ultrasonic treatment, the abrupt bursting of the bubbles generates microjets and shock waves on the surfaces of suspended cellulose particles, causing the scission effect on micron-sized cellulose particles (Ni et al., 2021). This scission action has the potential to disrupt the comparatively weak interfaces between the fibres, which are mostly held together by van der Waals forces and hydrogen bonds. According to Ni et al. (2021), the increases of sonication duration will lead to higher energy density to break the structure of the biomass and disintegrate the cellulose particles. Also, Xu and Pan (2013) studied that the increasing of sonication duration will promote the interaction of biomass with the solvent due to the cavitation impact of ultrasound on the surface of plant matrix.

4.2.1.1 Effect of Sonication Duration Using Ethanol as Solvent

The comparison of the FTIR results of raw OPEFB with treated samples using ethanol solution at various duration; Sample 1, Sample 2 and Sample 3 is portrayed in Figure 4.4. The adsorption bands of each sample are tabulated in Table 4.3. When sonication duration increases from 120 minutes to 240 minutes, the changes of the functional group are not much significant compared to the raw OPEFB. However, the intensity of treated samples is reduced. The presence of cellulose in Sample 1, Sample 2 and Sample 3 is still could be observed. The absorption peak which is the β-Glycosidic linkage located at 896 cm^{-1} to 899 cm^{-1} in Sample 1, Sample 2 and Sample 3 prove that the cellulose structure is not destroyed when the treatment duration increases. With the increasing of sonication duration, the OH stretching at 3333 cm^{-1} shifts to 3336 cm^{-1} as the removal of the hemicellulose leads to the increase of OH content in the treated samples (Wang et al., 2022). Also, the stretching vibration peak of CH_2 at 2921 cm^{-1} shifts to 2906 cm^{-1} due to the structural changes in the treated

OPEFB since specific levels of lignin and hemicellulose were eliminated (Wong et al., 2021). Furthermore, lignin structure with peak of C=O stretching at 1734 cm^{-1} shifts to 1726 cm^{-1} and C=C stretching with aromatic ring at 1512 cm^{-1} shifts to 1509 cm^{-1} when the time increases to 240 minutes. It proves that the lignin structure is affected and some of the lignin components are reduced (Sayakulu and Soloi, 2022).

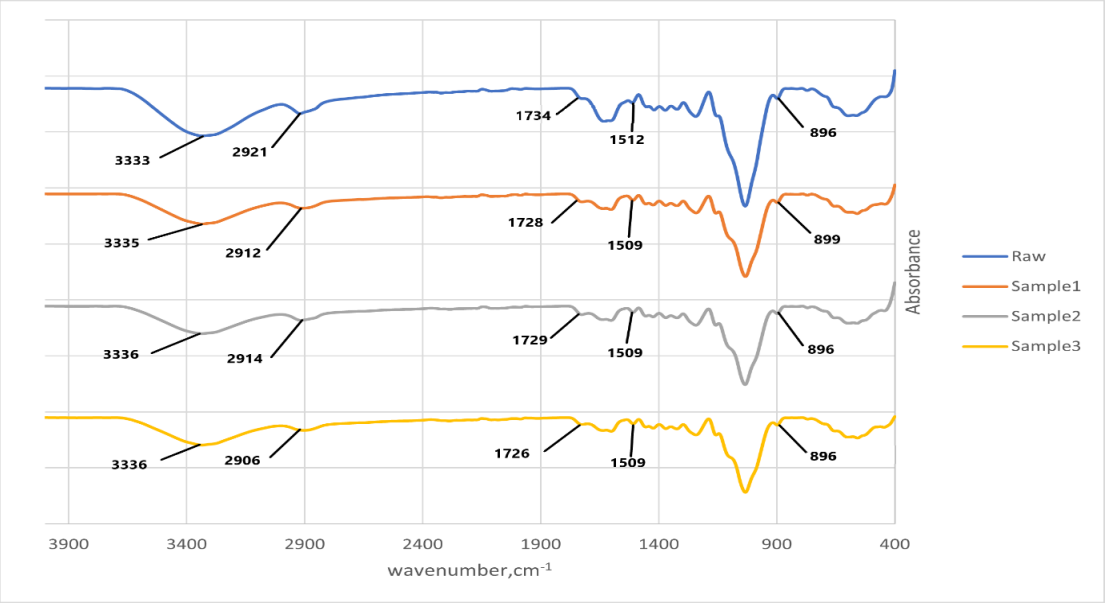


Figure 4.4: FTIR Result of Raw OPEFB and Treated Samples Using Ethanol as Solvent at Various Sonication Duration.

Table 4.3: Absorption Band of Raw OPEFB and Treated Samples Using Ethanol as Solvent at Various Sonication Duration.

Wavenumber (cm^{-1})				Assignment
Raw	**Sample 1**	**Sample 2**	**Sample 3**	
3333	3335	3336	3336	O-H stretching
2921	2912	2914	2906	C-H_2 asymmetric stretching
1734	1728	1729	1726	C=O stretching
1634	1646	1643	1640	OH bending
1601	1602	1602	1601	C=C stretching with aromatic ring

1512	1509	1509	1509	C=C stretching with aromatic ring
1423	1423	1423	1423	-CH_2 bending
1369	1369	1372	1369	C-H bending
1321	1325	1322	1322	C-O bending
1244	1242	1243	1242	C-O-C stretching
1034	1033	1034	1033	C–O symmetric stretching
896	899	896	896	β -Glycosidic linkage

4.2.1.2 Effect of Sonication Duration Using Chloroform as Solvent

Figure 4.5 shows the spectra of Sample 6, Sample 7 and Sample 8 while Table 4.4 summarizes the difference in the adsorption band of Sample 6, Sample 7 and Sample 8. Although the Sample 6, Sample 7 and Sample 8 are using chloroform with the sonication duration increases from 120 minutes to 240 minutes respectively, the changes between these three samples are not much. Nevertheless, the cellulose structure with peak at 2918 cm^{-1} shifts to 2909 cm^{-1} when the sonication time increases to 240 minutes in the view of fact that long sonication time strengthen the acidity of chloroform and it degrades the amorphous structure of cellulose (Kong-Win Chang et al., 2018).

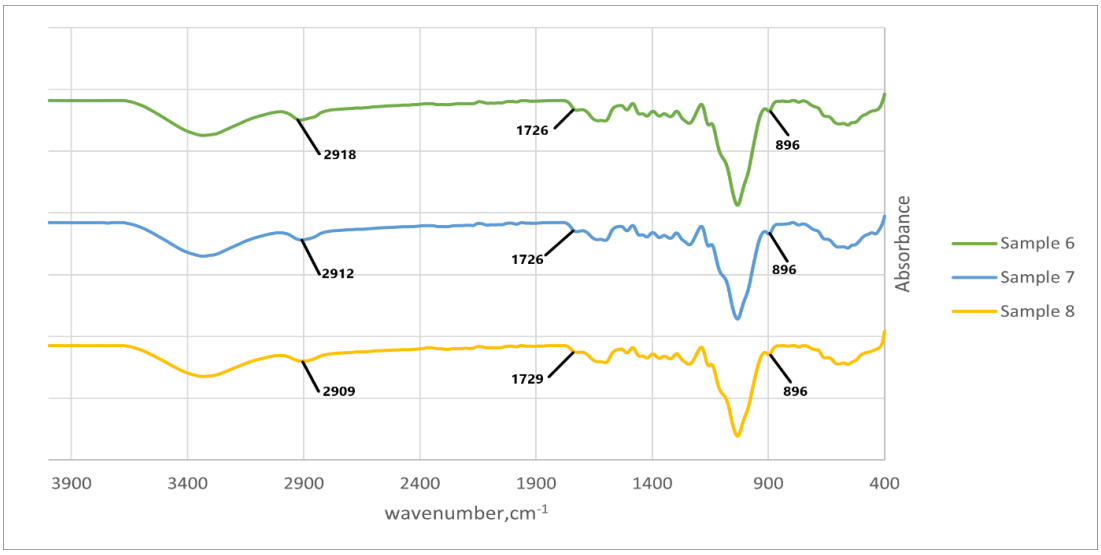


Figure 4.5: FTIR Result of Treated Samples Using Chloroform as Solvent at Various Sonication Duration.

Table 4.4: Absorption Band of Treated Samples Using Chloroform as Solvent at Various Sonication Duration.

Wavenumber (cm^{-1})			Assignment
Sample 6	**Sample 7**	**Sample 8**	
3333	3334	3333	O-H stretching
2918	2912	2909	C-H_2 asymmetric stretching
1726	1726	1729	C=O stretching
1640	1643	1643	OH bending
1604	1603	1603	C=C stretching with aromatic ring
1509	1509	1506	C=C stretching with aromatic ring
1423	1423	1423	-CH_2 bending
1369	1369	1369	C-H bending
1322	1322	1322	C-O bending
1241	1241	1242	C-O-C stretching
1033	1033	1033	C–O symmetric stretching
896	896	896	β -Glycosidic linkage

4.2.1.3 Effect of Sonication Duration Using Aqueous NaOH as Solvent

Figure 4.6 compares the FTIR result of Sample 9, Sample 10 and Sample 11. It should be noted that Sample 9, Sample 10 and Sample 11 are using NaOH as solvent in extraction of cellulose with increases of sonication duration. By comparing these three samples to the raw OPEFB, it can observe a lot of differences in the FTIR spectra for NaOH compared to other solvents used before. The further explanation about the effect of solvent towards the cellulose extraction process is discussed in Section 4.2.4. Through Table 4.5 which is comparing the FTIR result by varying the sonication duration, it can be seen that the delignification effect is obvious for Sample 9, Sample 10 and Sample 11 which are using NaOH because the peak range in 1725– 1710 cm^{-1} which denote the lignin and hemicellulose is absence for these three samples (Wong et al., 2021).

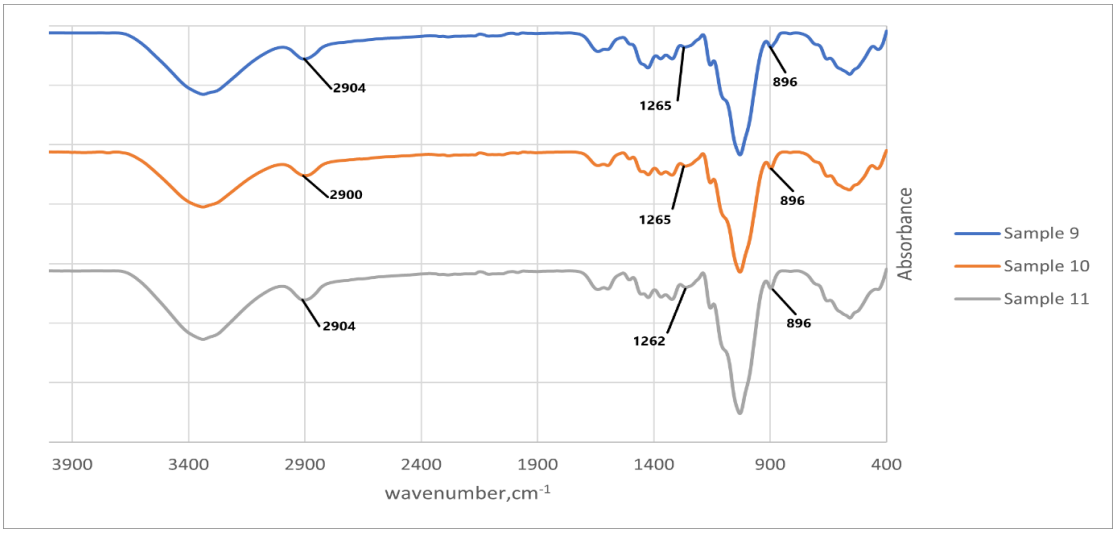


Figure 4.6: FTIR Result of Treated Samples Using NaOH as Solvent at Various Sonication Duration.

Table 4.5: Absorption Band of Treated Samples using NaOH as Solvent at Various Sonication Duration.

Wavenumber (cm^{-1})			Assignment
Sample 9	**Sample 10**	**Sample 11**	
3337	3338	3338	O-H stretching
2904	2900	2904	C-H_2 asymmetric stretching
-	-	-	C=O stretching
1640	1640	1640	OH bending
1595	1598	1595	C=C stretching with aromatic ring
1506	1502	1506	C=C stretching with aromatic ring
1426	1423	1426	-CH_2 bending
1372	1369	1369	C-H bending
1322	1322	1322	C-O bending
1265	1265	1262	C-O-C stretching
1030	1030	1030	C–O symmetric stretching
896	896	896	β -Glycosidic linkage

To recap, the increasing of sonication duration from 120 minutes to 240 minutes can increase cellulose contents. Although the FTIR spectra of the samples for each investigated solvent do not show significant changes. According to Methrath Liyakathali et al. (2016), a short period of sonication duration will cause ineffective delignification. Yet, prolonged ultrasound may promote particle collision and aggregation, as well as condensation of certain solubilized chemicals, reducing the action of the ultrasound (Ivetić et al., 2017). In short, the increasing of sonication duration will increase the cellulose content, but the effect is not that significant after a certain time. It is important to find the optimum extraction time for different solvent. Thus, the optimal sonication duration used is 180 minutes to 240 minutes for following extraction parameters.

4.2.2 Effect of Sonication Temperature on Cellulose Content

The effect of the temperature on cellulose content was investigated by varying the temperature for samples using ethanol as solvent. At the same time, the sonication duration is fixed as 240 minutes. The result of the cellulose content of the samples is tabulated as Table 4.6.

Table 4.6: Cellulose Content of Samples Using Ethanol as Solvent with Various Temperature.

Sample	Type of Solvent	Sonication Duration (min)	Temperature (ºC)	Concentration of Solution (M)	Cellulose Content (%)
4	Ethanol	240	30	-	40.97
3	Ethanol	240	40	-	57.51
5	Ethanol	240	50	-	46.34

Previous research has established that the ultrasonic treatment with high temperature can cause cavitation which is adequate to loosen the microstructure of biomass by dislocating the lignin fibre, exposing the cellulose layer (Hafid et al., 2023). When the temperature is elevated, the kinetic energy in the biomass mixture can be boosted and accelerate the diffusion between the solvent and solute. Thus, it clearly observes the cellulose content increases from 40.97 % to 57.51 % when the temperature rises from 30 ^{o}C to 40 ^{o}C in Table 4.6. On the contrary, the cellulose content is decreases when the temperature further increases to 50 ^{o}C because the ethanol is possible to evaporate at 50 ^{o}C and it reduces the cellulose extraction efficiency (Abdel-Banat et al., 2009). Moreover, high temperature could contribute to the combination of acoustic bubbles and natural vapour bubbles which can reduce the efficacy of ultrasonic effects (Ali et al., 2014).

