PREPARATION AND CHARACTERIZATION OF NANOCELLULOSE FROM ORANGE PEEL WASTE

Submitted in accordance with requirement of the Universiti Teknikal Malaysia Melaka (UTeM) for Bachelor Degree of Manufacturing Engineering (Hons.)

by

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DECLARATION

I hereby, declared this report entitled “Preparation and Characterization of Nanocellulose From Orange Peel Waste” is the result of my own research except as cited in references.

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APPROVAL

This report is submitted to the Faculty of Manufacturing Engineering of Universiti Teknikal Malaysia Melaka as a partial fulfilment of the requirement for the Degree of Bachelor of Manufacturing Engineering (Hons). The member of the supervisory committee is as follow:

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ABSTRAK

Kulit oren ialah serat semula jadi yang boleh didapati secara komersil dengan minat yang semakin meningkat untuk menggunakannya sebagai bahan mentah dalam pelbagai aplikasi kerana kepekatan selulosa yang tinggi dalam tumbuhan. Penyelidikan ini bertujuan untuk menentukan penyediaan nanoselulosa daripada sisa kulit oren dengan menggunakan kaedah rawatan kimia iaitu kaedah hidrolisis asid. Bagi menyediakan keadaan optimum nanoselulosa sisa kulit oren yang mempunyai kehabluran tinggi dalam struktur kristalnya dengan menggunakan kaedah hidrolisis asid dan kesan terhadap pelbagai kepekatan asid terhadap indeks kehabluran, saiz kristal dan morfologi nanohablur selulosa telah dikaji. Walau bagaimanapun, berdasarkan kajian terdahulu, penggunaan sisa kulit oren sebagai kajian merupakan suatu kajian yang terhad terhadap jenis asid dan kepekatan asid optimum yang merupakan parameter penggunaan kaedah hidrolisis. Oleh itu, dalam kajian ini, proses penyediaan nanoselulosa sisa kulit oren dengan menggunakan kaedah hidrolisis asid dengan jenis asid terbaik menggunakan asid sulfurik (H_2SO_4) dan asid hidroklorik (HCl), kepekatan asid optimum 30wt% pada masa hidrolisis malar (120min) dan suhu malar (45°C) dikaji secara menyeluruh. Daripada keputusan yang diperolehi, H_2SO_4 adalah kaedah terbaik untuk hidrolisis asid yang diperlukan untuk menghasilkan selulosa kristal yang tersebar dengan baik dengan kesan pengagregatan yang minimum dan kepekatan asid optimum ialah 30wt% dengan masa hidrolisis 120min dan suhu 45°C dengan indeks kehabluran tertinggi dan saiz kristal iaitu masing-masing 87.69% dan 3.19nm. Nanoselulosa sisa kulit oren boleh menjadi bahan hijau yang menjanjikan dimana ianya sesuai dengan trend reka bentuk dan pembangunan kemampanan global.

ABSTRACT

Orange peel is a commercially available natural fiber with an increasing interest in using it as a raw material in a variety of applications due to its high cellulose concentration in the plant. This research aims to determine the preparation of nanocellulose from orange peel waste by using chemical treatment of acid hydrolysis method. In order to provide the optimum conditions of nanocellulose of orange peel waste which are possessed high crystallinity in their crystal structure by using acid hydrolysis method and the effects of various acid concentrations on the crystallinity index, crystallite size and morphology of cellulose nanocrystals were studied. However, based on the previous study, using orange peel waste there is limited study has been reported on type of acid and optimum acid concentration as a parameter of using hydrolysis method. Therefore, in this study, the process of preparation orange peel waste nanocellulose by using acid hydrolysis method with the best type of acid used sulphuric acid (H_2SO_4) and hydrochloric acid (HCl), optimum acid concentration 30wt% at constant hydrolysis time (120min) and constant temperature (45°C) is comprehensively studied. From the result obtained, H_2SO_4 is the best method for acid hydrolysis needed to generate well-dispersed crystalline cellulose with minimal aggregation affect and the optimum acid concentration is 30wt% with 120min hydrolysis time and 45°C temperature with the highest crystallinity index and crystallite size which is 87.69% and 3.19nm respectively. Orange peel waste nanocellulose can be promising green material that fits well with global sustainability design and development trends.

DEDICATION

Only

my beloved father, Saad bin Na'aman

my appreciated mother, Azizah binti Zairan

my adored sister, Siti Sazira, Noor Ayuni, Nur Syazwani, Ainur Fazliana

my adored brother, Muhammad Faizal

my partner, Abdul Aziz bin Samin

for giving me moral support, money, cooperation, encouragement and also understandings

Thank You So Much & Love You All Forever

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Last but not least, I want to express my affection to beloved family members with their prayers, constant love, sacrifice, and strength which build the perfect version of this project that boost my courage which I have to face various obstacles in finishing this project. Thanks to all my friends and everybody who helped by encouraging my work and helping me in advance with their knowledge and valuable suggestion during this project. May Allah s.w.t shower His godsend in their live with respect and honour. I hope that all the deed and effort every single day will benefits us with revolutionary learning that can be practised for the future. I have no valuable words to express my thanks, but my heart is still full of favours received from every person. Thank you very much.

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LIST OF ABBREVIATIONS

XRD	-	X-Ray Diffraction
FTIR	-	Fourier Transform Infrared
SEM	-	Scanning Electron Microscope
TEM	-	Transmission Electron Microscopy
CNC	-	Cellulose Nanocrystals
CNF	-	Cellulose Nanofibers
BNC	-	Bacterial Nanocellulose
BC	-	Bacterial Cellulose
H_2SO_4	-	Sulphuric Acid
HCl	-	Hydrochloride Acid
NaOH	-	Sodium Hydroxide
NaCl	-	Sodium Chloride
PVA	-	Polyvinyl Alcohol
CrI	-	Crystallinity Index
H_3PO_4	-	Phosphoric Acid
HNO_3	-	Nitric Acid
OH	-	Hydroxide
Na^+	-	Natrium
H_2O	-	Water
OPW	-	Orange Peel Waste

LIST OF SYMBOLS

α	-	Alpha
°C	-	Degree Celsius
°	-	Degree
%	-	Percent
g	-	Gram
GPa	-	Gigapascal
kv	-	Kilovolt
kN	-	Kilonewton
mA	-	Milliampere
µm	-	Micrometer
nm	-	Nanometer
cm^{-1}	-	$100m^{-1}$
mg	-	Milligram
MPa	-	Megapascal
nm	-	Nanometre
wt%	-	Weight Percentage
µm	-	Micrometre
min	-	Minutes
ml	-	millimetre
pH	-	Potential of Hydrogen

CHAPTER 1

INTRODUCTION

Chapter 1 covers the background of the study, problem statement, objectives, scope, the importance of study and organization of the report. Background of the study explains how this project is significant to make a nanocellulose from orange peel waste (OPW). The problem statement demonstrates the problem that inspired the idea for this project. Following that, objectives depict the overall goal of this project, whereas scopes depict the constraints and method used to create this project.

1.1 Background of Study

Citrus fruits, which include oranges, limes, grapefruits, and lemon, are among the most popular and well-known types of fruits worldwide. Due to its waste material, orange peel is one of the underutilised waste materials. Citrus fruits are high in vitamin C, a nutrient that boosts the immune system and keeps skin looking youthful. They also contain vitamin A and B, dietary fibres, folic acid, amino acids, and minerals like calcium, potassium, and phosphorus, all of which are beneficial to health. This study is supported by (Kerri *et al*. 2017).

Alkaline treatment can be used to extract nanocellulose, a natural fibre, from cellulose that contains cementing materials such as lignin and hemicellulose. The alkaline treatment is critical for the production of highly pure cellulose nanocrystals (Ng *et al*. 2017).

Alkaline treatment increases the density of fiber by removing the nanocellulosic component that is hemicellulose and lignin by using sodium hydroxide (NaOH). This treatment remove lignin and hemicellulose and it also increase the amount of cellulose exposed on the fiber surface. Consequently, alkaline treatment increases the degree of crystallinity.

All plant materials, including citrus fruits and natural lignocellulosic materials, are made up of three organic components that are cellulose, hemicelluloses, and lignin. Cellulose is a linear D-glucose that form microfibrils which is responsible for the polymer's strength and resistance. While hemicellulose is a form of polysaccharide whose structure varies depending on the source, such as the type of plant and plant tissue. Lignin is a high molecular weight complex and amorphous polymer with three-dimensional network which is linked by phenylpropane monomers. It provides support to the plant cell wall; microbial resistance and it has hydrophobicity of the cell wall (Monika *et al.* 2017).

The hydrolysis process conditions, such as type of acid, optimum acid concentration, constant hydrolysis temperature, and constant hydrolysis time are critical in the production of cellulose nanocrystals. As a result, the motivation of this project is to investigates the type of acid used, as well as the optimum acid concentration used during the hydrolysis treatment, constant temperature and constant hydrolysis time, as a parameter in order to develop nanocellulose from orange peel waste. The temperature and time will be considered a constant parameter and two different acidic is used during acid hydrolysis as a parameter.

As a result, the purpose of this research is to develop a strategy for the preparation and characterization of nanocellulose from orange peel waste via chemical treatment methods such as acid hydrolysis. Due to the limitations of the previous study, only a few studies have been published on the effect of different type of acid and acid concentration as a parameter of hydrolysis of nanocellulose from orange peel waste. Additionally, the orange peel waste nanocellulose will be analysed using X-ray Diffraction (XRD) analysis, Fourier Transform Infrared (FTIR) analysis and Scanning Electron Microscope (SEM) .

1.2 Problem Statement

This study emphasizes the preparation and the characterization of nanocellulose from orange peel waste (OPW) via chemical treatment. In order to prepare nanocellulose OPW several procedures need to be done which start from the orange peel soaking in the distilled water to separate the albedo and flavedo, while in this study albedo was chosen as a raw material, then will be subjected to alkaline treatment, bleaching process and acid hydrolysis treatment. The outcome of this studies is dependent on the chosen parameters such as type of acid, optimum acid concentration, constant temperature, and constant hydrolysis time. OPW is one of the underutilized waste materials, however, there are possibility to produce high-yield and high crystallinity due to its high fibre content. In order to achieve high-yield and high-quality nanocellulose, an efficient method for producing of nanocellulose with excellent properties at low cost is still a challenges.