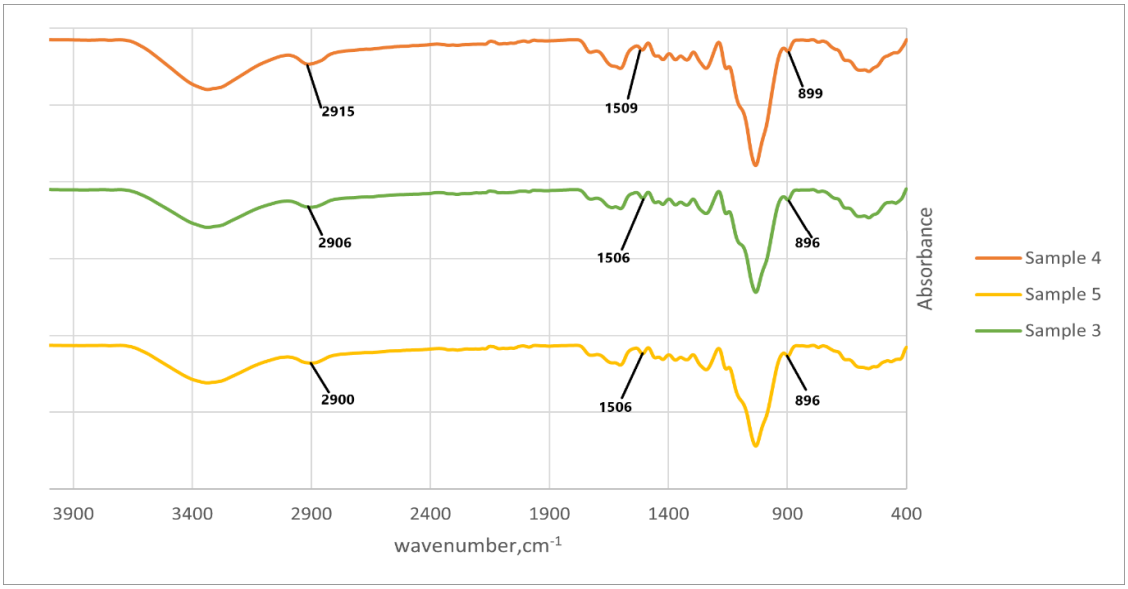


Figure 4.7: FTIR Result of Treated Samples Using Ethanol as Solvent at Various Temperature.

Table 4.7: Absorption Band of Treated Samples Using Ethanol as Solvent at Various Temperature.

Wavenumber (cm^{-1})			Assignment
Sample 4	**Sample 3**	**Sample 5**	
3336	3336	3336	O-H stretching
2915	2906	2900	C-H_2 asymmetric stretching
1729	1726	1729	C=O stretching
1641	1640	1646	OH bending
1603	1601	1602	C=C stretching with aromatic ring
1509	1509	1506	C=C stretching with aromatic ring
1420	1423	1426	-CH_2 bending
1366	1369	1369	C-H bending
1322	1322	1325	C-O bending
1242	1242	1242	C-O-C stretching
1034	1033	1033	C–O symmetric stretching
899	896	896	β -Glycosidic linkage

According to Figure 4.7 and Table 4.7, there are a lot of similar peaks for Sample 4, Sample 3 and Sample 5. When the temperature increases from 30 ^{o}C to 50 ^{o}C, it does not make an obvious change in the functional group of the samples. Most of it has been explained in Section 4.2.1. There is a slight shifting for 1509 cm^{-1} to 1506 cm^{-1} as the lignin and hemicellulose structure is disrupted when the temperature rises.

Normally, during the treatment with an ultrasonic bath, the ultrasonic cavitation energy is transformed to heat, and a long duration operation will increase the temperature of the water bath (Kohn, 2016). In this research, the temperature of the ultrasonic water bath is usually higher than the temperature set after it operates for more than one hour. Although the temperature set for the ultrasonic water bath is 50 ^{o}C, it could elevate to around 56 ^{o}C. This phenomenon should be considered when selecting the temperature for ultrasonic treatment to prevent the solvent evaporated due to high temperature. Due to this reason, the temperature set for samples using ethanol as solvent is lower than its boiling point which is 78 ^{o}C and the temperature set for samples using chloroform as solvent is 40 ^{o}C which is below its boiling point, 61 ^{o}C. The temperature selected for samples using sodium hydroxide as solvent is the highest, 80 ^{o}C since it has a high boiling point. The ideal temperature for usage in the ultrasonic water bath is roughly 65% of the solution's boiling point (Zenith Ultrasonics, 2020). In short, the optimum temperature is 40 ^{o}C for treating OPEFB using ethanol as it yields the highest cellulose content.

4.2.3 Effect of Concentration of NaOH on Cellulose Content

In this research, the effect of concentration of NaOH on cellulose content was studied and the other two parameters, sonication duration and temperature, are constant. Table 4.8 illustrates the cellulose content of samples using NaOH with different concentration 0.5 M, 0.75 M and 1.0 M.

Table 4.8: Cellulose Content of Samples Using NaOH as Solvent with Various Concentration.

Sample	Type of Solvent	Sonication Duration (min)	Temperature (°C)	Concentration of Solution (M)	Cellulose Content (%)
12	NaOH	180	80	0.50	37.89
13	NaOH	180	80	0.75	67.08
14	NaOH	180	80	1.00	78.12

With the support of Table 4.8, it can observe that higher concentration of the NaOH solution extract higher yield of cellulose content. The cellulose content in Sample 14 is relatively higher than other samples. Generally, the concentrated NaOH solution provides a better delignification effect since the solution is strong enough to degrade more lignin and reduce the crystallinity of cellulose in the biomass. In addition, lignin is soluble in an alkaline environment, thus dissolving them once the linkage is broken and leaving behind solid cellulose. When the concentration of the NaOH solution is low, it is difficult for the solution to penetrate through the OPEFB to remove the lignin and hemicellulose. The linkages between lignin macromolecule units are broken by the NaOH solution and it increases the biomass porosity as well as exposes more cellulose content (Chen and Wang, 2017). Additionally, at pH greater than 12, NaOH lowers super oxide radicals ($-O_2$), causing lignin and hemicellulose to hydrolyse (Rojas, Bedoya and Ciro, 2015). Consequently, the cavitation phenomenon created by the ultrasonic bath will enhance the accessibility of the NaOH solution towards the biomass, resulting in a greater amount of cellulose (Subhedar et al., 2015). However, Sayakulu & Soloi (2022) states that an extreme concentration of NaOH solution will disrupt portions of the crystalline area in the cellulose and cause the dissolution of cellulose in the solution treatment, thus reducing cellulose content.

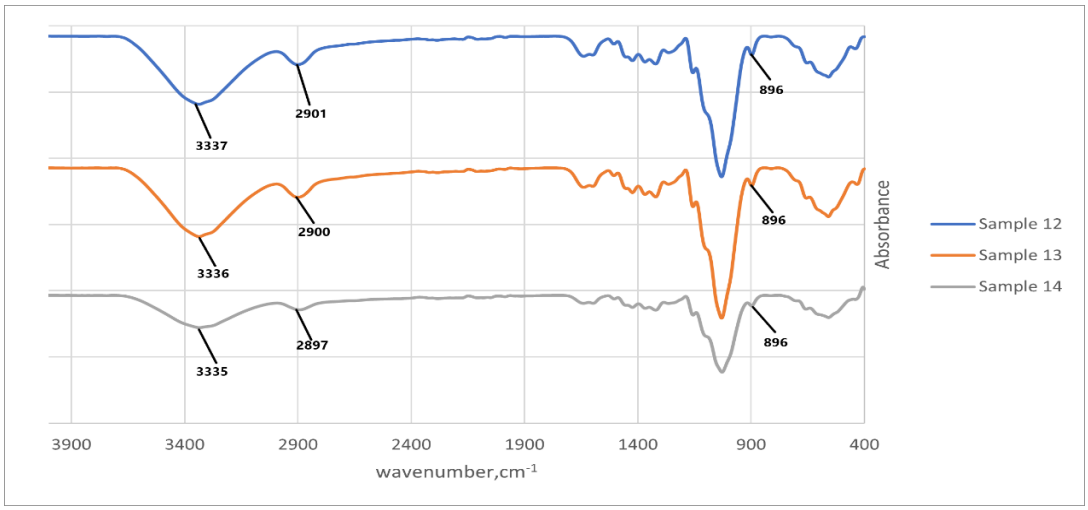


Figure 4.8: FTIR Result of Treated Samples Using NaOH as Solvent at Various Concentration.

Table 4.9: Absorption Band of Treated Samples Using NaOH as Solvent at Various Concentration.

Wavenumber (cm^{-1})			Assignment
Sample 12	**Sample 13**	**Sample 14**	
3337	3336	3335	O-H stretching
2901	2900	2897	C-H_2 asymmetric stretching
-	-	-	C=O stretching
1639	1639	1643	OH bending
1598	1598	1595	C=C stretching with aromatic ring
1506	1509	1506	C=C stretching with aromatic ring
1423	1426	1423	-CH_2 bending
1366	1366	1369	C-H bending
1322	1321	1321	C-O bending
1265	1265	1268	C-O-C stretching
1030	1030	1029	C–O symmetric stretching
896	896	896	β -Glycosidic linkage

Figure 4.8 displays the FTIR spectra of Sample 12, Sample 13 and Sample 14 while the comparison of the FTIR result is tabulated in Table 4.9. After the ultrasonic

treatment with NaOH solution, the peak at around 1734 cm^{-1} which is assigned as C=O stretching due to lignin is totally vanished for these three samples under the alkaline treatment (Melesse et al., 2022). For Sample 12 and Sample 13, their C-H_2 asymmetric stretching at around 2900 cm^{-1} have higher intensity than Sample 14 due to the high crystallinity of cellulose. According to Aini et al. (2019), the strength of this peak reflects the cellulose crystallinity as the biomass with higher cellulose crystallinity will have higher intensity at this peak. The high concentration of NaOH cellulose makes the cellulose more swelled for Sample 14.

As a result, it can summarize that 1.0 M NaOH is the most suitable concentration used for the cellulose extraction process due to the higher cellulose content produced compared to other samples. The selection of the concentration for the solvent is critical to the cellulose extraction process to prevent cellulose degradation as well as promote solubility of unwanted lignin. An ideal concentration of solution will ease the treatment and increase the cellulose content in the OPEFB.

4.2.4 Effect of Solvent Used on Cellulose Content

Table 4.10 reveals the optimum cellulose content for each studied solvent and their respective extraction condition.

Table 4.10: Cellulose Content of Treated Samples Using Different Solvents.

Sample	Type of Solvent	Sonication Duration (min)	Temperature (ºC)	Concentration of Solution (M)	Cellulose Content (%)
3	Ethanol	240	40	-	57.51
6	Chloroform	120	40	-	52.50
14	NaOH	180	80	1.00	78.12

Through Table 4.10, it is noticed that ethanol, chloroform and sodium hydroxide (NaOH) are able to increase the cellulose content of treated samples compared to raw OPEFB (33 wt%) when extraction operated under the ultrasonic water bath at optimum condition. Among these solvents, NaOH has the highest cellulose content, 78.12 wt%, followed by the ethanol, 57.51 wt% and the lowest is chloroform, 52.50 wt%. The solvents chosen have their own properties. The NaOH solution is an alkaline while both ethanol and chloroform are organic solvents. Although both ethanol and chloroform are organic solvents, they have different pH values. The pH of ethanol used is 7.30 and the pH of chloroform used is 4.6.

The NaOH solution yields the highest cellulose content from OPEFB as the alkaline treatment with ultrasonication is effective to solubilize the lignin and hemicellulose by cleaving their intermolecular ester bond through saponification reaction (Sun et al., 2015). The hydroxyl ion cluster in NaOH solution creates small surface tension in the solution and it causes less energy required to produce the cavitation bubbles (Hemwimol et al., 2006). Moreover, the presence of NaOH solution can eliminate acetyl and the different uronic acid substitutions on hemicellulose, which reduces the enzyme's accessibility to the hemicellulose and cellulose surfaces (Harmsen et al., 2010). Therefore, the application of NaOH solution in cellulose extraction process is satisfactory when removal of lignin is needed.

Furthermore, the using of ethanol as a solvent in sonication extraction manages to get 57.51 wt% of cellulose. The organic solvent such as ethanol can be utilised to dissolve the extractives, leaving the cellulose residue. The cavitation collapses are violent in ethanol solution primarily because of its low vapour pressure properties (Hemwimol et al., 2006). However, the delignification strength of ethanol is not stronger compared to alkaline solution; thus, it is usually coupled with a catalyst to increase its rate of delignification in the organosolv treatment for cellulose extraction (Baruah et al., 2018). Moreover, chloroform which acts as an organic solvent with acidic properties has the least cellulose content compared to NaOH solution and ethanol. The study by Wu et al. (2001) stated that the chloroform is expected to undergo destruction by oxidation or pyrolysis due to the cavitation bubbles produced

by ultrasonication. Also, chloroform has high vapor pressure, and it can affect the collapse of cavitation bubbles since it has smaller a internal and external pressure differential (Chemat et al., 2017). Through this evidence, the chloroform is found not a suitable solvent for cellulose extraction under sonication effect.

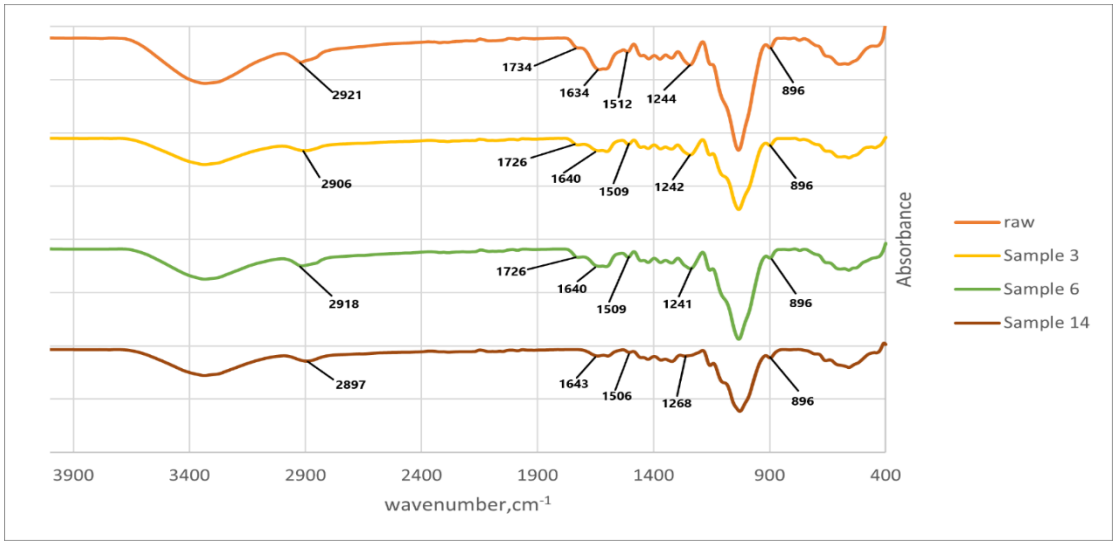


Figure 4.9: FTIR Result of Raw OPEFB and Treated Samples (Sample 3-ethanol; Sample 6-chloroform; sample 14-aqueous NaOH).

Table 4.11: Absorption Band of Raw OPEFB and Treated Samples.

Wavenumber (cm^{-1})				Assignment
Raw	**Sample 3 (Ethanol)**	**Sample 6 (Chloroform)**	**Sample 14 (Aqueous NaOH)**	
3333	3336	3333	3335	O-H stretching
2921	2906	2918	2897	C-H_2 asymmetric stretching
1734	1726	1726	-	C=O stretching
1634	1640	1640	1643	OH bending
1601	1601	1604	1595	C=C stretching with aromatic ring
1512	1509	1509	1506	C=C stretching with aromatic ring

1423	1423	1423	1423	-CH_2 bending
1369	1369	1369	1369	C-H bending
1321	1322	1322	1321	C-O bending
1244	1242	1241	1268	C-O-C stretching
1034	1033	1033	1029	C–O symmetric stretching
896	896	896	896	β -Glycosidic linkage

Figure 4.9 exhibits the FTIR spectra among the raw OPEFB, Sample 3 (ethanol), Sample 6 (chloroform) and Sample 14 (aqueous NaOH), and their absorption bands are sorted in Table 4.11. As seen from Figure 4.9 and Table 4.11, the cellulose in treated samples do not be affected much as the peak at 896 cm^{-1} which indicates β -Glycosidic linkage is available in all samples. In accordance to Ngadi & Lani (2014), it is difficult to fully remove the water even though the samples are dried due to cellulose water interaction. The wavenumber corresponding to 1734 cm^{-1} in raw OPEFB is the presence of lignin and hemicellulose. The intensity of the peak is reducing when the samples are treated with ethanol and chloroform while the peak is completely disappeared when the sample is treated with NaOH solution. This specifies that NaOH solution has stronger delignification effect compared to other solvents and it can eliminate most of the hemicellulose and lignin (Ching & Ng, 2014). Lignin presented characteristic peak at 1512 cm^{-1} and 1244 cm^{-1} also changed much after the OPEFB is treated with different solvents, especially NaOH solution which has strong delignification effect (Ching & Ng, 2014). The C-O-C stretching of lignin at 1244 cm^{-1} is almost disappear in Sample 14 owing to the strength of NaOH in removing the lignin from the raw OPEFB.