Therefore, acid hydrolysis with concentrated mineral acids is the most frequently used method for the preparation of nanocellulose. However, the method has several critical flaws, including being hazardous to the environment and human body, corroding process equipment and causing excessive degradation of raw cellulose material, and being expensive. sulphuric acid (H_2SO_4) and hydrochloric acid (HCl) was able to overcome the difficulties associated with hydrolyzing cellulose due to its medium acidity, and OPW nanocellulose with good nanoscales and high yield were successfully produced (Ji *et al*. 2019). Therefore, this current problem must be improved by developing the optimal design parameter for the acid hydrolysis treatment on this experiment by varying type of acid used and optimum acid concentration and the constant hydrolysis time and temperature. Throughout the process of nanocellulose from OPW, a diverse set of experiments with varying parameters is used.

In contrast, recent acid hydrolysis studies only focused on the optimal hydrolysis time to get the best crystal structure of nanocellulose OPW. Nevertheless, only a few studies have been done on the various type of acid used and optimum concentrations used in hydrolysis. Hence, this study will investigate the effect of type of acid as well as optimum acid concentration on nanocellulose preparation from OPW in term of crystallinity and crystallite size.

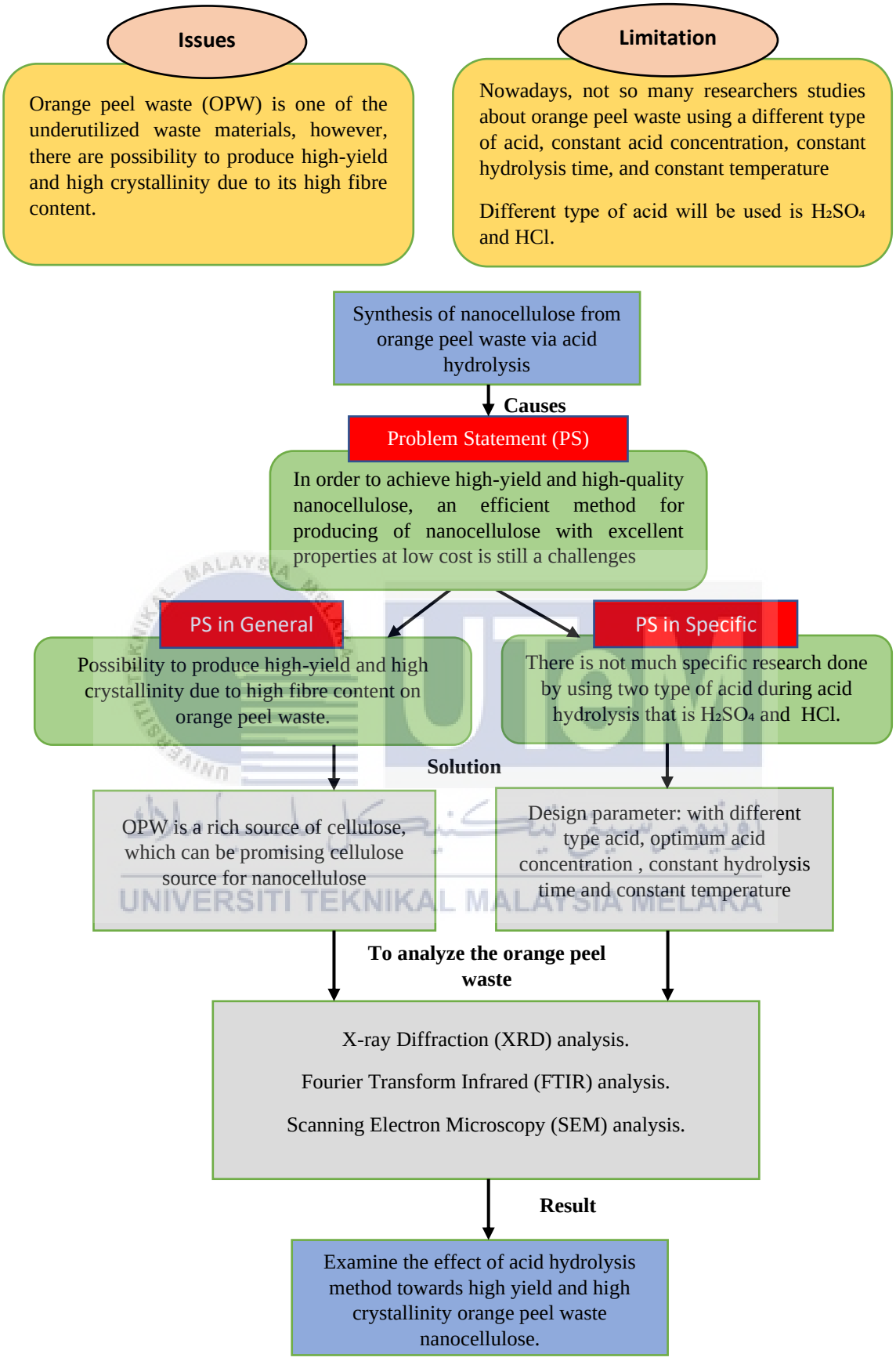

Figure 1.1: The summarization of problem statement and research gap

1.3 Objective

The objectives are as follows:

1. To prepare nanocellulose from orange peel waste (OPW) using acid hydrolysis method using different type of acid.
2. To determine the crystallinity of nanocellulose OPW by using X-ray Diffraction (XRD) and Fourier Transform Infrared (FTIR) analysis.
3. To characterize the surface morphology and structure of OPW waste by using Scanning Electron Microscopy (SEM) analysis.

1.4 Research Scope

This study focuses on the chemical fractionation of orange peel. For the preparation of nanocellulose from OPW, from the raw material preparation followed by alkaline treatment and acid hydrolysis treatment is used to extract the cellulose fiber. To obtain highly purified cellulose, lignin is removed using an alkaline treatment. The samples were subjected to concentration, time, and temperature variations. The primary objective is to characterize and apply OPW via acid hydrolysis with the acid concentration, as well as to investigate the crystallinity and crystallite size and characterization of cellulose nanocrystals. To achieve the objective, research was carried out using a variety of scopes.

The first objective of this research is to investigate the method of preparing nanocellulose from OPW via acid hydrolysis. To extract the cellulose nanocrystals, the acid hydrolysis method was used. This is due to the fact that acid hydrolysis is the most efficient method for dissolving glycosidic bonds in cellulose and consumes the least amount of energy. The type of acid, optimum acid concentration, constant temperature, and constant hydrolysis time is the parameters that will be optimized in this method. In this study, various type of sulfuric acid (H_2SO_4) and hydrochloric acid (HCl) will be used

Then, as mentioned in the second objective, to analyze the crystallinity and crystallite size of cellulose extracted from orange peel waste by using X-ray Diffraction (XRD) analysis. By calculating the crystallinity index (Crl) by using Segal equation and crystallite size by using Scherer equation, the XRD analysis is used to investigate the crystallinity of the nanocellulose OPW. Following that, the experiment result is supported by Fourier Transform Infrared (FTIR) analysis. After acid hydrolysis has accomplished, FTIR is used to determine the presence of lignin and hemicellulose structures. Finally, the surface morphology changes and structure of nanocellulose OPW will be characterized. Scanning Electron Microscopy (SEM) analysis was used to investigate the surface morphology changes and structure of nanocellulose OPW in order to accomplish this final objective.

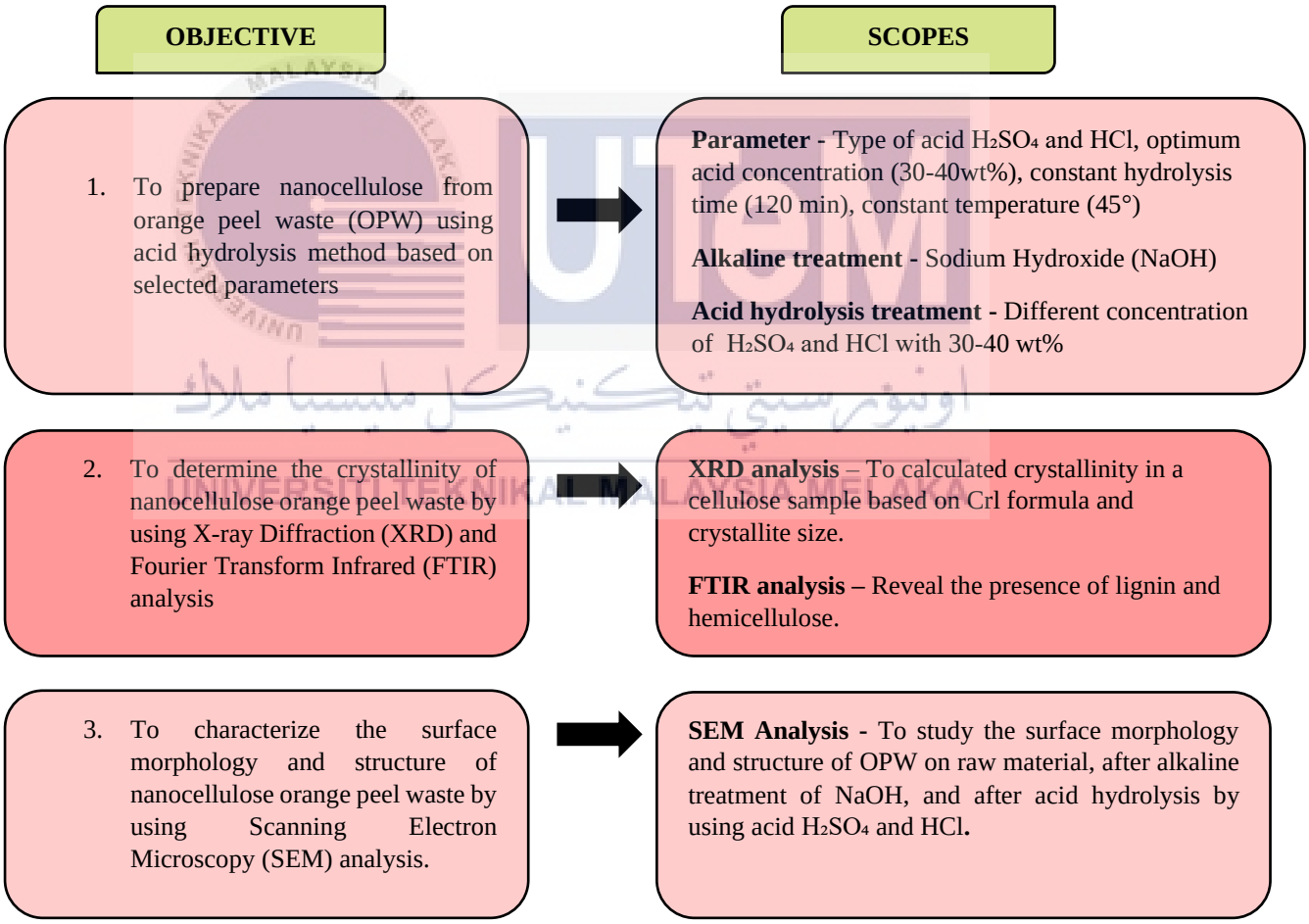

Figure 1.2: The mapping matrix of the scopes and objectives

1.5 Significant of Research

Citrus fruit processing industries produce massive amounts of waste materials like peel and pulp that are not properly managed. In the current study, citrus waste was chosen to extract cellulose and nanocellulose. The goal of this study is to find out how crystallinity of OPW nanocellulose can be successfully produced and its crystallite size. The hydrolysis parameters, such as optimum acid concentration, constant hydrolysis time, and constant temperature, have recently been investigated. However, limited research has been done on the use of an appropriate type of acid used with optimum acid concentration during hydrolysis process. Thus, the nanocellulose OPW extraction studies will be carried out by using parameter of type of acid used, optimum acid concentration, constant hydrolysis time and constant temperature during the hydrolysis process.