The DSC result of the raw OPEFB, Sample 3 (ethanol), Sample 6 (chloroform) and Sample 14 (aqueous NaOH) are compared in Figure 4.10 while their onset temperature, peak temperature and specific enthalpy are shown in Table 4.12. Apparently, it can notice that three treated samples also have broad endothermic peak which denote the evaporation of water molecules in the samples. A sample with a greater endothermic peak temperature indicates that it contains a greater amount of amorphous material (Yeng et al., 2015). The treated samples have higher peak temperature than the raw OPEFB; thus, it can assume that the lignin and hemicellulose

structure are disrupted, and more cellulose is appeared after the samples are treated under chemical treatment with ultrasonication. In addition, the specific enthalpy of the samples of Sample 3 is -25.35 W/g and it is slightly higher than the raw OPEFB which is -23.89 W/g and the other two treated samples. It means that more heat is required for Sample 3 to remove the moisture content (Bryś et al., 2016). On the other hand, the secondary endothermic peak is absent in all treated samples when the temperature becomes higher. The secondary endothermic peak was associated with the crystallinity of cellulose (Yeng et al., 2015). The crystallinity comparison between the samples cannot be conducted without the second endotherm transition after 250℃. Kim et al. (2010) identified that the thermal degradation of cellulose switched to higher temperatures as the crystallinity cellulose index increased. In other word, higher amount of cellulose can be found in the treated samples compared to the raw OPEFB.

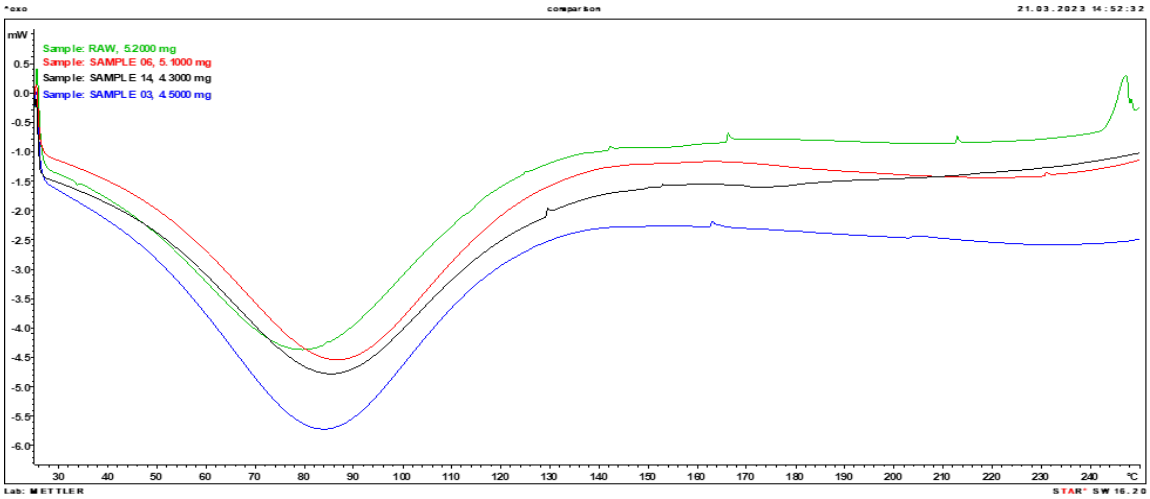


Figure 4.10: DSC Curves of Raw OPEFB and Treated Samples.

Table 4.12: DSC Analysis of Raw OPEFB and Treated Samples.

Samples	1st Endothermic Peak		
	T_o, ℃	T_p, ℃	Δh, W/g
Raw OPEFB	43.00	81.17	-23.89
Sample 3	48.00	83.83	-25.35
Sample 6	49.03	86.83	-21.87
Sample 14	47.62	85.00	-23.09

T_o – Onset temperature, T_p – Peak temperature, Δh – Specific enthalpy

In summary, treatment with NaOH is the best solvent used in this research because it removes most of the lignin and hemicellulose content compared to other solvents. In spite of that, the concentration of the NaOH needs to be controlled to prevent the cellulose degradation. Although the ethanol treatment brings out higher amount of amorphous cellulose in the DSC result, the specific enthalpy of NaOH treated sample is only slightly lower than the specific enthalpy of ethanol treated sample. Evidently, the NaOH treatment works well with the ultrasonic water bath, and it shows effective extraction of cellulose.

4.3 CMC Characterization

Throughout the investigation of treatment behaviour on cellulose content of OPEFB, the manipulation of sonication duration, sonication temperature, concentration of solvent and type of solvent have profound effect to the cellulose extraction process. In order to get high amount of cellulose to produce CMC, the experiment is repeated twice to provide sufficient evidence that the cellulose extracted can be utilised in the production of CMC. The parameters that set for the cellulose extraction process are decided through the previous investigation. On top of that, the solvent used is the NaOH solution due to its outstanding performance to recover high amount of cellulose compared to ethanol and chloroform. The concentration of NaOH solution remains as 1.0 M and it provides strong delignification effect towards the raw OPEFB. Other than that, the sonication temperature is set as 80 ^oC while the sonication duration is fixed as 120 minutes. The effect of sonication duration is not that significant for the NaOH solution. The duration set is sufficient for the solvent to penetrate through the plant cell with the aid of cavitation bubbles for releasing the cellulose as well as it is more cost and time saving.

The two sets of new treated OPEFB samples at sonication temperature of 80 ^{o}C using 1.0 M of NaOH for 120 minutes undergoes carboxymethylation to produce the CMC. The two CMCs formed are labelled as CMC 1 and CMC 2 to ensure the result consistency. Cellulose has partly crystalline structure and strong inter- and intramolecular hydrogen bonding; hence, it is difficult to melt and dissolve in water or the majority of solvents (Haqiqi et al., 2021). The conversion of cellulose to CMC via alkalization and etherification manages to increase the value of original cellulose to be employed in a variety of industrial applications. Figure 4.11 illustrates the appearance of treated samples and the synthesised CMC. The treated samples are more brownish while the synthesised CMC is a little yellowish and light brownish in colour. Despite of changing of appearance, the conversion of treated OPEFB to CMC can be verified in Table 4.13. The mass of the treated OPEFB increases after the carboxymethylation process. The process is capable to yield about 192.98 wt% and 195.22 wt% of CMC, , the addition of weight is due to the successful bonding of carboxymethyl group with treated samples.

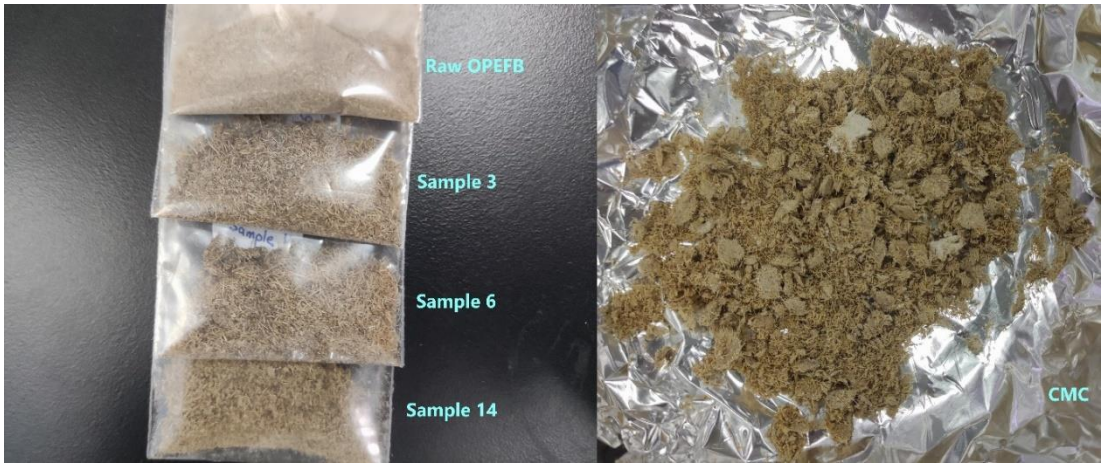


Figure 4.11: Appearance of Raw OPEFB (at the left), Treated Samples (at the left) and Synthesised CMC (at the right).

Table 4.13: The Yield of CMC.

Sample	Weight before Carboxymethylation (g)	Weight after Carboxymethylation (g)	Yield of CMC (wt%)
CMC 1	2.85	5.50	192.98
CMC 2	2.93	5.72	195.22

In the research, the commercial CMC (cCMC) in the market was used to compare with the raw OPEFB and CMCs that were produced by the treated OPEFB. The FTIR spectra comparison is presented in Figure 4.12 and their absorption band is shown in Table 4.14. Referring to the Figure 4.12 and Table 4.14, there is some similar bands can be observed in the samples. For instance, the absorption bands ranging from 3333 cm^{-1} to 3252 cm^{-1} are detected in all samples. It represents the stretching of hydroxyl group (OH) in the cellulose (Muhamad Parid et al., 2017). Similarly, the peaks at around 2921 cm^{-1} to 2894 cm^{-1} for the samples are attributed to the stretching of CH_2 in the lignocellulose (Faizi et al., 2019). Additionally, the CO bending at 1321cm^{-1} to 1325 cm^{-1}, C-O stretching at 1025 cm^{-1} to 1054 cm^{-1} and β -Glycosidic linkage at 896 cm^{-1} to 920 cm^{-1} are available in all samples. In contrast, the presence of an extra sharp peak at 1590 cm^{-1} and 1589 cm^{-1} are found in all CMCs, when compared to raw OPEFB, demonstrated that CMC was effectively synthesised from OPEFB cellulose via carboxymethylation process. The bands around 1590 cm^{-1} and 1415 cm^{-1} in CMCs are assigned to the emergence of asymmetrical and symmetrical stretching of the -COO group from -CH_2COONa of SMCA to substitute the -OH groups on the cellulose when meet with high alkalinity NaOH solution (Tuan Mohamood et al., 2021). There is not much difference between the synthesised CMCs from the OPEFB which are CMC 1 and CMC 2 with the commercial CMC. Accordingly, the cellulose extracted from OPEFB can be utilised in the production of CMC.

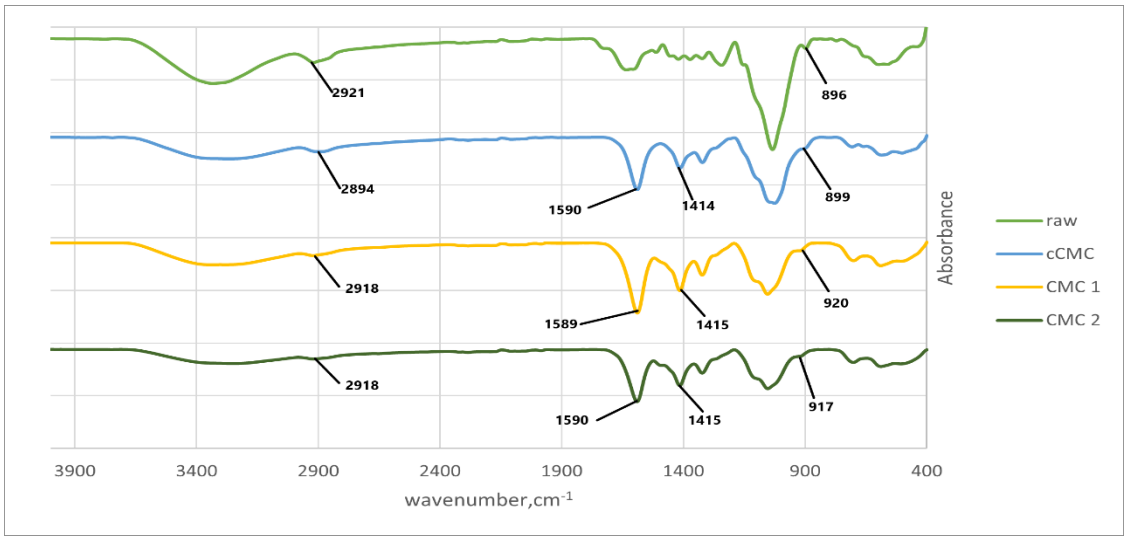


Figure 4.12: FTIR Result of Raw OPEFB and CMCs.

Table 4.14: Absorption Band of Raw OPEFB and CMCs.

Wavenumber (cm^{-1})				Assignment
Raw	**cCMC**	**CMC 1**	**CMC 2**	
3333	3263	3343	3252	O-H stretching
2921	2894	2918	2918	C-H_2 asymmetric stretching
1734	-	-	-	C=O stretching
1634	-	-	-	OH bending
1601	-	-	-	C=C stretching with aromatic ring
-	1590	1589	1590	asymmetric stretching of -COO group
1512	-	-	-	C=C stretching with aromatic ring
1423	-	-	-	-CH_2 bending
-	1414	1415	1415	symmetric stretching of -COO group
1369	-	-	-	C-H bending
1321	1322	1323	1325	C-O bending
1244	-	-	-	C-O-C stretching
1034	1025	1054	1054	C–O symmetric stretching
896	899	920	917	β -Glycosidic linkage

The DS values of CMCs are measured, and standard deviation is calculated. The DS calculated (Appendix I) for CMC 1 is 0.5884 while the DS of CMC 2 is 0.4517. The average DS for the CMC is 0.5201 with standard deviation of 0.0967. When the value of standard deviation is low, it implies that data is grouped around the mean, whereas a large standard deviation shows that data is more spread out. Thus, the mean value of DS is reliable because the standard deviation calculated is closer to zero.

The value of DS of CMC denotes the number of hydrogens in the hydroxyl group of a glucose unit that have been replaced by carboxymethyl group (Anon, 2013). The solubility, thickening property, stability, acid resistance, and salt tolerance of CMC are all affected by the DS. The average value of DS of the CMCs synthesised from OPEFB is 0.5201. The CMC products are usually no less than a DS of 0.4 so that they can be solubilized in the water (BeMiller, 2019). According to Haqiqi et al. (2021), the DS of commercial CMC is 1.13. The DS of the CMC synthesised is relatively lower than the commercial CMC in the market due to low purity of cellulose. The treated OPEFB in the experiment does totally remove all the hemicellulose and lignin. The presence of lignin will result in a reduced efficiency in CMC synthesis (Haqiqi et al., 2021). Consequently, the extracted cellulose can be bleached to remove the lignin especially the acid-soluble lignin and have a better appearance if converted to CMC (Eliza et al., 2015). Although the DS of CMC produced is only 0.5201, it is soluble in water and have certain market value.

The CMCs in the experiment is further modified to become a film. The CMC films are created successfully as shown in Figure 4.13. The CMC film has the potential to be applied as packaging material and it can be further developed using bioactive compound (Łopusiewicz et al., 2021). The glycerol to CMC solution enables the increase of flexibility and mechanical properties (Tabari, 2017). As illustrate in Figure 4.13, the CMC film produced by commercial CMC is colourless, transparent while the CMCs produced by the OPEFB are a bit brownish with some insoluble OPEFB fibres. It is because the presence of lignin and fibres in the CMC produced by the OPEFB brings out the brown colour characteristics (Haqiqi et al., 2021). Also, the treated OPEFB is not fully carboxymethylated and it is the reason why the DS of CMC is not

that high. All in all, CMC can be produced by the treated OPEFB using ultrasonic bath coped with the NaOH solution.

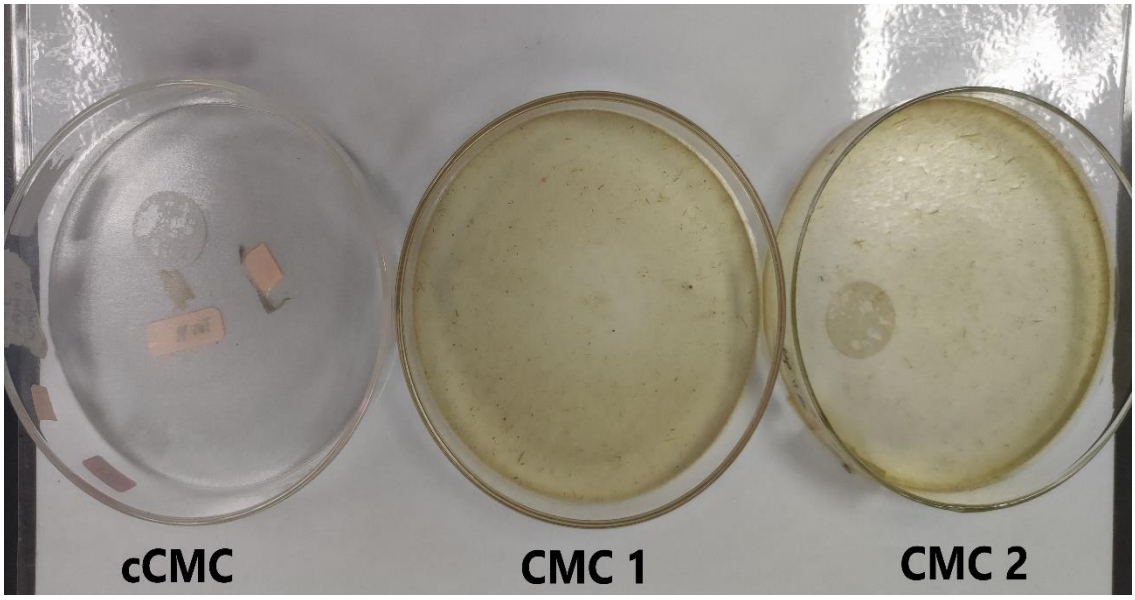


Figure 4.13: The Appearance of Commercial CMC (cCMC), CMC 1 and CMC 2.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

To conclude, the cellulose is extracted successfully from the OPEFB with ultrasonic-assisted treatment. The FTIR analysis have been carried out and the outcomes display the presence of cellulose, hemicellulose and lignin in the raw OPEFB. The cellulose content in the raw OPEFB is determined through the HPLC analysis and it was 33.77 wt%. The thermal stability of the raw OPEFB is estimated to find out the thermal degradation of the raw OPEFB via the DSC.

The extraction of cellulose from the OPEFB is conducted under various parameters in the ultrasonic water bath. This research investigation involves the manipulation of solvent used and three operational parameters: sonication duration, sonication temperature, and concentration of NaOH solution. Following a series of trials, it found that the raw OPEFB treated with 1.0 M NaOH solution with 80 ^{o}C for 180 minutes can yield the highest cellulose content, 78.12 wt%. The NaOH solution works well with the ultrasonic water bath as it can remove most of the lignin and hemicellulose in the raw OPEFB compared to other solvents. The FTIR analysis shows the disappearance of hemicellulose and lignin in term of functional group after the raw OPEFB was treated with NaOH solution. The employing of ultrasonication increases the efficiency of the alkali medium to break the ether bonds between lignin and hemicellulose from the cell wall, exposing more cellulose content. Also, the DSC

thermographs prove higher cellulose content in the treated OPEFB with the existence of higher endothermic peak.

With the optimum parameters found, the OPEFB is treated with the ideal conditions: 1.0 M NaOH solution, 80 ^{o}C and 120 minutes and the treated OPEFB further undergoes carboxymethylation to improve its application. The CMC produced has DS value of 0.5201 and it can be solubilized in the water. The absorption peak at 1589 cm^{-1} in the FTIR spectra proves the substitution of -COO group from -CH_2COONa of SMCA on glucose unit of the cellulose to form CMC. To enhance the CMC application, it can be utilized in the manufacturing of CMC film which is extensively employed in a variety of sectors.

In this study, the ultrasonic-assisted treatment is an alternative method to extract the cellulose from the OPEFB. The OPEFB can be a great raw material for cellulose extraction and further be modified to more value-added product. With the usage of alkali solution in cellulose extraction, the lignin and hemicellulose can be removed to improve the purity of cellulose. The cellulose extraction process is simple to achieve with the combination of ultrasonication and alkali solution at substantially lower operating temperatures, treatment durations and solvent quantity. This research is essential to find out the ultrasonic-assisted extraction is a promising and economical way to overcome the biomass waste problem.

5.2 Recommendation

The ultrasonic-assisted extraction is successful to extract the cellulose from the OPEFB using different solvents. Because of the differences in potential and efficiency, both forms of high-power ultrasonic devices, such as probes and bath systems, are frequently employed in industry. The choice of system will rely on the matrix and the

application needed. In the experiment, the ultrasonic water bath is utilized. Thus, the using of ultrasonic probe in cellulose extraction process can be investigated to determine a better ultrasonic device to extract the cellulose from OPEFB. The choice of the technique is possible to affect the efficiency as well as cost-effectiveness of whole cellulose extraction process at industry level.

Other than that, the characteristics of the treated OPEFB may be further identified through X-ray diffraction (XRD) and scanning electron microscopy (SEM) to know more properties about the products. The using of XRD can determine the crystalline portion of the sample while the using of SEM provides a better surface morphology of the samples. The adding of the characterization methods can bring out a more accurate and detailed result for deciding an ideal condition for the ultrasonic-assisted extraction.

Last but not least, the combination of ultrasonication with alkali medium can extract high amount of cellulose but the alkali medium is considered expensive, and its waste treatment is a necessary issue if the method is widely employed in the industry. Hence, the further research on more solvent properties under the ultrasonication can be done. The selection of a solvent should be based not only on its productivity but also on other essential characteristics such as its economic evaluation and environmental effect.

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APPENDICES

APPENDIX A: HPLC Analysis Calibration Curve for Simple Sugars.

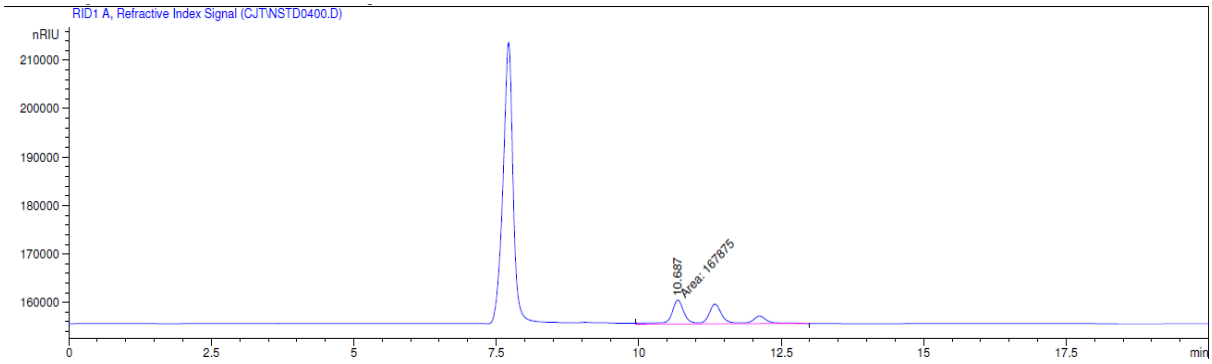

Figure A.1: HPLC Analysis for Arabinose, Xylose, and Dextrose at 0.4 g/L.

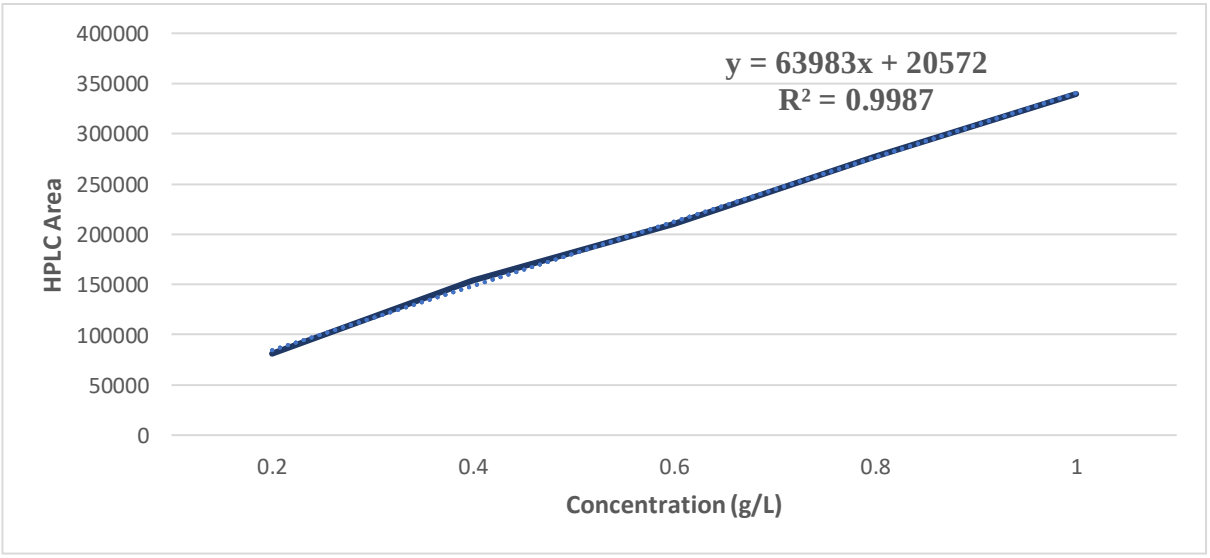

Figure A.2: HPLC Calibration Curve for Arabinose, Xylose, and Dextrose.

APPENDIX B: Calculation Step for the Total Cellulose Content of the Raw OPEFB.

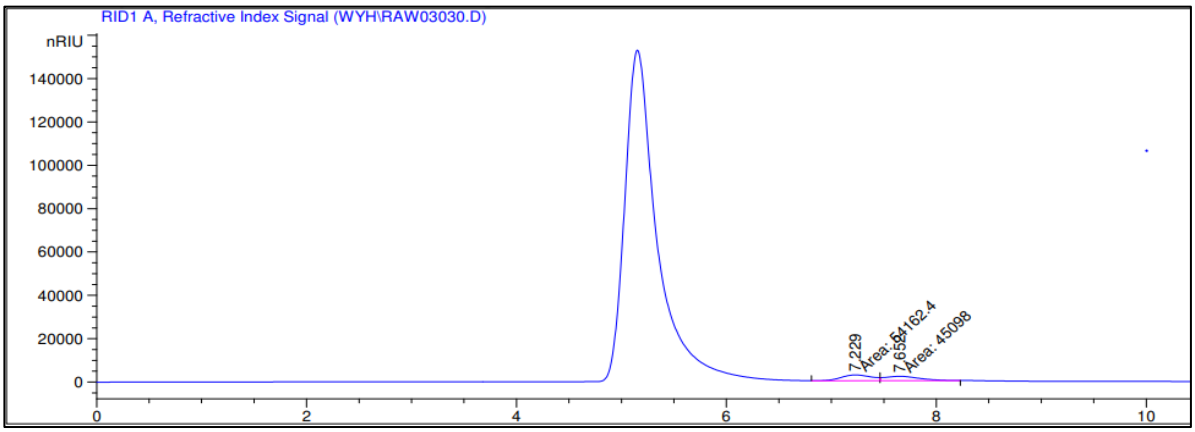

Figure B.1: HPLC Analysis for Raw OPEFB.

HPLC Area of OPEFB = (54162.4 + 45098) *0.57 = 56578

From HPLC Calibration Curve for Arabinose, Xylose, and Dextrose:

y = 63983x + 20572

56578 = 63983x + 20572

x = 0.5627 g/L

$M_1V_1 = M_2V_2$

M_1 (1 mL) = (0.5627 g/L) (2 mL)

M_1 = 1.1254 g/L

Because of 90 mL of sample solution prepared:

Mass of Arabinose, Xylose, and Dextrose = 1.1254 g/L × 0.090 L

= 0.1013 g

Because of 0.3 g of sample used:

Total Cellulose wt% = (0.1013g / 0.3 g) × 100%

= 33.77 wt %

APPENDIX C: HPLC Analysis for Treated OPEFB using Ethanol.

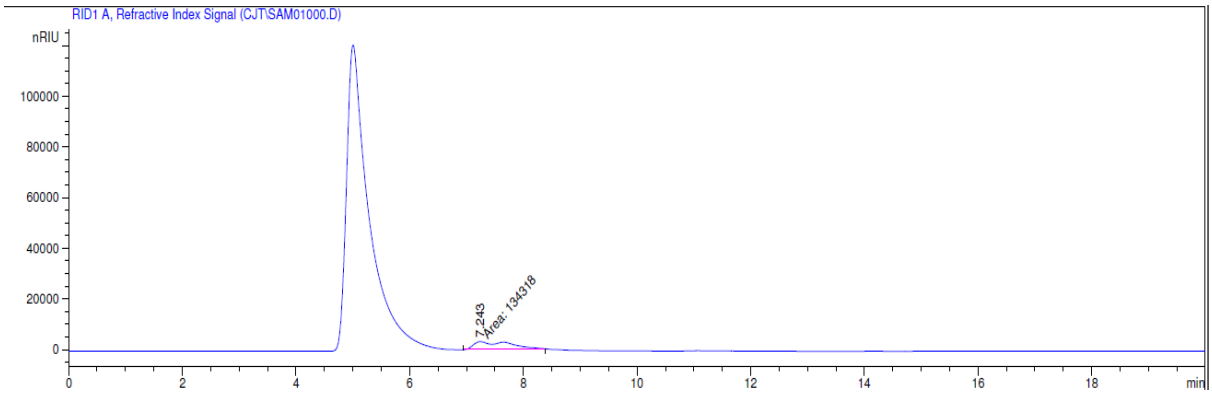

Figure C.1: HPLC Analysis for Sample 1 which is using Ethanol for 120 minutes.