Finally, the use of this eco-friendly material aligns with technology trends toward sustainable design and development, energy efficiency, and water conservation. The production of natural fibers does not emit greenhouse gases into the atmosphere, which contribute to global warming. However, the manufacturing processes for synthetic fibers like glass fiber or carbon fiber emit carbon dioxide, which can contribute to ozone depletion.

CHAPTER 2
LITERATURE REVIEW

This chapter presents a literature review of previous research that is aligned to this topic, which has been defined and carried out over an interval of time. Based on the research into the preparation of nanocellulose from orange peel waste (OPW), the characterisation and analysis of the cellulose from OPW, related information from earlier studies is extracted for use as references and discussion.

2.1 Nanocellulose Fiber

Cellulosic fibres are found in all natural fibers and are made up of cellulose, hemicellulose, lignin, and pectin. Lignin is a complex and amorphous polymer with a three-dimensional network of phenylpropane monomers of high molecular weight. It helps to strengthen the plant's cell wall. Cotton, hemp, flax, jute, ramie, and wood are all examples of plant cells that contain cellulose. Agricultural by-products containing cellulose include sugarcane bagasse, jute, ramie, banana, orange, and corncob. Furthermore, cellulose, which is made up of proteins and carbohydrates, can be found in a wide variety of bacterial species, tunicates, algae, and sea creatures.

Next, George *et al.* (2015) found that cellulose is made up of monomers linked together by glycosidic oxygen bridges and formed by condensation. The chemical structure of cellulose are shows on Figure 2.1. due to the abundance of cellulose sources, there has been a surge in interest in studying cellulose fibers in recent years. Nanocellulose research began because natural fibers can be used to create new ecologically and biodegradable products.

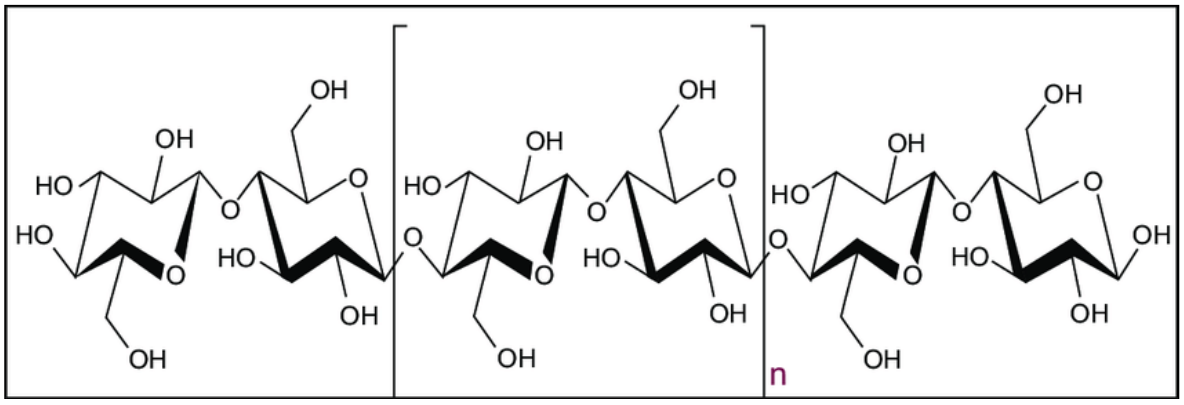

Figure 2.1: Molecular structure of cellulose (George, *et al.* 2015)

2.1.1 Types and Structure of Nanocellulose

As illustrated in Figure 2.2, nanocellulose can be classified into three types of cellulose nanofibers (CNF), cellulose nanocrystals (CNC), and bacterial nanocellulose (BNC). They show how they are changed on the surface and how they can be used in a variety of applications.

CNC have nano dimensions in length and diameter, whereas CNF have micro dimensions in length and nano dimensions in diameter, CNC have nanoscale length and diameter, whereas CNF have microscale length and nanoscale diameter; their size varies according on the source from which they are created, but it is typically between 100 and 1,000 nm in length and 4 to 25 nm in diameter. For BNC nanocellulose, entanglement results in the formation of stable network architectures with average diameters of 20–100 nm and lengths of micrometres. Following that, mild acid hydrolysis combined with steam explosion can be used to produce CNF, whereas a medium acid, such as sulfuric acid (H_2SO_4) can be used to produce CNC, which eliminates the amorphous section (disordered region) and results in the nanocrystal structure, whereas BNC is produced extracellularly by microorganisms (Syafri *et al.* 2022).

According to the research study, acid pretreatment is a way of breaking the rigid structure of lignocellulosic materials in the presence of hydronium ions by targeting intermolecular and intramolecular connections between cellulose, hemicellulose, and lignin.

concentrated acids such as H_2SO_4, HCl, H_3PO_4, and HNO_3 are different types of acid used during hydrolysis process. Therefore, based on the previous study, CNC will be used in this study. The target was to find nanocellulose in the form of fibrilled fibers. Therefore, based on the previous study, CNC will be used in this study. Next, CNC that were obtained through the chemical reaction that deteriorated the amorphous region and only left the crsytalline region. CNC is highly crystalline in terms of its structure. Table 2.1 below shows the type of nanocellulose by (Gumrah et al. 2017).

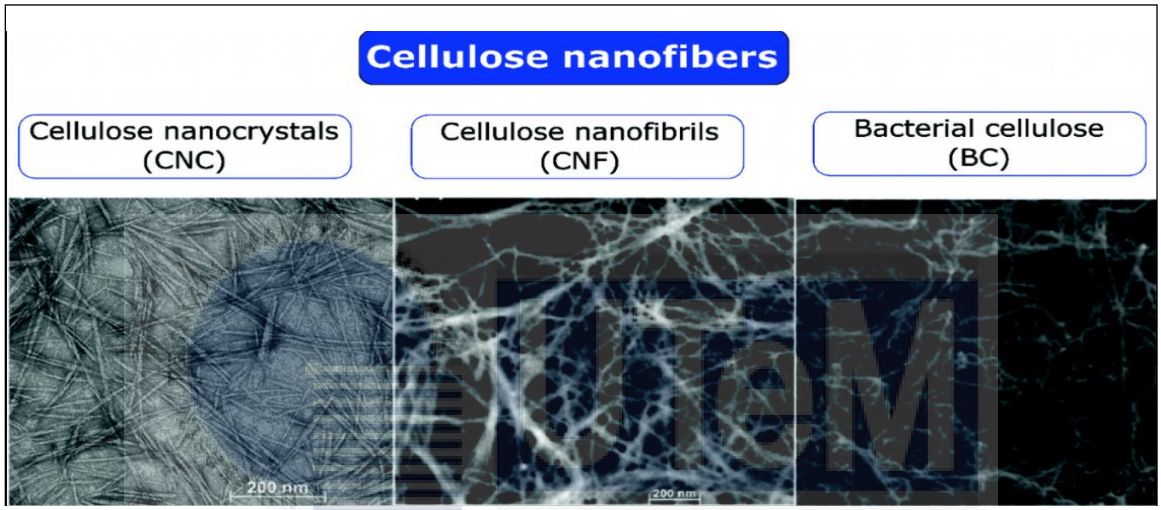

Figure 2.2: Representative electron microscope images of the three types of nanocellulose: CNC, CNF and BC (Hazwan *et al.* 2019).

Table 2.1: Type of nanocellulose

Type of nanocellulose	Typical sources	Method of production	Average Dimensions	References
Cellulose nanocrystals (CNC)	Wood, cutton, bamboo, tunicate	Acid Hydrolysis	Diameter : 3-50nm Length: 30nm-300nm	(Gumrah *et al*. 2017)
Cellulose nanofibers (CNF),	Wood, cotton,flax, potato hemp	High pressure homoginzation, grinding,	Diameter : 5-10nm Length: several microns	
Bacterial nanocellulose (BNC)	Acetobacter/ glucobacter xylinum	Biosynthesis of glucose and alcohol	Diameter : 10-100nm Length: mostly several tens of micrometer up to a mm.	

2.1.2 Characteristic of Nanocellulose

The characteristics of nanocellulose have been reported in several studies in the literature. Nanocellulose is a nanoscale material made up of cellulose nanocrystals (also referred to as nanocrystalline cellulose) or cellulose nanofibrils (also referred to as nanofibrils cellulose) with exceptional chemical, mechanical, biological, optical, and thermal properties. Therefore, on Table 2.2 shows characteristic of cellulose nanocrystals (CNC) by (Jitan *et al.* 2021).