HPLC Area of OPEFB = 134318 *0.57 = 76561.26

From HPLC Calibration Curve for Arabinose, Xylose, and Dextrose:

y = 63983x + 20572

76561.26 = 63983x + 20572

x = 0.8751 g/L

$M_1V_1 = M_2V_2$

M_1 (1 mL) = (0.8751 g/L) (2 mL)

M_1 = 1.7502 g/L

Because of 90 mL of sample solution prepared:

Mass of Arabinose, Xylose, and Dextrose = 1.7502 g/L × 0.090 L

= 0.1575 g

Because of 0.3 g of sample used:

Total Cellulose wt% = (0.1575g / 0.3 g) × 100%

= 52.5 wt %

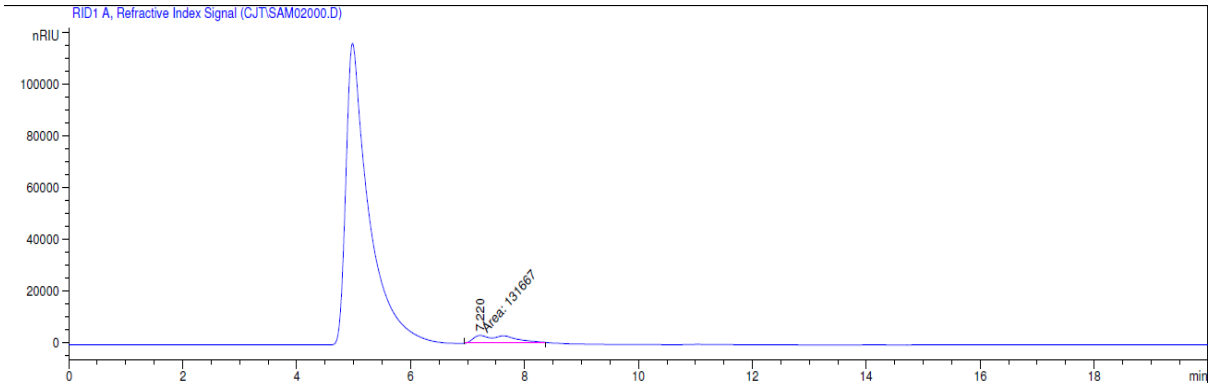

Figure C.2: HPLC Analysis for Sample 2 which is using Ethanol for 180 minutes.

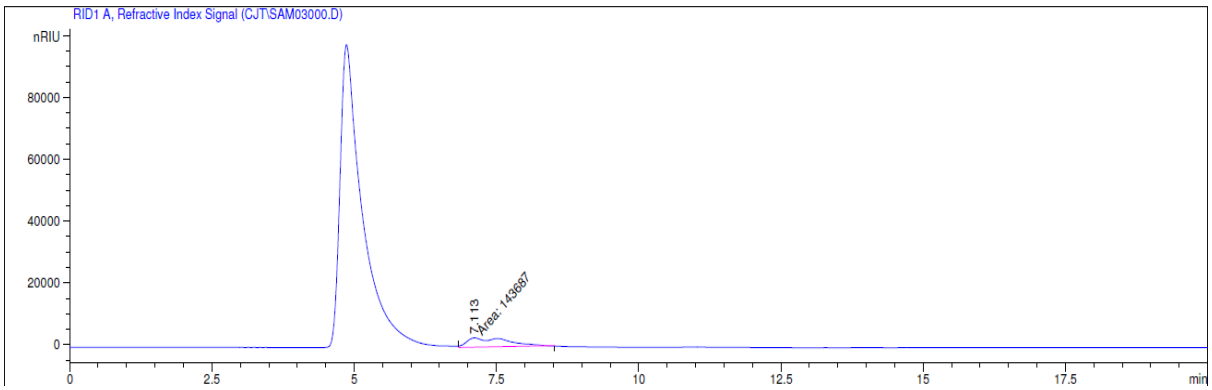

Figure C.3: HPLC Analysis for Sample 3 which is using Ethanol for 240 minutes.

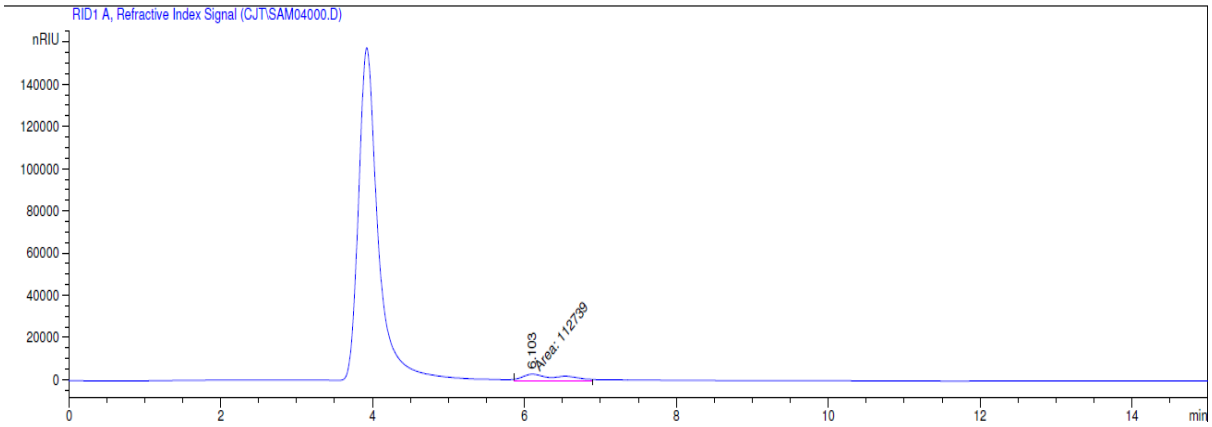

Figure C.4: HPLC Analysis for Sample 4 which is using Ethanol at 30 ^{o}C.

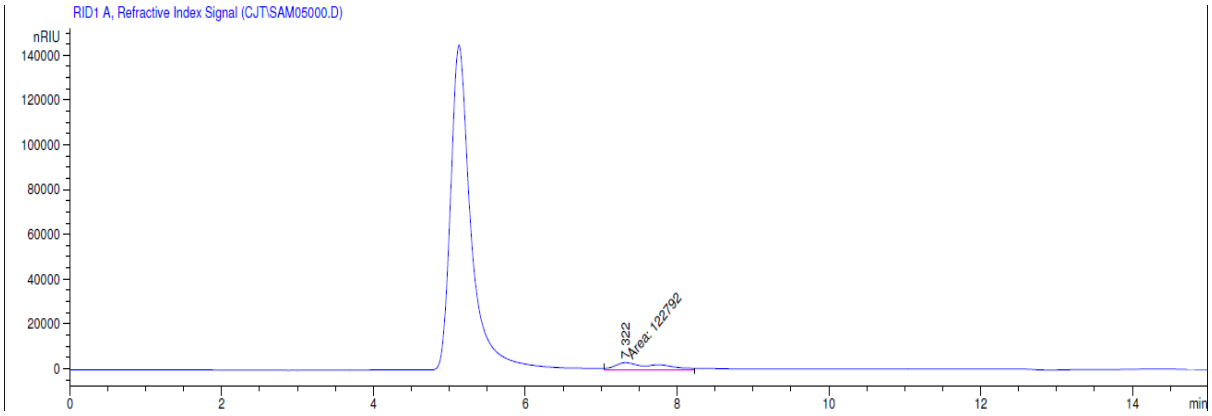

Figure C.5: HPLC Analysis for Sample 5 which is using Ethanol at 50 ºC.

APPENDIX D: HPLC Analysis for Treated OPEFB using Chloroform.

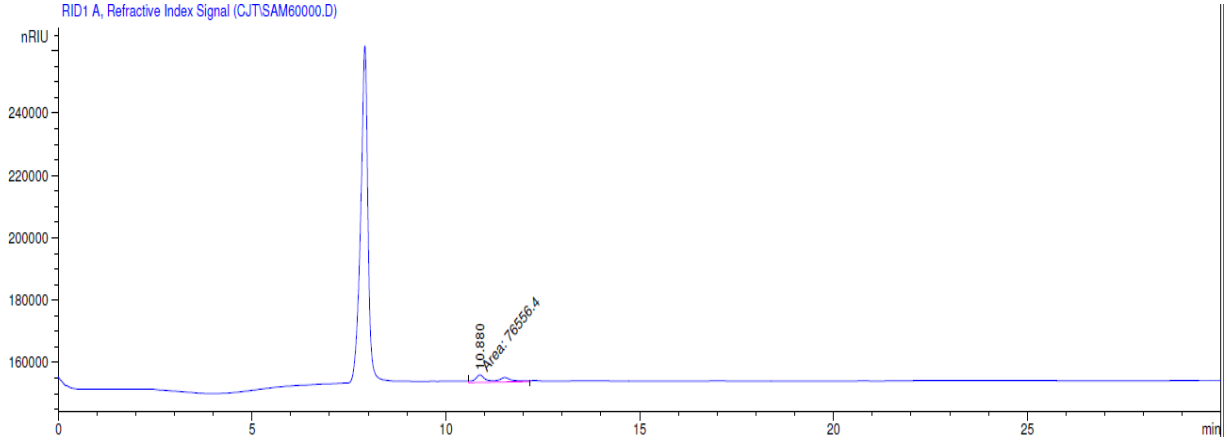

Figure D.1: HPLC Analysis for Sample 6 which is using Chloroform for 120 minutes.

From HPLC Calibration Curve for Arabinose, Xylose, and Dextrose:

$y = 63983x + 20572$

$76556.4 = 63983x + 20572$

$x = 0.8750$ g/L

$M_1V_1 = M_2V_2$

M_1 (1 mL) = (0.8750 g/L) (2 mL)

M_1 = 1.75 g/L

Because of 90 mL of sample solution prepared:

Mass of Arabinose, Xylose, and Dextrose = 1.75 g/L × 0.090 L

= 0.1575 g

Because of 0.3 g of sample used:

Total Cellulose wt% = (0.1575g / 0.3 g) × 100%

= 52.5 wt %

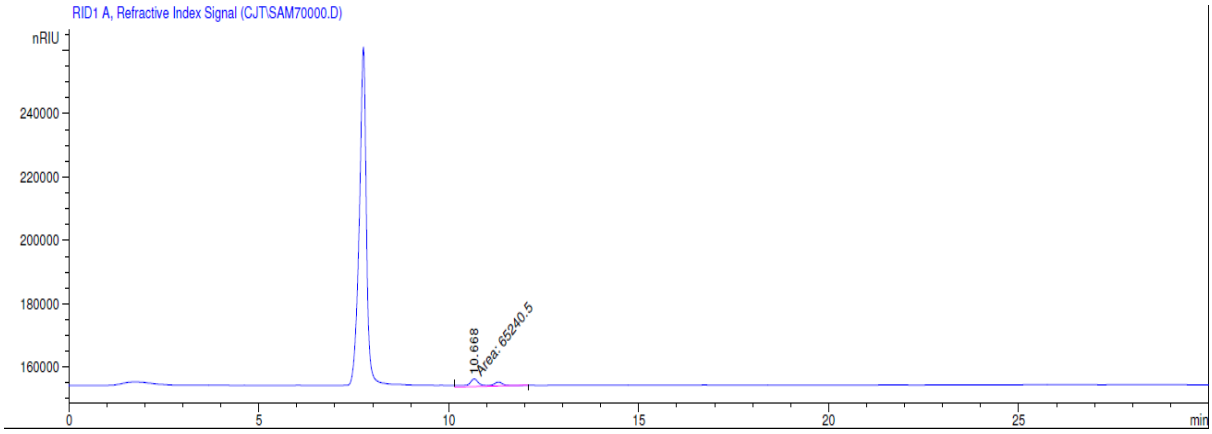

Figure D.2: HPLC Analysis for Sample 7 which is using Chloroform for 180 minutes.

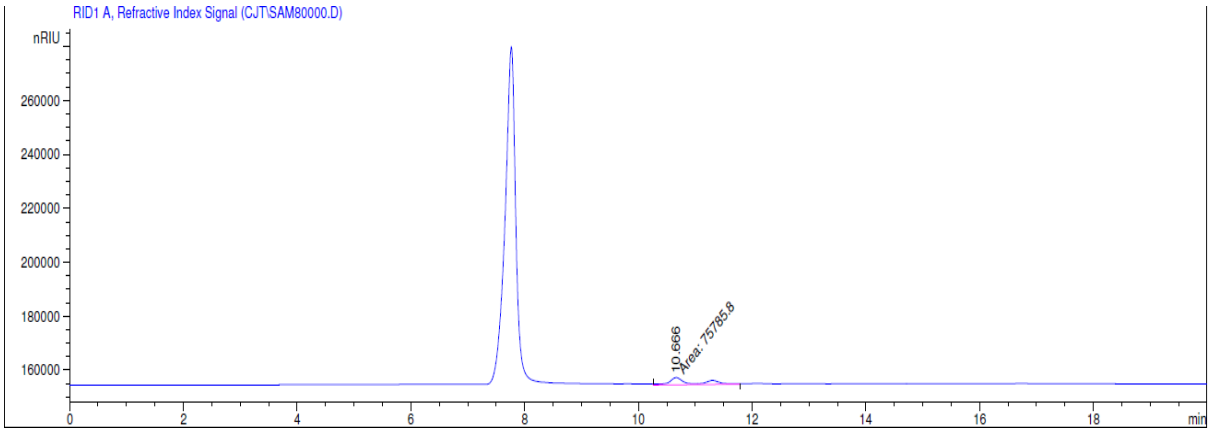

Figure D.3: HPLC Analysis for Sample 8 which is using Chloroform for 240 minutes.

APPENDIX E: HPLC Analysis for Treated OPEFB using Sodium Hydroxide (NaOH)

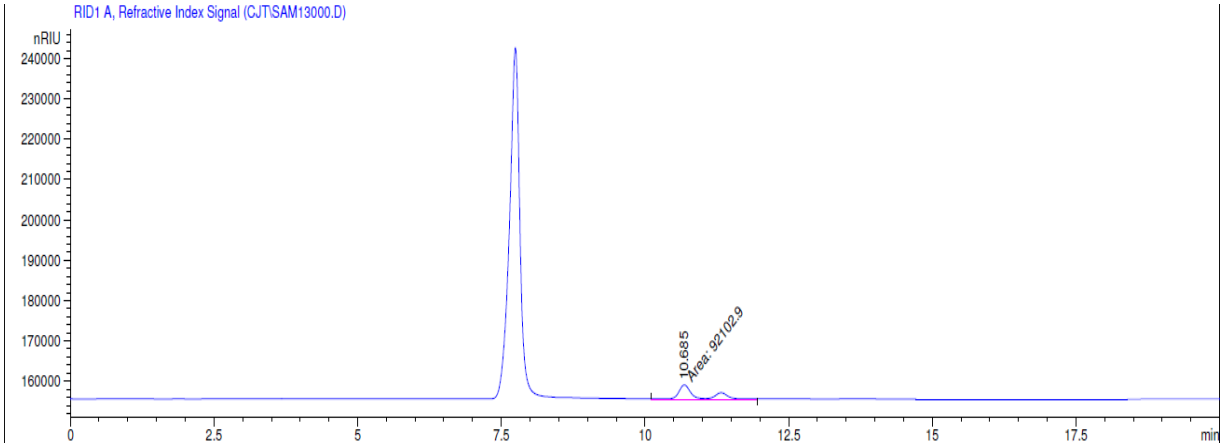

Figure E.1: HPLC Analysis for Sample 13 which is using 0.75 M NaOH for 180 minutes.