Table 2.2: Characteristic of cellulose nanocrystals (CNC)

Reference	Properties	Main finding
(Jitan *et al.* 2021)	Cellulose Nanocrystals (CNC) Length/diameter = 10-100nm Tensile strength : 10Gpa Cellulose Nanofibrils (CNF) Length/diameter = 100-150nm	Low aspect ratio Young Modulus: 8.3 ± 0.9 Gpa High aspect ratio Young Modulus: 17.2 ± 1.2 Gpa

2.1.3 Summary of Literature Review of Nanocellulose

In conclusion, many intriguing results demonstrating the potential of cellulose nanocrystals have been reported. CNC has received attention as a good choice for many applications, such as reinforcing material in nanocomposites, due to its many positive characteristics. Based on the three types of nanocellulose presented in previous studies, cellulose nanocrystals (CNC) will be used in this study due to the ease of preparation and the good characteristics they produce. Table 2.3 compiles a summary of the literature on nanocellulose.

Table 2.3: Summary literature review of nanocellulose

References	Sub-topic	Main Findings
Inder *et al.* 2001	Nanocellulose Orange peel waste	Cellulosic fibres are made up of cellulose, hemicellulose, polysaccharide called lignin, and pectin and are found in all natural fibres.
George *et al.* (2015)		Cellulose is a chemical structure made up of monomers connected by glycosidic oxygen bridges that is formed through condensation.
Syafri et al. (2022)	Type and Structure nanocellulose	The three types of nanocellulose are cellulose nanofibers (CNF), cellulose nanocrystals (CNC), and bacterial nanocellulose (BNC)
(Jitan *et al.* 2021)	Characteristic of Nanocellulose	Nanocellulose is a nanoscale substance composed of cellulose nanocrystals or cellulose nanofibrils (also known as nanofibrils cellulose)

2.2 Orange Peel Waste

Orange peel waste (OPW) is one type of citrus waste. There are numerous techniques for extracting cellulose from the most appropriate and allowable source selection for cellulose leftovers, such as vegetable and fruit trash. As a result of its low cost and great volume of production, as well as its favourable nutritional properties, orange is one of the most extensively consumed fruits in the world. Oranges are high in vitamins C, A, and B, as well as minerals calcium, phosphorus, and potassium (Salem *et al.* 2021).

Orange has a lot of soluble and insoluble carbohydrates in it, thus it has a lot of potential for fibre recovery and can be employed as a functional food ingredient. In general, orange peel waste is separated into two parts: albedo and flavedo. Albedo is the inner portion of the mesocarp, and the main orange peel is white, spongy, and cellulosic tissue. Due to its high fibre content, albedo as a component and a potential fibre supply, while flavedo is the outer part or epicarp. Therefore, in this study, we will be using albedo as a peel waste because it contains low lignin and has the possibility to produce high yields and high crystallinity cellulose. Figure 2.3 shows a orange peel waste.

Figure 2.3: Orange Peel Waste (Salem et al. 2021)

2.2.1 Structure and Composition of Orange Peel Waste

Citrus processing waste from orange was explored for the production of nanocellulose. Oranges are citrus organic fruits utilized in high extent as a natural fruit. In order to harness the maximum value from waste peel, it is essential to have reliable information regarding its chemical composition.Orange peel waste is rich in biomolecules of economic interest. Briefly orange peel contains soluble sugar, starch, fiber including cellulose, hemicellulose, lignin and pectin, ash, fat and protein, and the quantitative composition in percentage as stated in Table 2.4.

Table 2.4: The chemical composition of orange peel waste (Ayala *et al.* 2021)

Analysis	**Composition (% in weight)**
Moisture	73.530%
Volatiles	99.261%
Ash	0.052%
Fixed carbon	0.687%
Lignin	19.801%
Holocellulose	78.110%
cellulose	69.096%
Hemicellulose	5.433%

2.2.2 Fiber structure of Orange Peel Waste

The finest of fiber were depended on the outermost and innermost layer of the orange peel waste (OPW). It is mean that, the innermost layer of OPW are more rough fiber than the outermost layer. Orange contains three different layer which are: (i) External layer

formed by flavedo (epicarp) consisting (ii) albedo (mesocarp), and (iii) inner layer material called endocarp that contains vesicles with juice. The quality and quantity of the fibers may be depended on the location where the fibers were extracted. Therefore on OPW, the fiber more contains in the albedo. OPW is low in calories,sugar, and fats and free from cholesterol. Because of its special characteristics such as high specific strength, superior thermal properties, and low cost. Therefore, it can produce high crsytallinity cellulose. (Ranganagowda *et al*. 2019). On Table 2.5 it shows a chemical composition of orange peel fiber at 110°C-130°C.

In order to make nano and micro forms, they can be made by alkaline tratment, bleaching process, and acid hydrolysis. Cellulose would be extracted from plant fibers using some of the chemical and mechanical methods. OPW is made of a biodegradable fibre that can be used as an alternative material that is better for the earth. Figure 2.4 shows a squeezer type juice extractor and orange processing system.

Table 2.5: Chemical composition of orange peel fiber at 110°C-130°C (Jongaroontaprangsee *et al.* 2018)

Citrus by-product (Lime)	Yield of solid content (%)	cellulose	Hemicellulose	Lignin
Raw Material	100.00	11.46±1.86	10.18±5.36	7.29±1.87
110°C	35.84±1.13	41.66±3.41	10.06±0.71	7.91±1.77
120°C	31.01±0.62	47.07±2.24	3.94±1.21	9.19±0.71
130°C	29.35±0.35	46.58±2.53	3.55±1.27	7.90±1.83

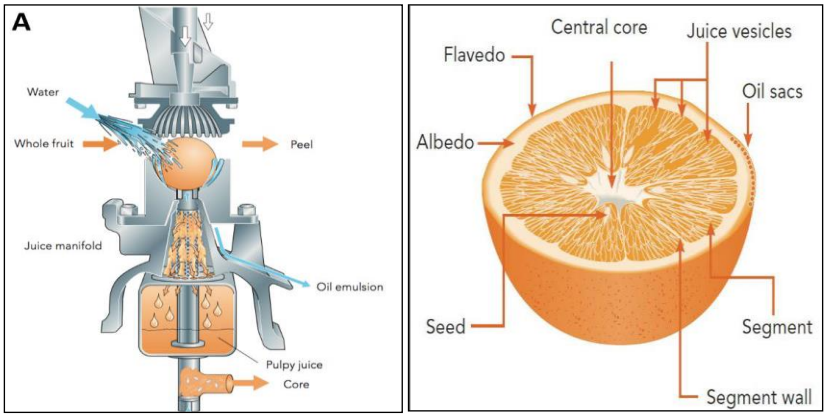

Figure 2.4: A squeezer type juice extractor and orange processing system (Rivas *et al.* 2008)

2.2.3 Application of Orange Peel Waste

Orange peel waste can be a potential raw fiber for nanocellulose application due to its outstanding properties and biodegradability, nanocellulose is advantageous in a range of applications, including nanocomposite materials, surface-modified materials, and translucent paper with exceptional capabilities. Nanocellulose has been used in variety of sector such as, nanocomposites which can be used in other situation, and it also create a windmill blade with a high-strength structure and flexible batteries among other features. Apart from that, nanocellulose-based nanocomposites are an emerging application of catalytic degradation of organic contaminants for water pollution treatment (Wei *et al.* 2019).

In recent years, there has been also great interest in the use of OPW especially on production of solid biofuels and bio sorbents of heavy metal. According to research study by Khan *et al*. (2021) indicated that solid waste management contributes to the sustainability of waste creation from the manufacturing process This discovery has increased interest in the development of technologies to improve the efficiency of biomass conversion processes, resulting in increased value through the addition of byproducts such as biofuels.

In a different study, Sachidhanandham (2020) reported about to convert of OPW into textile. The grounded OPW was then processsed and cellulose were separated. Based on the result on their study, its shows that the orang peel waste is processed with a patented technology 'pastazzo' that separated the cellulose from the material. Microencapsulation technique was used to trap the vital element of orange peel waste.

According to Rathinavel *et al.* (2021), OPW powder is used as a filler material in biocomposite films, together with polyvinyl alcohol (PVA) as a matrix.The combination of these green composites is suitable to improves the thermal stability. Research finding by Charles (2016) also points to the creation of a brake pad made from an orange peel reinforcement polymer composite. The hardness of brake pads was measured with a Shore D hardness tester to guarantee equal mixing and curing during the manufacture of brake pads with orange peels, carbon, aluminium oxide, and resin reinforced composite.

2.2.4 Summary of Literature Review of Orange Peel Waste

According to the review, it was found that OPW is one of the natural fibers that contain high amount of cellulose which making it both economic and ecological advantages. The orange peel waste is selected in this study due to its potential to be used as the alternative for the non-renewable resource. The summary of finding on OPW is presented in Table 2.6.

Table 2.6: Summary literature review of OPW

References	Sub-topic	Main Findings
(Salem *et al.* 2021)	Citrus Waste OPW	Orange contains vitamins C, A, and B, as well as minerals calcium, phosphorus, and potassium
(Rivas, *et al* 2008)	Structure and Composition of OPW	Nanocellulose can be divided into three groups that are cellulose nanofibers (CNF), cellulose nanocrystals (CNC), and bacterial nanocellulose (BNC)
(Ayala et al. 2021)	The chemical composition of OPW	Cellulose: (69.096 %) Hemicellulose: (5.433%) Lignin: (19.801%)
(Moon *et al.* 2011)	Fiber structure of OPW	Orange contains three different layer which are: (i) External layer formed by flavedo (epicarp) (ii) albedo (mesocarp), and (iii) inner layer material called endocarp that contains vesicles with juice.
Marshall *et al.* (2013)	Application of OPW	OPW especially on production of solid biofuels and bio sorbents of heavy metal
Sachidhanandham (2020)		Convert of OPW into textile
Rathinavel *et al.* (2021)		OPW powder and polyvinyl alcohol are used as filler materials (PVA)
Charles (2016)		The development of brake pad using orange peel reinforcement polymer composite the hardness of brake pads

2.3 Preparation of Cellulose Nanocrystals

Based on the previous study, only a few studies reporting on the preparation of nanocellulose from orange peel waste. Based on research done by Naz et al. (2016), the CNC is prepared by extracted cellulose fiber from the raw material of OPW purification process such as alkaline treatment before the chemical treatment can proceed. The experimental method for CNC preparation from orange peel waste is represented in Figure 2.5.