From HPLC Calibration Curve for Arabinose, Xylose, and Dextrose:

$y = 63983x + 20572$

$92102.9 = 63983x + 20572$

$x = 1.118$ g/L

$M_1V_1 = M_2V_2$

M_1 (1 mL) = (1.118 g/L) (2 mL)

$M_1 = 2.236$ g/L

Because of 90 mL of sample solution prepared:

Mass of Arabinose, Xylose, and Dextrose = 2.236 g/L × 0.090 L

= 0.20124 g

Because of 0.3 g of sample used:

Total Cellulose wt% = (0.20124g / 0.3 g) × 100%

= 67.08 wt %

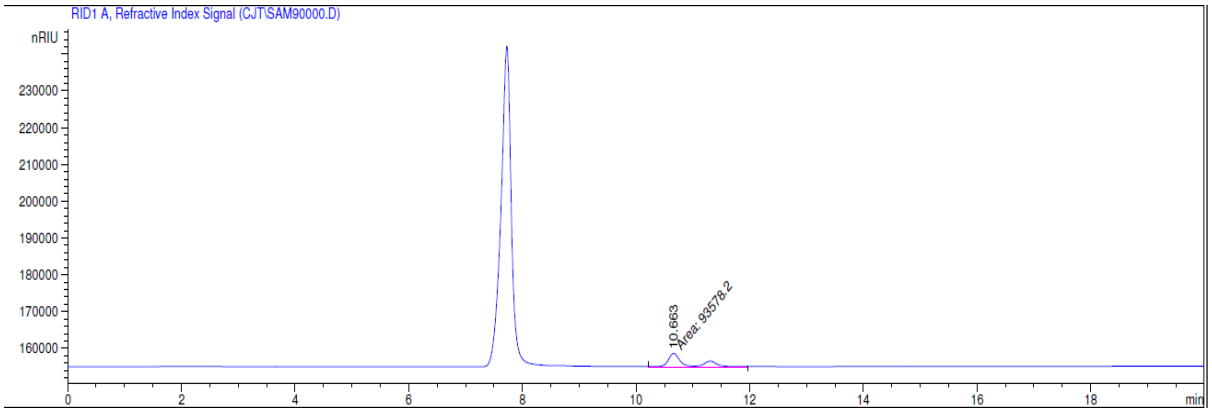

Figure E.2: HPLC Analysis for Sample 9 which is using 0.75 M NaOH for 120 minutes.

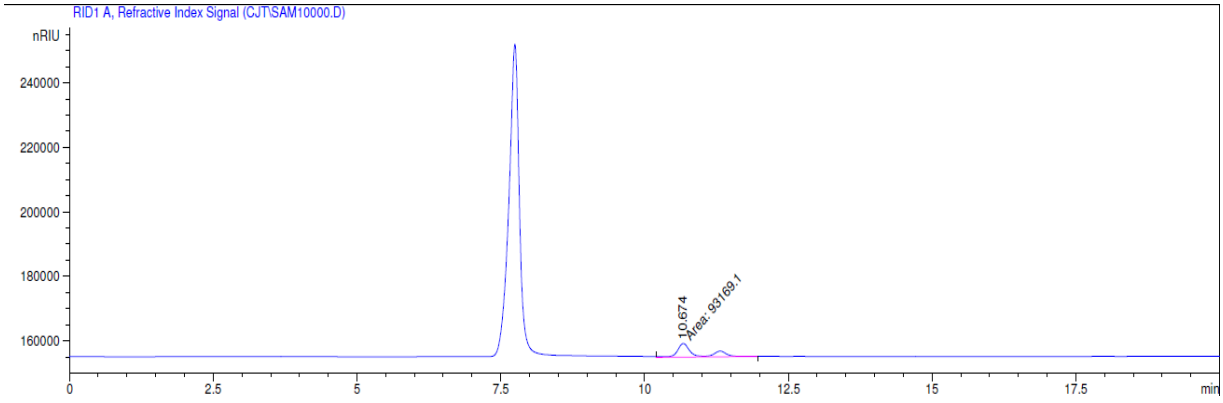

Figure E.3: HPLC Analysis for Sample 10 which is using 0.75 M NaOH for 180 minutes.

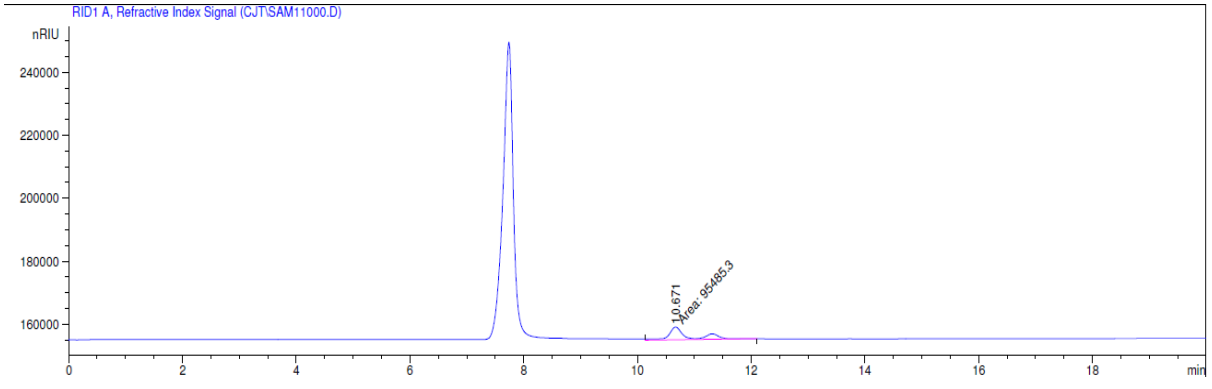

Figure E.4: HPLC Analysis for Sample 11 which is using 0.75 M NaOH for 240 minutes.

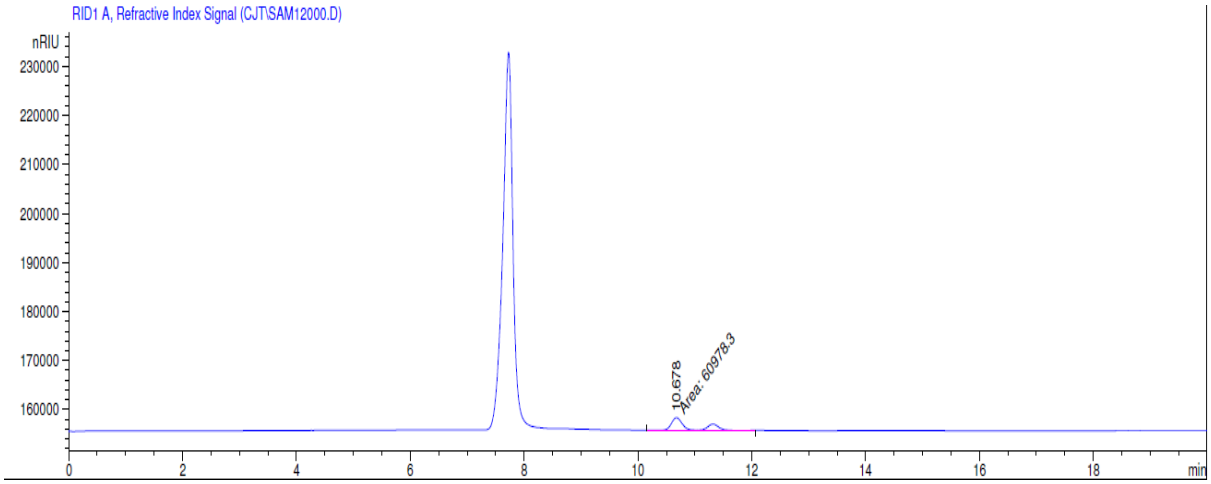

Figure E.5: HPLC Analysis for Sample 12 which is using 0. 5 M NaOH for 180 minutes.

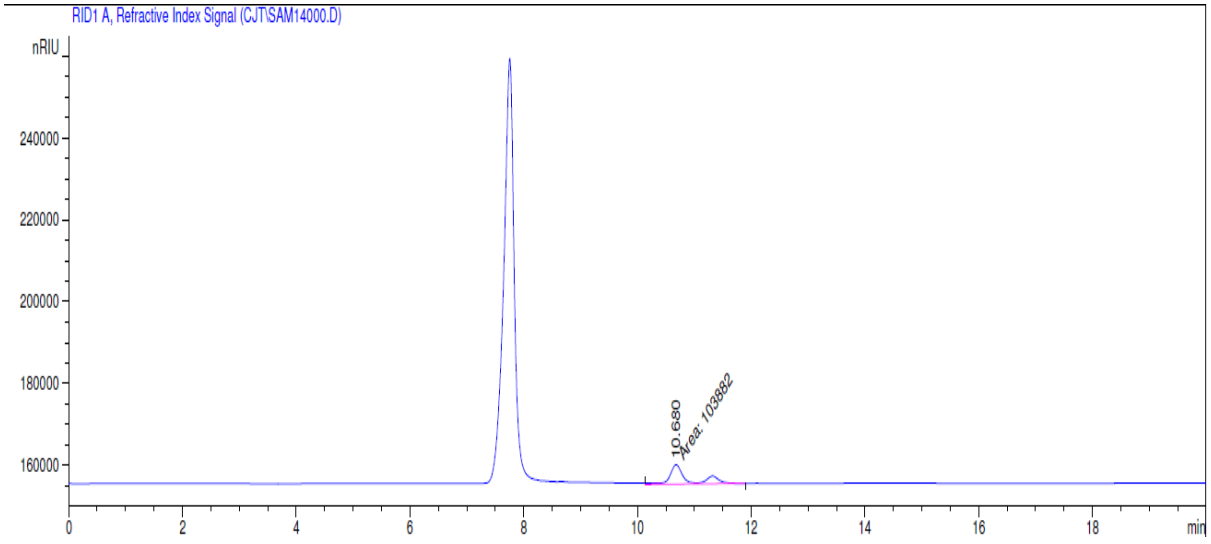

Figure E.6: HPLC Analysis for Sample 14 which is using 1.0 M NaOH for 180 minutes.

APPENDIX F: Summary of the Cellulose Content for all Samples

Table F.1: The Cellulose Content of the Samples Through HPLC Analysis.

Sample	Type of Solution	Sonication Duration (min)	Temperature (^{o}C)	Concentration of Solution (M)	Mass of Arabinose, Xylose, and Dextrose (g)	Cellulose Content (%)
1	Ethanol	120	40	-	0.158	52.50
2	Ethanol	180	40	-	0.153	51.09
3	Ethanol	240	40	-	0.173	57.51
4	Ethanol	240	30	-	0.123	40.97
5	Ethanol	240	50	-	0.139	46.34
6	Chloroform	120	40	-	0.157	52.50
7	Chloroform	180	40	-	0.126	41.89
8	Chloroform	240	40	-	0.155	51.78
9	NaOH	120	80	0.75	0.205	68.46
10	NaOH	180	80	0.75	0.204	68.08
11	NaOH	240	80	0.75	0.210	70.25
12	NaOH	180	80	0.50	0.114	37.89
13	NaOH	180	80	0.75	0.201	67.08
14	NaOH	180	80	1.00	0.234	78.12

APPENDIX G: FTIR Spectra of Treated OPEFB

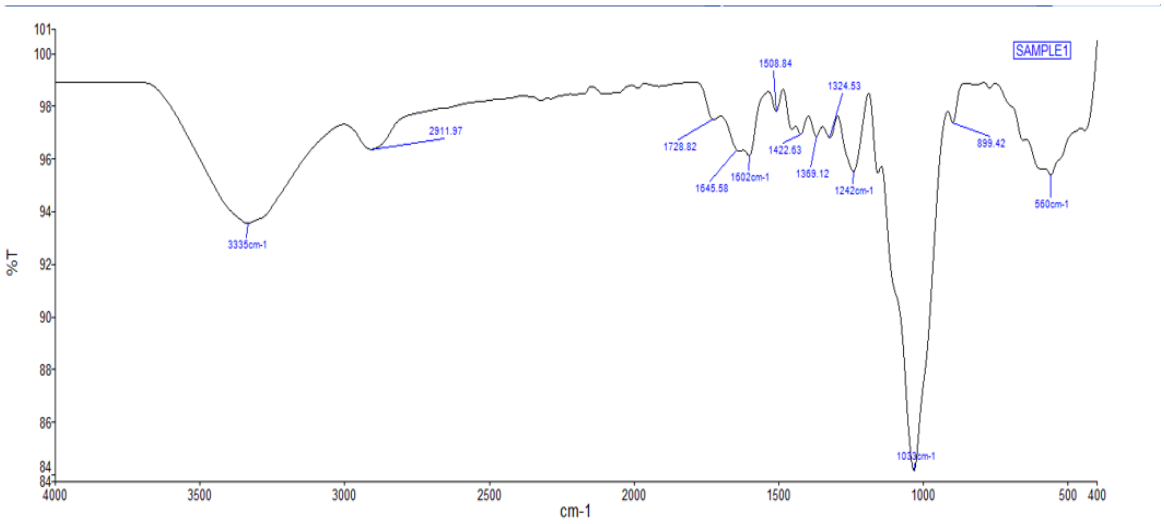

Figure G.1: FTIR Spectra of Sample 1.

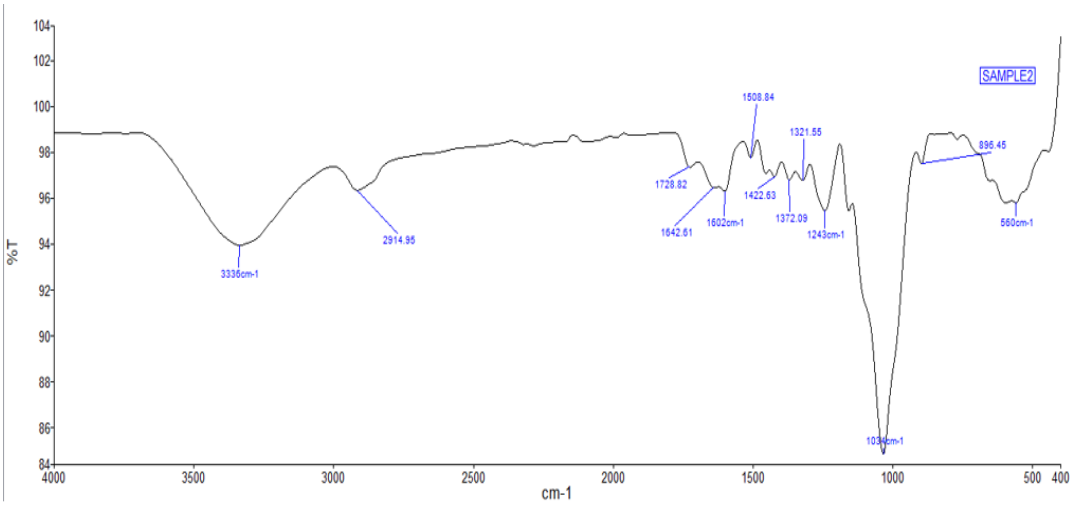

Figure G.2: FTIR Spectra of Sample 2.

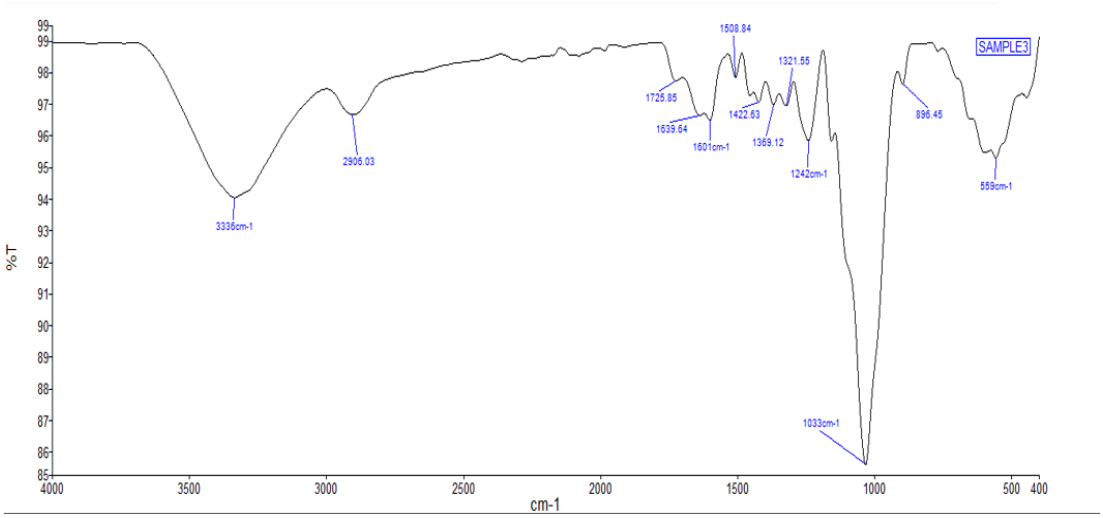

Figure G.3: FTIR Spectra of Sample 3.