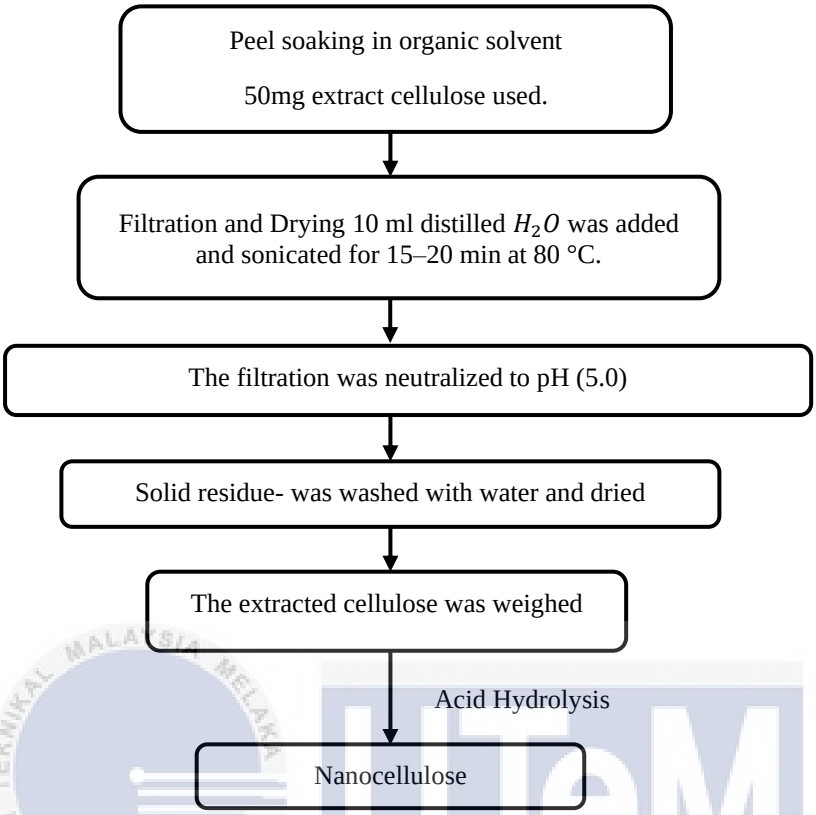

Figure 2.5: Diagrammatic scheme of different steps involved in extraction of cellulose and conversion to nanocellulose (Naz *et al.* 2016)

However, in recent years, to explain the method of preparing nanocellulose from OPW via alternative method, a study by Eduardo *et al*. (2017) also reported about conventional acid hydrolysis and acid-free microwave processing. In addition, Ribeiro *et al.* (2019) also stated that in his review article, the chemical treatment of the OPW production of nanocellulose by enzymatic hydrolysis.

2.3.1 Maceration Process

Orange peel waste (OPW) can be extracted from different types of methods or treatments, but usually OPW is extracted by using a maceration process. According to a previous study, the maceration process is a solvent extraction, and it is a method for the isolation of cellulose (Abdurrahman *et al.* 2019). Typically, during the maceration process, distilled water is the most popular solvent because, due to its water-soluble properties, the high water level makes the soluble nutrients in the food easily dissolve in the solution. Therefore, maceration is must during the process so that it will remove the flavedo that had accumulated on the surface of the albedo, and to soft shape that help for the sample to peel easily.

However, Wakeel et al. (2019) stated that the selection of the best solvent for extraction is a key issue. The type of solvent in terms of its polarity will cause the extraction of different groups of compounds that have different final uses. It is critical to emphasise that the extraction technique has a significant impact on the active ingredients and nutrients contained in the final product. Thus, utilising distilled water for the maceration procedure is the optimal technique to remove the flavedo and obtain only the albedo that used in the study.

2.3.2 Alkaline Treatment Method

In recent years, there has been a growing interest in the chemical treatment of orange peel waste to produce nanocellulose. One of the chemical treatments used to remove cementing components such as lignin and hemicellulose is alkaline treatment. Prior to the extraction of cellulose nanocrystals, an alkaline treatment is required to obtain highly purified cellulose fibre. According to Ramesh (2016), the reaction of NaOH with natural fibre (Cell–OH) is assumed to occur as shown in Equation 2.1.

Cell—OH + NaOH Cell—O- Na+ + H2O + surface impurities (Equation 2.1)

Experimental investigations into this method have been carried out by many researchers. Abdurrahman *et al.* (2019) found that alkaline treatment would affect the thermal degradation. The fiber is immersed in a 6 wt.% concentration of sodium hydroxide (NaOH) solution for 24 hours at room temperature, and then the fibres are washed with tap water until all traces of NaOH are removed from the fibres, followed by a drying process for 24 hours in an oven at a temperature of 40 °C.

2.3.3 Bleaching Process

After removing the hemicellulose with an alkaline solution, the bleaching procedure removes the remaining lignin present in the fibre. The majority of experiments employed sodium chloride (NaCl) solution as a bleaching agent since cellulose can be bleached to a high whiteness without degrading (Mzimela *et al.* 2018). Bleaching solution containing NaCl is a universal bleaching agent and is simple because it requires only mild rinsing as fibre-soluble alkali is not used. NaCl is a moderate-strength acid that is only active in an acidic medium, and sodium chlorite aqueous solutions are stable under alkaline conditions. In order to activate sodium chlorite and cause it to decompose, one has to turn the medium to the acid range.

2.3.4 Acid Hydrolysis Method

There have been several studies in the literature reporting about the use of acid hydrolysis method to obtain nanocellulose. Acid hydrolysis method is the most used method in extracting the cellulose nanocrystal (CNC) from the cellulose fiber since it is promising highly crystalline. According to Kusmono *et al.* (2020) had stated that CNC are high crystalline, defect-free needle-like particles with an average diameter of 5–10 nm and an average length of roughly 100 nm with the remove of amorphous parts in cellulose fibers via acid hydrolysis this is because, lignin and hemicellulose are sensitive due to the acid.

Based on the study by Mekala *et al.* (2014) hydrolysis of nanocelluse concentration acid used usually are sulfuric acid (H_2SO_4), hydrochloric acid (HCl), nitric acid (HNO_3) and

phosphoric acid (H_3PO_4). In producing a surface charges on cellulose nanocrystals, H_2SO_4 is highly recommended acid than others. It is because H_2SO_4 was able to overcome the difficulties associated with hydrolyzing cellulose due to its medium acidity, and OPW nanocellulose with good nanoscales and high yield were successfully produced. Acid hydrolysis is the most efficient method for dissolving glycosidic bonds in cellulose and consumes the least amount of energy.

Fitriani *et al.* (2021) on her research regarding on the agricultural waste had investigated the effect of hydrolysis time at 1-3 hours by using acid hydrolysis and the acid used are H_2SO_4 and the properties of the produced cellulose nanocrystals (CNC). The microcrystalline cellulose as the starting material was hydrolyzed in different condition and the properties of nanocrystalline cellulose obtained were compared.

As a result, this study employs a variety of acid concentrations, including H_2SO_4 and HCl, as well as combinations of parameters such as hydrolysis time and temperature. Therefore, the type of acid used is variable with respect to the acid concentration in this study. The influence of type of acid and acid concentration on this method has not been much studied.

Thus, the optimization of acid hydrolysis with an influence on type of acid, acid concentration as a parameter of using the hydrolysis time and constant temperature was determined in this study. The parameter on this study should be controlled in conducting acid hydrolysis method which are; (i) type of acid (ii) concentration of acid (iii) hydrolysis time (iv) constant temperature. The findings of the literature on the acid hydrolysis method can be summarised in Table 2.7.

Table 2.7: Summarization of the acid hydrolysis from previous studies

References	Type of acid used	Acid concentration (wt. %)	Time (hours)	Temperature (°C)	Findings
Kusmono *et al.* (2020)	H_2SO_4 HCl	64 37.5 64	1-3 hours	45	The influence of acid concentration
Mekala *et al.* (2014)	H_2SO_4 HNO3	64 80	3 hours	45	The influence of acid concentration
Fitriani *et al.* (2021)	H_2SO_4	64	1 hours	45	The influence of optimization conditions

2.4 Material Characterization and Analysis

Generally, the element crystal structure and the morphology of nanocellulose orange peel waste (OPW) can be determined by using various kinds of microscopy testing and analysis, such as X-ray Diffraction (XRD) analysis, Fourier Transform Infrared Spectrometry (FTIR), Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM). However, for the purposes of morphology characterisation and analysis, TEM was not employed in this work due to a number of constraint issues. The characterisation will be focused on XRD, FTIR, and SEM analysis in order to meet the scope of the project within the time schedule and budget constraints.

2.4.1 X-Ray Diffraction (XRD)

Previous research has shown that X-Ray Diffraction (XRD) can be conducted to analyse the crystallinity and crystallite size of cellulose extracted from OPW. It has been reported that the size of the crystallites cellulose changes during acid hydrolysis. Cellulose crystallinity refers to the ratio of the amount of crystalline cellulose to the total amount of sample material. The amount of cellulose content (%) in the raw material (orange peel trash) is 16.46±0.84 and Crystallinity Index (CrI) is 0.16. While for NaOH-treated fibers is 52.50±1.35 %. The crystallinity in a cellulose sample can be measured by variety of methods such as using XRD methods, and the numerical result is referring as Crystallinity Index (Crl).

According to Park *et al.* (2010) X-ray Diffraction (XRD) method provide a more accurate measure of the crystallinity of cellulose. Crystallinity should affect cellulose accessibility, but it's also likely to be influenced by other factors like lignin/hemicellulose quantity and distribution, porosity, and particle size. Therefore, the degree of crystallinity is the main propertyof characterizing material. Cellulose crystallinity refers to the ratio of the amount of crystalline cellulose to the total amount of sample material.

Figure 2.6 below shows that previous study from Naz *et al.* (2016) two separate cellulose crystallinity peaks can be seen in a fine graph created from an ASCII file on the screen. XRD patterns shows one distinct intensified diffraction peak in the whole spectrum of 2θ degrees value 22.34° which shows crystalline behaviour of nanocellulose. Crystallanity

phase showing peaks at 22.34° and 34.53°. It has been reported that the cellulose changes the size of the crystallites during acid hydrolysis. Alternatively, it is possible to measure the % crystallinity by XRD. Therefore Equation 2.2 is used to find crystallinity.

$$\text{CrI (\%)} = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \qquad \text{(Equation 2.2)}$$

While crystallite size can be calculated by using Scherer equation on Equation 2.3 , where K is a shape factor, With K is the Scherrer constant most adjusted to the nanocrystal shape, λ is the wavelength (1.5418 Å) and β correspond to the full width at half maximum intensity (FWHM) and θ is half the Bragg angle at peak maximum given in radian.

$$D = \frac{K\lambda}{\beta \cos\theta} \qquad \text{(Equation 2.3)}$$

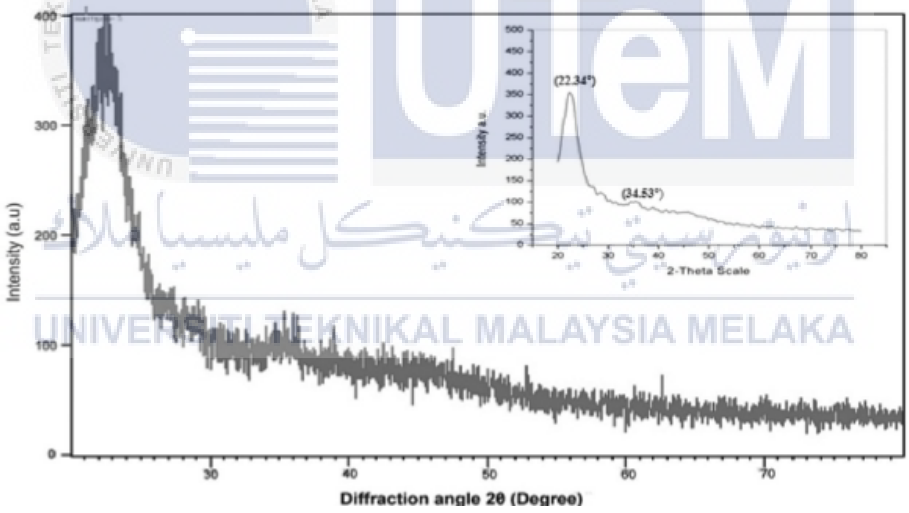

Figure 2.6: X-ray diffractogram of cellulose extracted from pamelo albedo as raw material (Razak *et al.* 2021)

From the result, it can be concluded that the pattern of XRD has increased upon the acid hydrolysis which is indicate higher crystallinity index but crystallanity phase showing peaks at 22.34° and 34.53°.

The crystalline arrangements were observed using XRD analysis that occur in the cellulose molecule during acid hydrolysis treatment. On the XRD pattern it will shows the

highest crystallinity index (Crl %) and crystallite size value. Borjesson et al. (2015) mentioned that the longer the hydrolysis time, the less amorphous region was remained which in turns, increases the crystallinity of the cellulose nanocrystal. Conversely, if the hydrolysis time is insufficient, thus more amorphous region left and the crystallinity index become lower. crystallinity index. However, if the hydrolysis time and temperature are both high, no crystallinity peak is detected due to acid degradation of the cellulose crystalline area.

The crystallite size of the nanocellulose from OPW were determined by using Scherer equation. From the calculation, it can be concluded that, the higher the temperature, the smaller the crystallite size of the cellulose nanocrystals. This is due to the higher reaction temperature, will leads to the effectiveness in obtaining shorten cellulose nanocrystals (George *et al.* 2015).

2.4.2 Fourier Transform Infrared Spectrometry (FTIR)

Fourier Transform Infrared Spectrometry (FTIR) is used to investigate the changes in functional groups on the fiber surfaces. The FTIR spectra were recorded on an attenuated total reflection. FTIR to analyse the chemical changes of the samples before and after each treatment, including alkaline treatment, bleaching and acid hydrolysis using a FTIR spectrophotometer. FTIR is used to analyse the primary functional groups contained in pomelo albedo. According to studies on nanocellulose from Razak *et al.* (2021) there are four samples of pomelo albedo at different condition were taken and being analyzed which are; (a) FTIR spectra of ground pomelo albedo, (b) alkali-treated albedo, (c) bleached, (d) acid hydrolysed pomelo albedo fibers. From the result on Figure 2.7 below, it was confirmed that lignin and hemicellulose was removed after the alkali treatment due to the disappeared of the band C=O stretching band that represent the amorphous non-crystalline region, lignin and hemicellulose.

Based on the FTIR analysis, lignin, hemicellulose, and other extractives have successfully removed by alkaline treatment. This analysis also supports the XRD analysis to confirm its chemical structure. This changed of functional groups can be referred to the different spectra shown by FTIR analysis as depicted in Figure 2.7. From the result, it was confirmed that lignin and hemicellulose was removed after the alkali treatment due to the

disappeared of the band C=O stretching band that represent the amorphous non-crystalline region, lignin and hemicellulose. Besides, the smaller shoulder peak at 1500 cm^{-1} slightly flattens down from orange peel waste which was due to the presence of C=O stretching. The presence of C=O stretching vanished after alkali treatment take place which was assigned to the presence of hemicellulose (Salem *et al.* 2021).

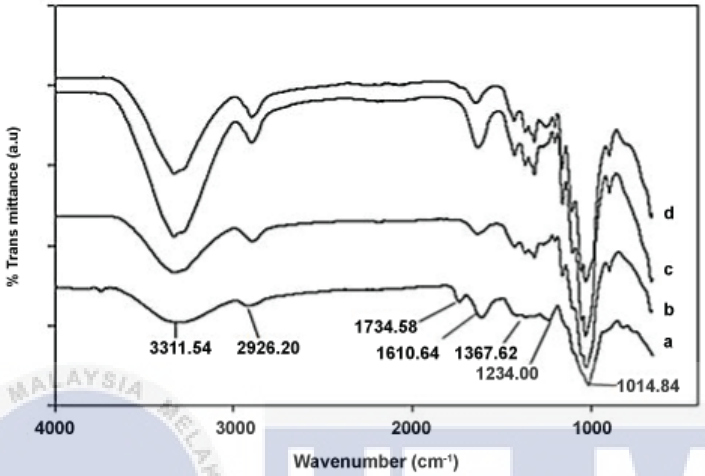

Figure 2.7: FTIR spectra of (a) ground pomelo albedo, (b) alkali-treated albedo, (c) bleached and, (d) acid hydrolysed pomelo albedo fibers (Razak *et al.* 2021)

2.4.3 Scanning Electron Microscopy (SEM) Analysis

Scanning electron microscopy (SEM) is a critical tool for determining the characteristics cellulose derived from the orange peel waste. SEM analysis is used to study the surface morphology OPW before and after chemical treatments is conducted. Research by Mariño *et al.* (2015) reported that the extent of acid hydrolysis was demonstrated through a variety of formations with various dimensions and morphologies. The morphology of the orange peel is shown in Figure 2.8

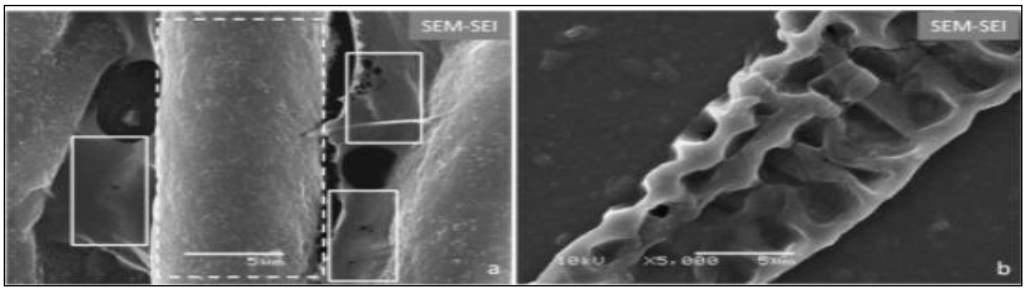

Figure 2.8: Scanning Electron micrographs (SEM) (a) before alkaline treatment (b) After alkaline treatment (Mariño *et al.* 2015)

The research was done using OPW samples. According to the electron microscopy results, the orange peel waste fibre changed after being treated with sodium hydroxide. Under SEM observation, it is obvious that some of the fiber tissue surface has been damaged, and the connections between the cells have become weaker than in the raw fibre. This is due to the removal of the amorphous portion such as lignin, hemicellulose, and some extractives like wax and impurities on the fibre surface by sodium hydroxide (NaOH). This treatment is an important in order to induce the swelling of the fibre structure to enhance the next chemical treatment stage.

CHAPTER 3
METHODOLOGY

Chapter 3 describes the step-by-step proposed method to carry out experiment for this research. The discussion will include the flowchart of experimental work concerned during this study, experimental materials, and experimental method by referring to previous study in Chapter 2. This chapter is proposing the suitable method, technique, tools, and apparatus to fulfil objective of this research.

3.1 Introduction

This chapter will be divided into two parts which are experimental materials and experimental methods for preparation of nanocellulose from orange peel waste (OPW) via acid hydrolysis method. Simultaneously, the experiment needs to fulfil the scope and objective of the research as stated in Chapter 1. An overview of the experimental studies and procedures are shown in Figure 3.1.