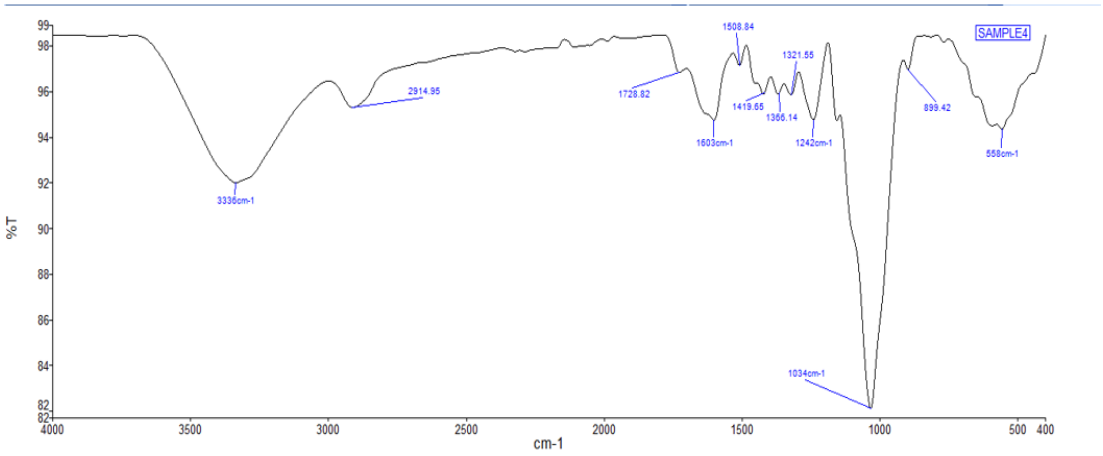

Figure G.4: FTIR Spectra of Sample 4.

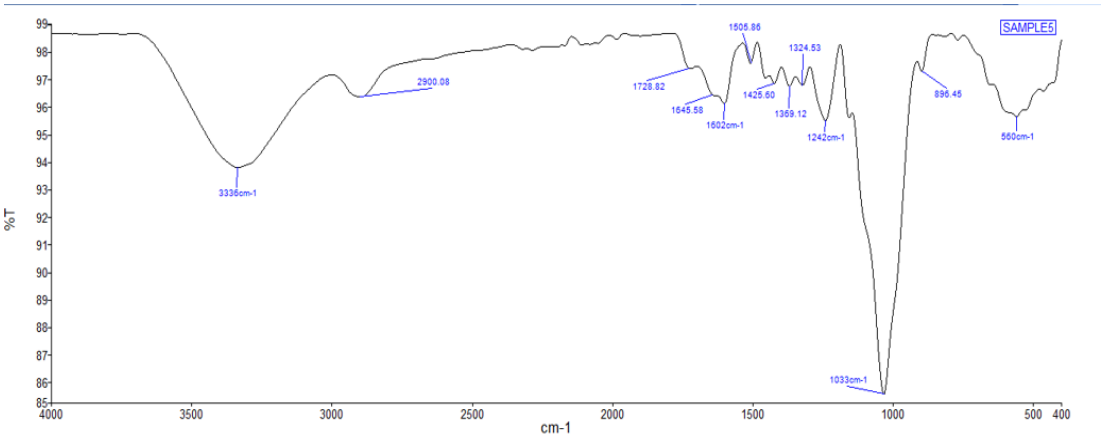

Figure G.5: FTIR Spectra of Sample 5.

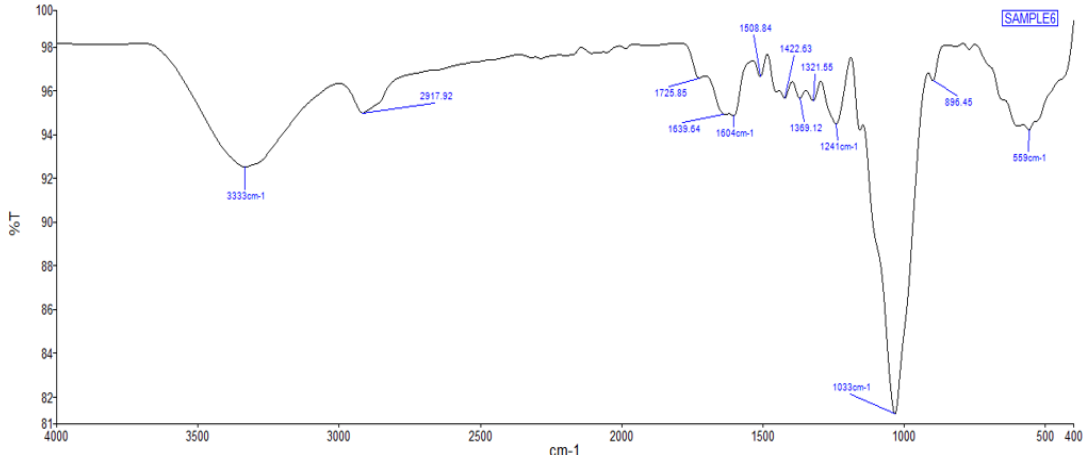

Figure G.6: FTIR Spectra of Sample 6.

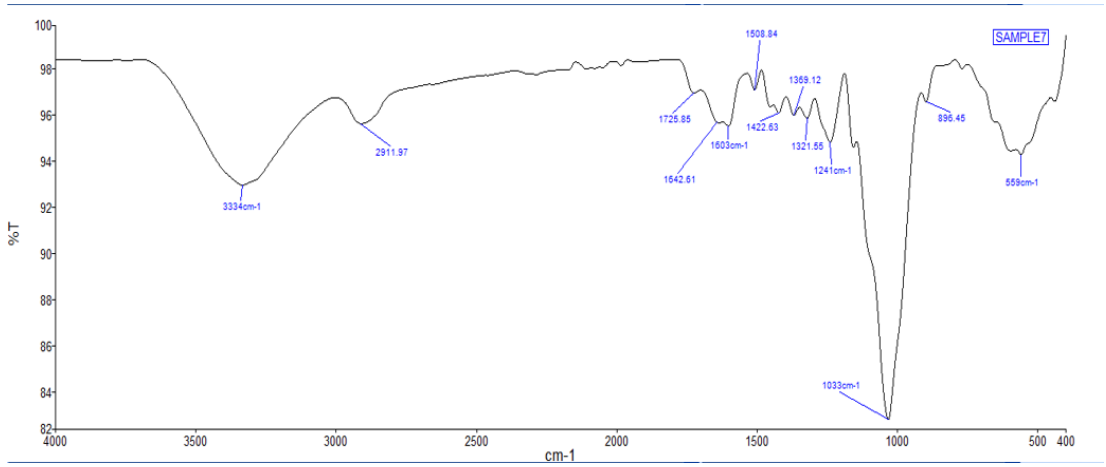

Figure G.7: FTIR Spectra of Sample 7.

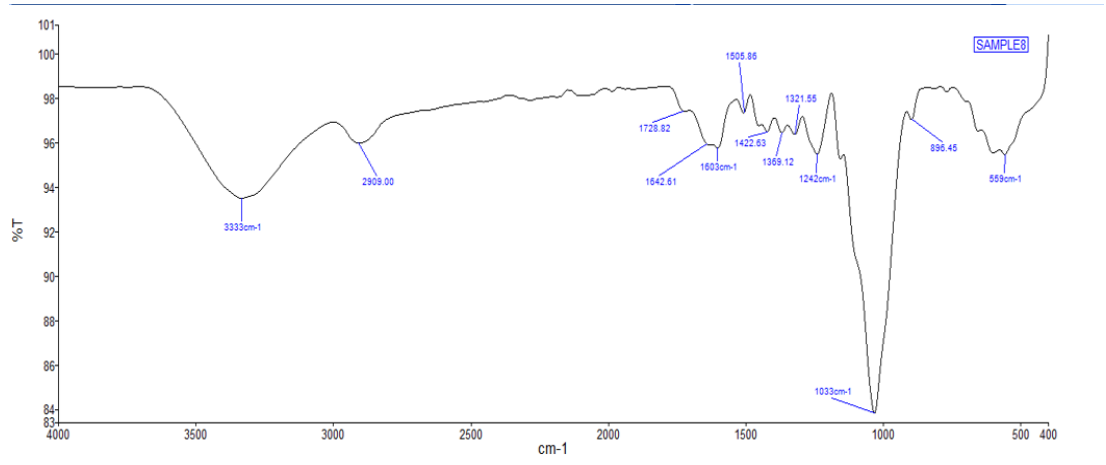

Figure G.8: FTIR Spectra of Sample 8.

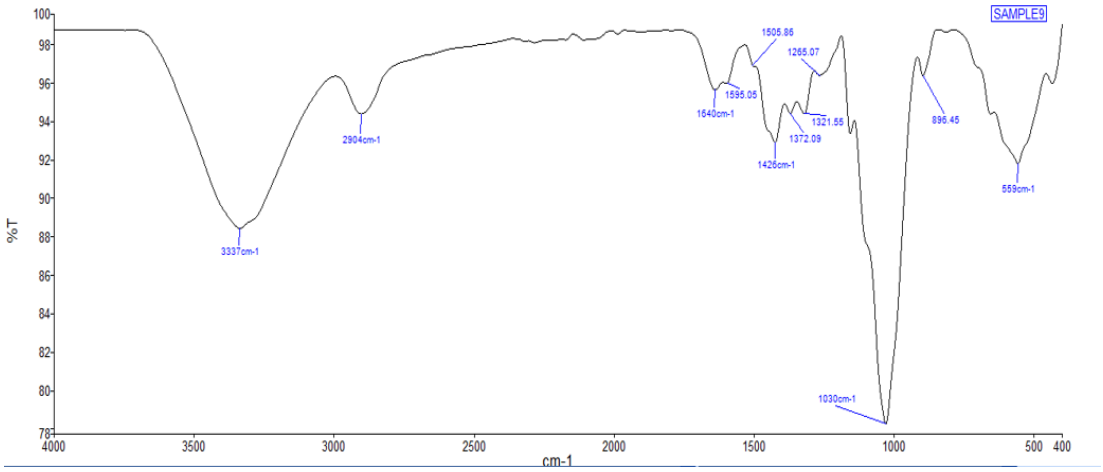

Figure G.9: FTIR Spectra of Sample 9.

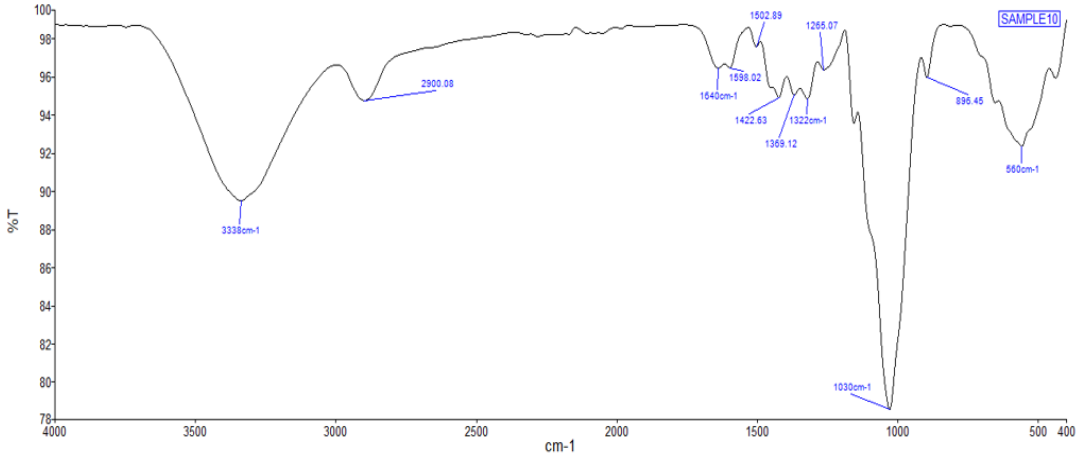

Figure G.10: FTIR Spectra of Sample 10.

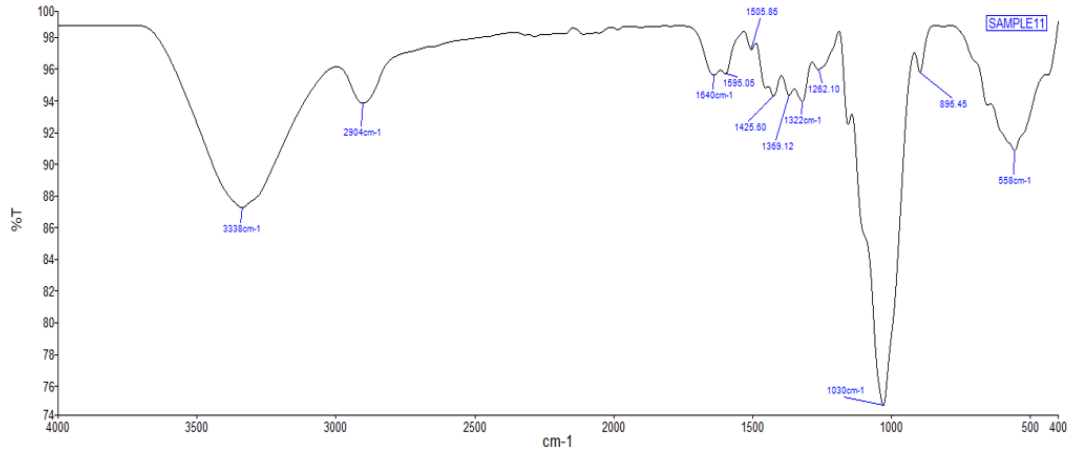

Figure G.11: FTIR Spectra of Sample 11.

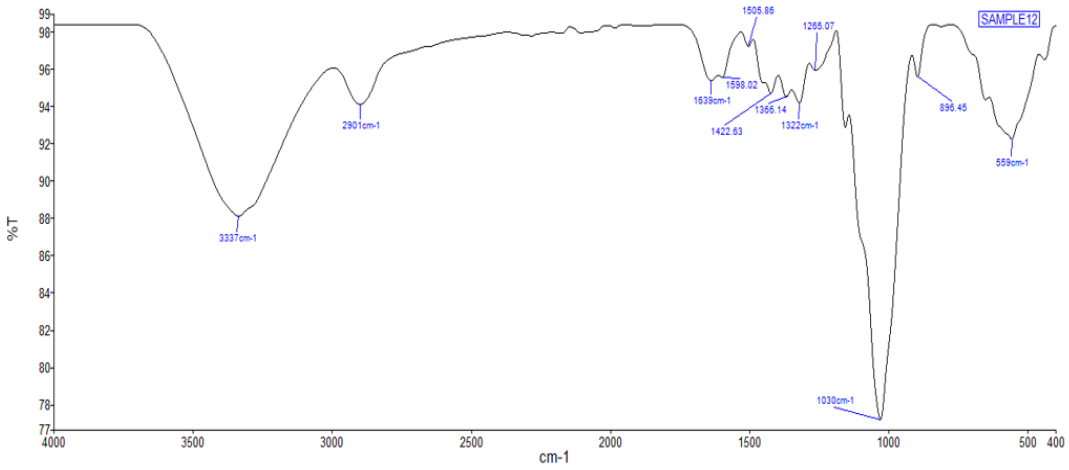

Figure G.12: FTIR Spectra of Sample 12.