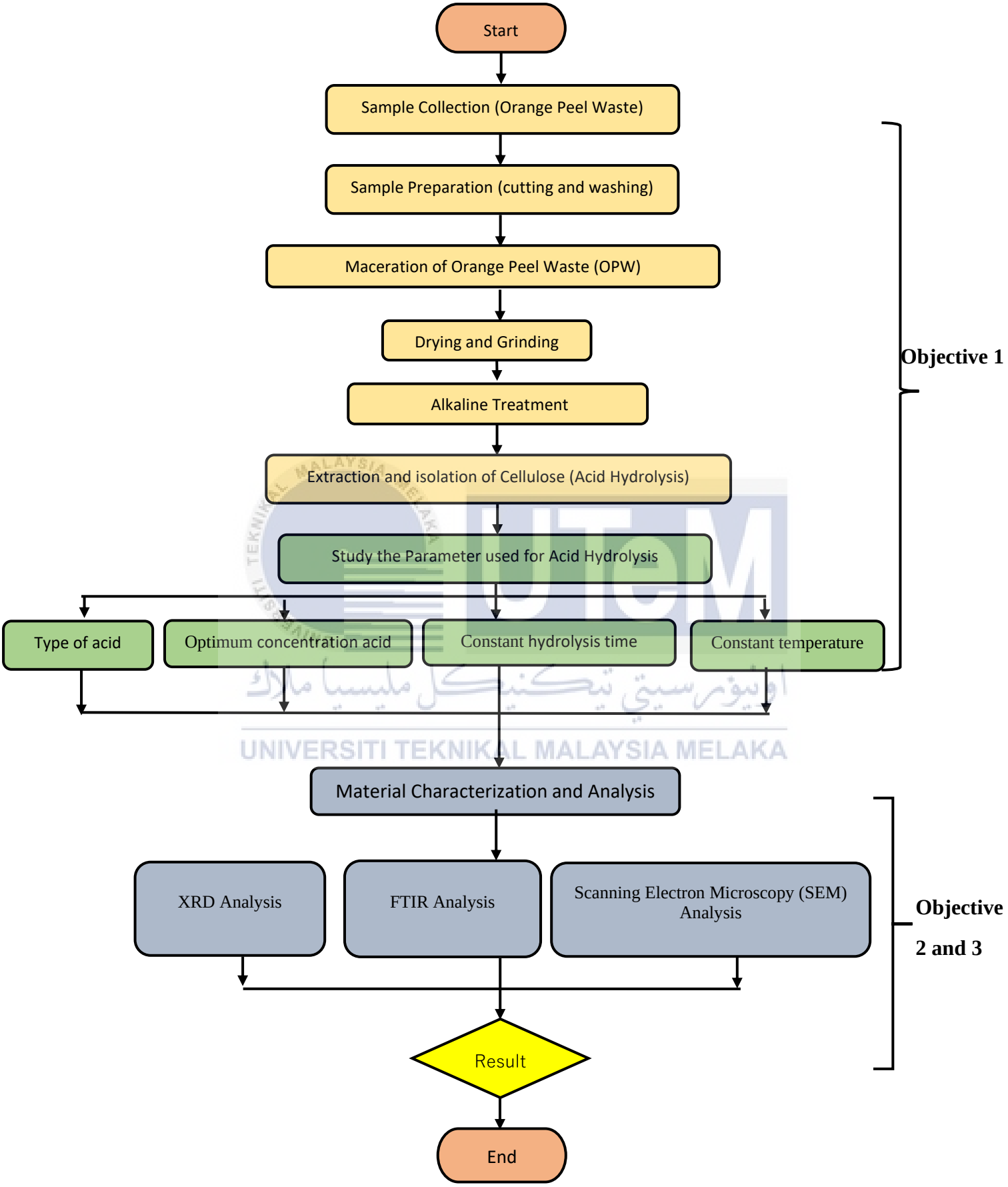

Figure 3.1: The flowchart of the experimental studies and procedures

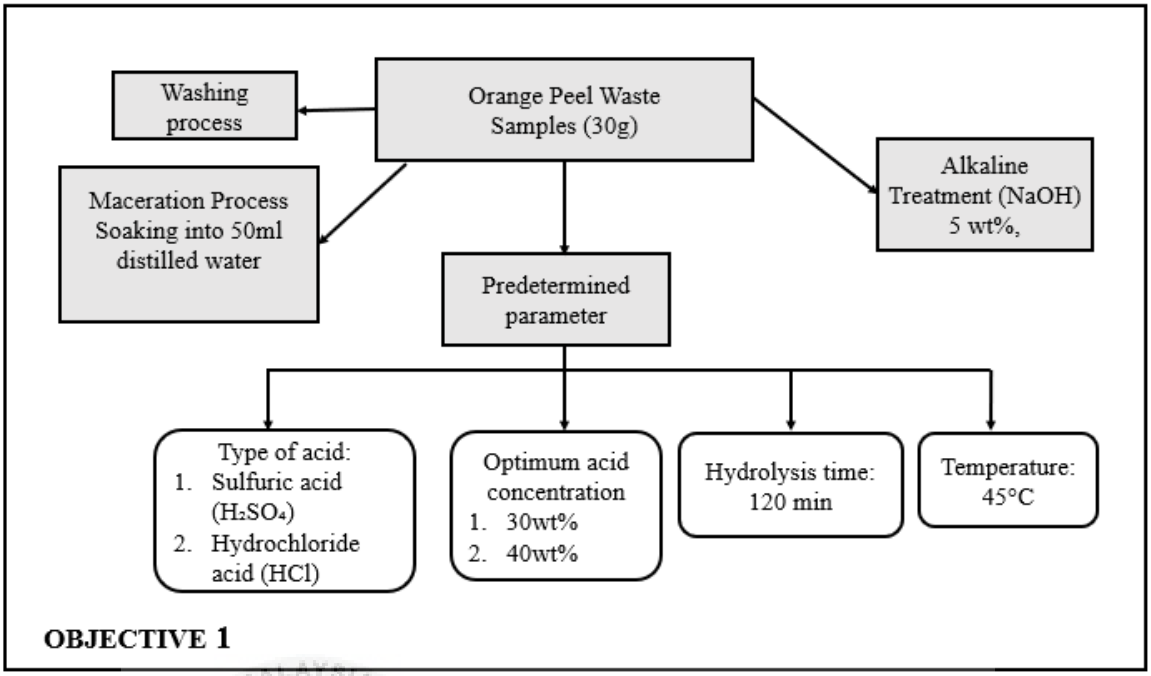

Figure 3.2 : Details on experimental works in Objective 1

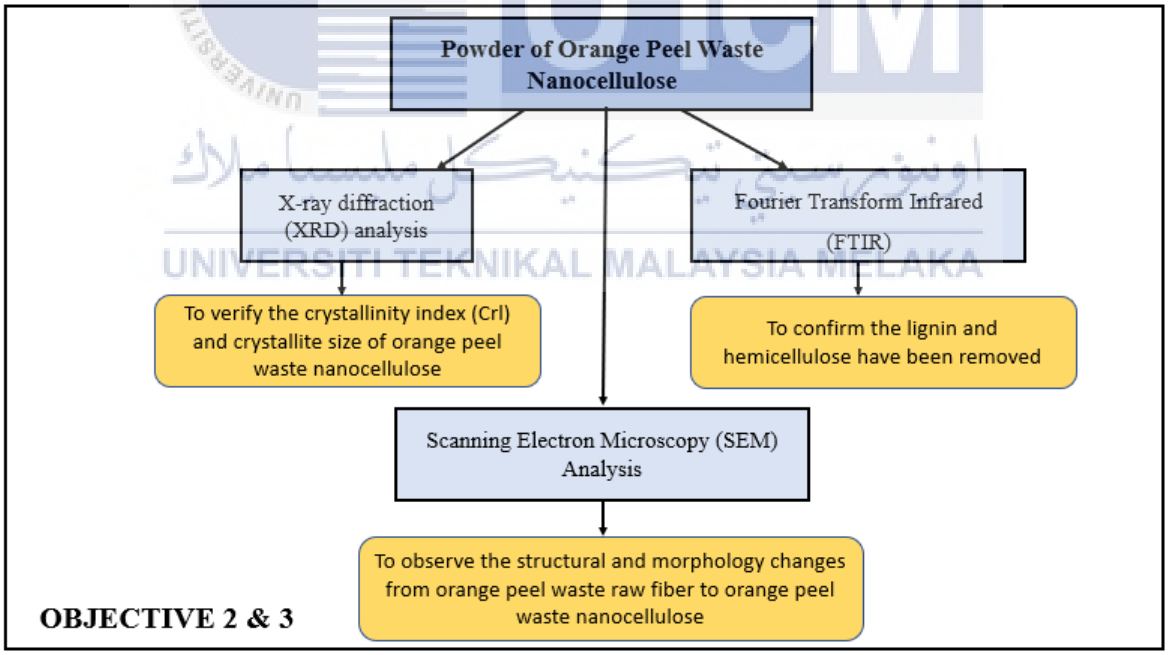

Figure 3.3 : Details on experimental works in Objective 2 & 3

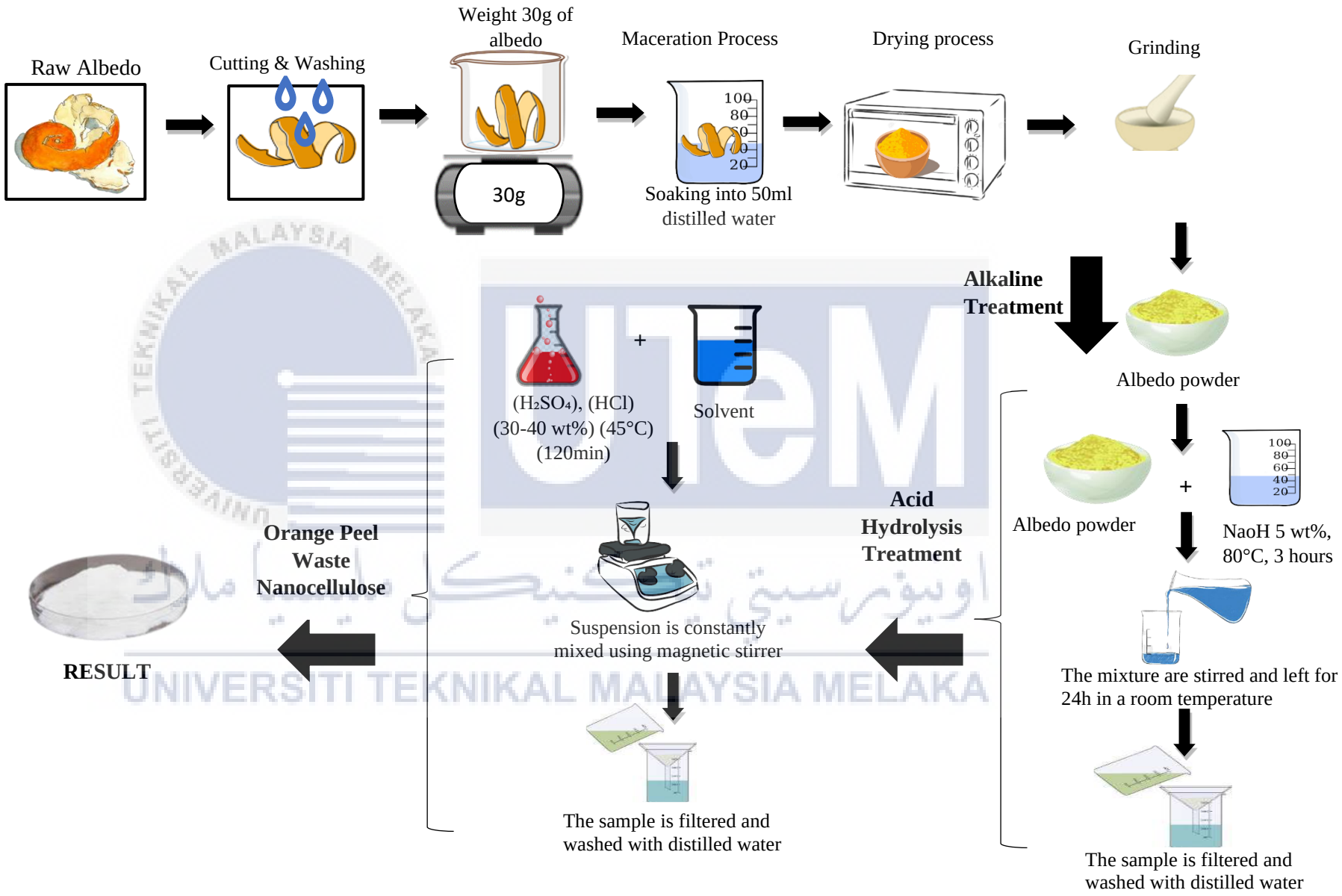

Figure 3.4: Illustration of Preparation of Nanocellulose from Orange Peel Waste

3.2 Experimental Work Regarding Objective 1

This section described the experimental activities involved in Objective 1, from raw material processing through the formation of orange peel waste nanocellulose (OPW).