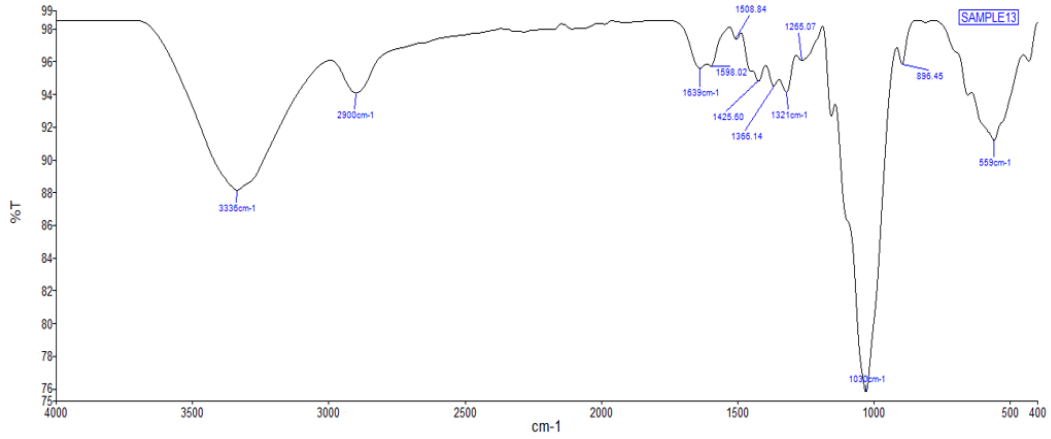

Figure G.13: FTIR Spectra of Sample 13.

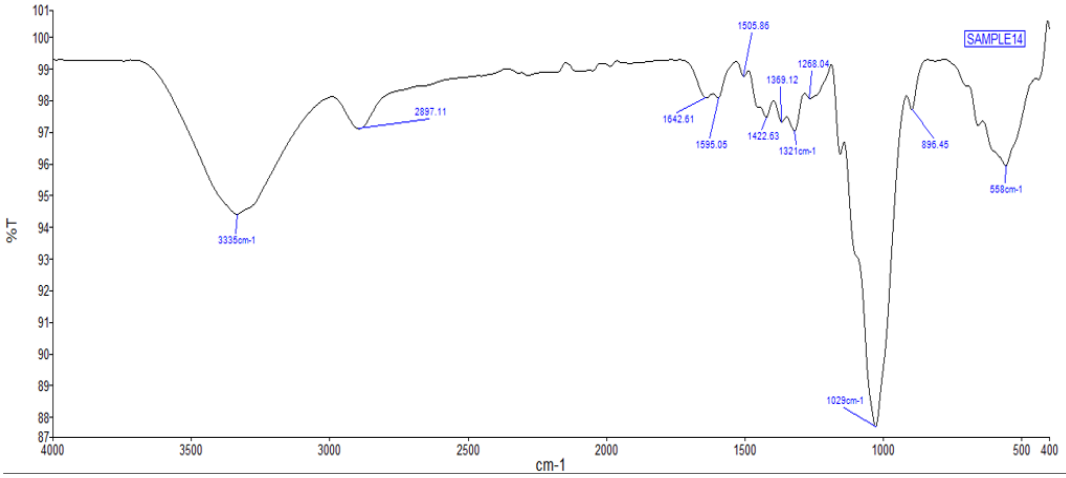

Figure G.14: FTIR Spectra of Sample 14.

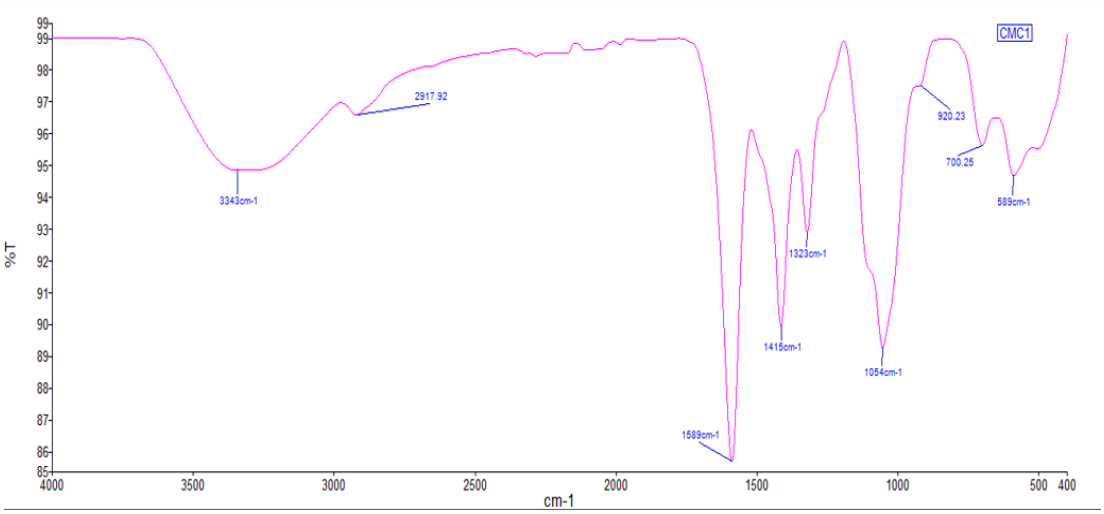

Figure G.15: FTIR Spectra of CMC 1.

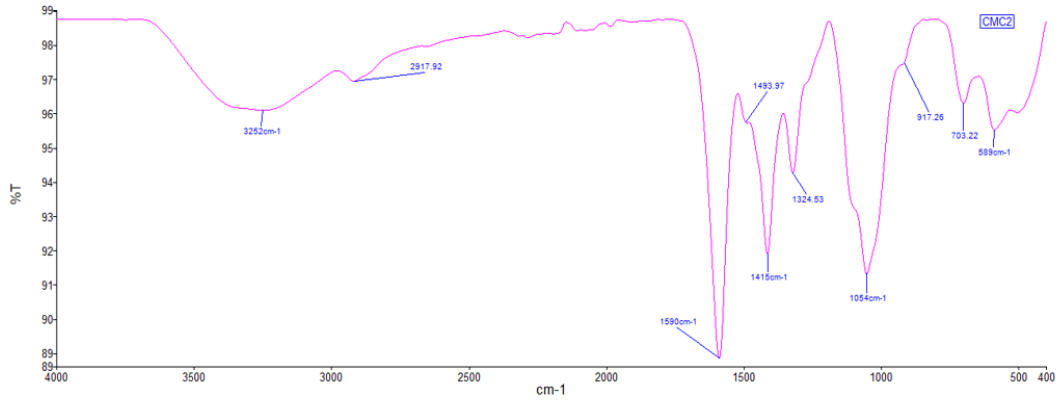

Figure G.16: FTIR Spectra of CMC 2.

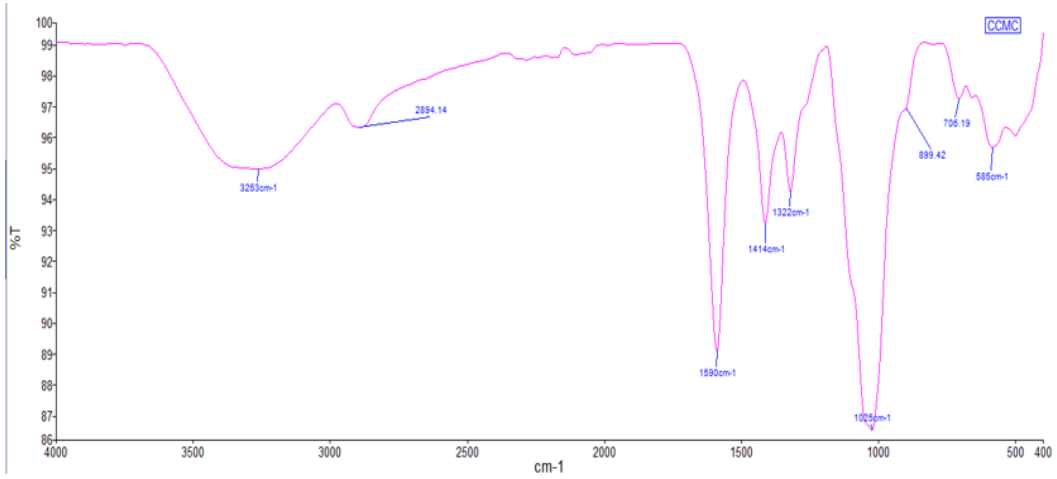

Figure G.17: FTIR Spectra of commercial CMC.

APPENDIX H: DSC thermograph of Treated OPEFB

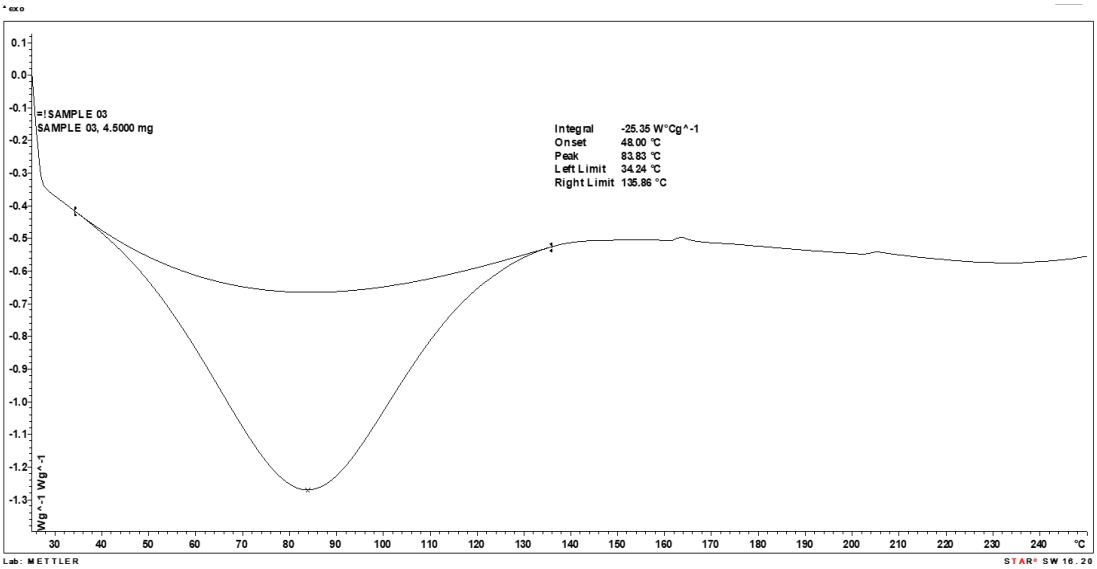

Figure H.1: DSC thermograph of Sample 3

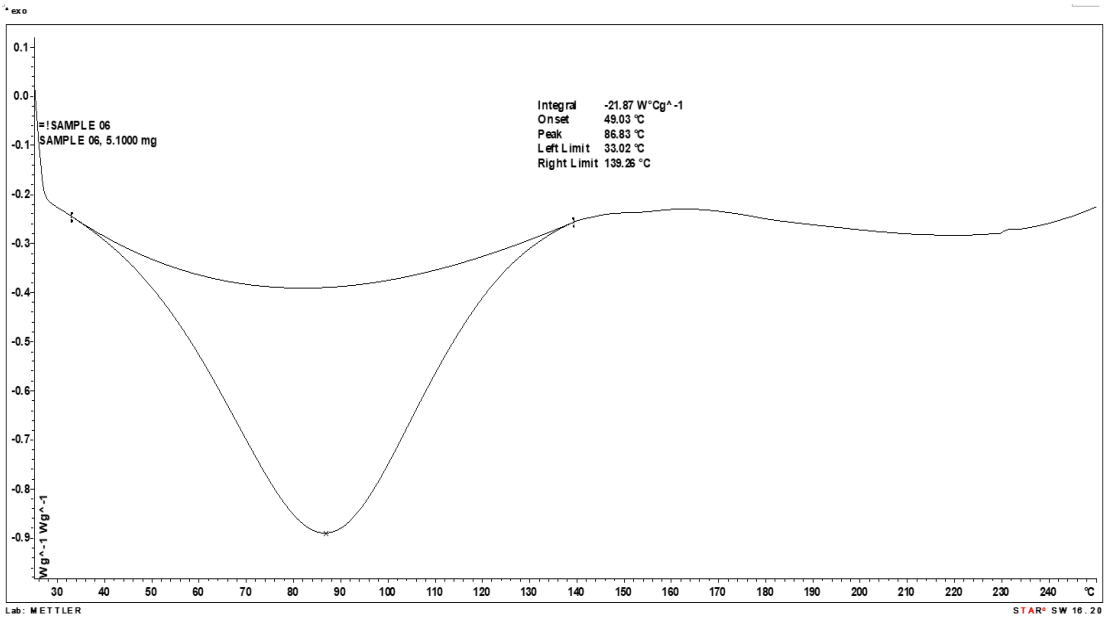

Figure H.2: DSC thermograph of Sample 6

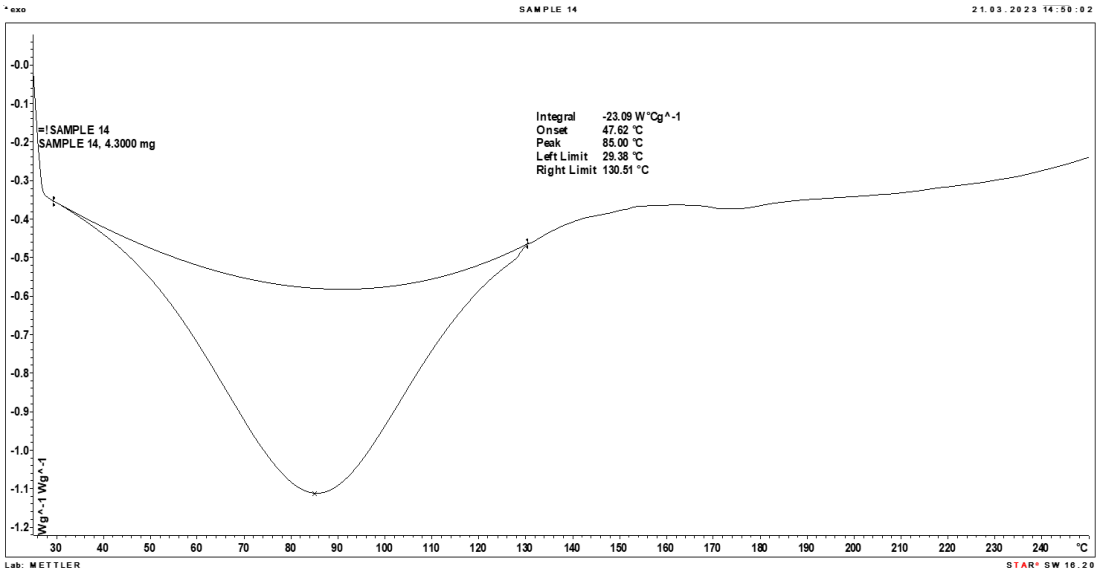

Figure H.3: DSC thermograph of Sample 14

APPENDIX I: Calculation Steps for DS of CMCs

From Equation 3.2, $A = \frac{BC-DE}{W}$

For CMC 1, D = Volume of HCl used = 15 ml

$$A = \frac{(25)(0.3) - (15)(0.3)}{1} = 3$$

Using Equation 3.1, $DS = \frac{0.162 \times A}{1 - 0.058 \times A}$

$$DS = \frac{0.162 \times 3}{1 - 0.058 \times 3} = 0.5884$$

DS of CMC 1 = 0.5884

For CMC 2, D = Volume of HCl used = 17 ml

$$A = \frac{(25)(0.3) - (17)(0.3)}{1} = 2.4$$

$$DS = \frac{0.162 \times 2.4}{1 - 0.058 \times 2.4} = 0.4517$$

DS of CMC 2 = 0.4517

Average DS of CMC = $\frac{0.5884+0.4517}{2} = 0.5201$