3.2.1 Experimental Materials

The following section discusses the critical experimental materials used in Objective 1, including raw materials, apparatus and equipment's, chemicals and reagents, gloves, and other variety of functional items.

3.2.1.1 Raw Materials

OPW was used in this study as a raw material to produce nanocellulose. The OPW was used to evaluate the preparation and characterization of nanocellulose, followed by an examination of the resulting materials and structural properties. As illustrated in Figure 3.5, OPW (albedo part) was chosen due to its high carbohydrate content, both soluble and insoluble, and its ability to recover fibres and albedo part also contains low lignin therefore, possibility to produce high yield ang high crystallinity cellulose.

Figure 3.5: Orange Peel Waste

3.2.1.2 Chemical and Other Substances

This project requires the use of a chemical component to produce nanocellulose from orange peel waste. The chemicals utilised included sodium hydroxide (NaOH), sulphuric acid (H_2SO_4), hydrochloride acid (HCl) and distilled water. NaOH was used to remove the cementing material that had dissolved in the solution during the alkaline treatment and bleaching procedure, such as lignin and hemicellulose. This project requires the use of a chemical component to produce nanocellulose from orange peel waste. The chemicals utilised included sodium hydroxide (NaOH), sulphuric acid (H_2SO_4), hydrochloride acid (HCl), and distilled water. NaOH was used to remove the cementing material that had dissolved in the solution during the alkali treatment and bleaching procedure, such as lignin and hemicellulose. Furthermore, the concentration of NaOH used in this experiment is 5 wt%. Various concentrations of H_2SO_4 and HCl will be used during the hydrolysis treatment. For this study, different acid concentration solvents were used to investigate the effect of acid concentration on the crystallinity of nanocellulose derived from OPW. H_2SO_4 and HCl was choose since it can overcome the difficulties associated with hydrolysing cellulose due to its medium acidity. Table 3.1 contains a list of the substances that were used.

Table 3.1 : The lists of substances

Material	Concentration (wt %)
Sulphuric Acid (H_2SO_4)	30-40
Hydrochloride Acid (HCl)	30-40

3.2.1.3 Experimental Equipment

Throughout the completion of the experiment regarding on Objective 1, equipment such as, round bottom flask, chemicals and reagents, gloves, spatula, stirring hot plate, magnetic stirrer, high speed blender and drying oven, Figure 3.6 and Figure 3.9. Next, for testing analysis, this experiment also will used FTIR-6100 machine, X-Ray diffraction machine, Scanning Electron Microscopy (SEM).

Figure 3.6 : Lab glassware

Figure 3.7 : Stirring hot plate

Figure 3.8 : Drying oven machine

Figure 3.9 : Gloves and spatula

3.2.2 Experimental Methods

There are numerous experimental procedures to be performed in order to produce nanocellulose from OPW, which are includes the samples preparation, maceration of OPW, alkaline treatment and the extraction of nanocellulose via acid hydrolysis method. This method performed in order to achieve the first objective of this research study.

3.2.2.1 Sample Preparation

The raw material used in this experiment are orange peel waste. Approximately 30g of the inner section of the mesocarp, which is albedo, orange peel waste, was peeled and cleaned by washing it in water to remove dirt, dust, and other contaminants as depicted in Figure 3.10. In this study, albedo was chosen because it contains low lignin therefore possibility to produce high yield and high crystallinity cellulose.

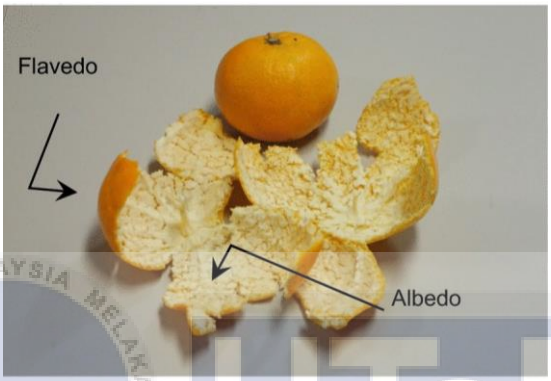

Figure 3.10: Peeled and cleaned orange peel waste.

3.2.2.2 Maceration Process

For albedo and flavedo extraction, during maceration process, orange peel waste was soaked in 50ml of distilled water to soften the surface that is flavedo and albedo for an hour. Theoretically, soaking is intended to remove the flavedo that had accumulated on the surface of the albedo, and to soft shape that help for the sample to peel easily.

Soaking is mainly used during the maceration process to remove germination inhibitors on the fiber such as, sand, dust and root that attached on the surface of fiber. maceration process can minimized the impurities that attached on OPW by minimized the dirt that may impede during maceration process.

3.2.2.3 Drying and Grinding

For 48 hours, the cutting orange peel was dried in a drying oven machine set to 50°C. The dried OPW was then powdered using an mortar and pestle, resulting in the powdered shown in Figure 3.11 (b). This process is repeated until the peel powder is formed. The powdered were then stored in a Schott bottle to prevent air moisture before further treatment and analysis.

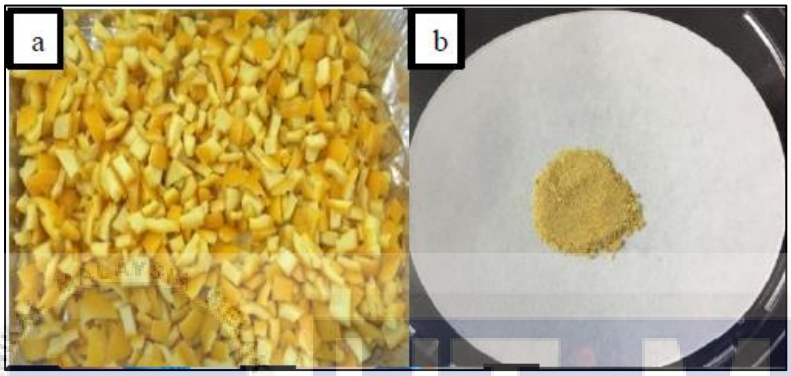

Figure 3.11: (a) orange peel cutting into pieces (b) grinded orange peel

3.2.2.4 Alkaline Treatment

About 30g of orange peel powder was treated by using a 5% of sodium hydroxide (NaOH) heated with 80°C and will stirred for 3 hours. After that, the mixture was filtered and washed with distilled water for several time to remove lignin and hemicellulose that dissolve in the solution. Then, the resultant fiber was dried before used for acid hydrolysis.

3.2.2.5 Preparation of Cellulose Nanocrystalline by Acid Hydrolysis

Acid hydrolysis was performed by using sulfuric acid (H_2SO_4) and hydrochloric acid (HCl) with different concentration were mixed with about 30g of orange peel powder. The parameters that involved during the hydrolysis were, different type of acid, optimum acid concentration, constant hydrolysis time and hydrolysis temperature with a constant temperature. The optimum acid concentration which was 30-40 wt% had been conducted. Then hydrolysis time were taken at 120min and at 45°C. The hydrolysis process had been

conducted by subjected the orange peel waste cellulose into the condition of parameter. The mixture was steadily stirred by the magnetic stirrer until the hydrolysis time was completed. The cellulose was dialyzed a few times by using distilled water until a constant pH was achieved. After that, the sample were dried in the oven for three days with the temperature 40°C. The cellulose nanocrystalline obtained will be kept at room temperature for further analysis.

3.2.3 Design of Parameter

Based on the previous studies, acid hydrolysis is a method used to extract the cellulose nanocrystals from various kinds of natural fibres. This is the reason why acid hydrolysis is chosen as chemical treatment on the orange peel waste due to its reliability and validity. Therefore, the conditions designed for conducting this chemical treatment was carried out at different acid concentration 30-40wt% by constant hydrolysis time and constant temperature. Table 3.2 shows experimental factors, and their stages of different type of acid involves are sulphuric acid (H_2SO_4) and hydrochloric acid (HCl).

Table 3.2: Experimental factors and their levels used in design of parameter

Variables	**Type of Acid**	
	H_2SO_4	HCl
Acid concentration (wt%)	30-40	30-40
Hydrolysis time (min)	120	120
Hydrolysis temperature (°C)	45	45

The variables used were fixed acid concentration (wt%) and constant hydrolysis time (min) while the fixed temperature at 45°C. The design consists of 4 runs. The responses measured were the Crystallinity Index (CrI) and crystallite size (nm). Table 3.3 shows sample conditions arrangement for acid hydrolysis method based on parameter selected.

Table 3.3: Sample conditions arrangement for acid hydrolysis method

No of run	Type of acid	Acid concentration (wt%)	Hydrolysis time (min)	Hydrolysis temperature (°C)
1	H_2SO_4	30	120	45
2	H_2SO_4	40	120	45
3	HCl	30	120	45
4	HCl	40	120	45

3.3 Experimental Work Regarding Objective 2

This section describes the experimental analysis used in Objective 2, including X-ray diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR).

3.3.1 X-ray Diffraction (XRD) analysis

OPW was analysed using XRD to determine the crystallinity of cellulose. In this analysis, the crystallinity of nanocellulose from OPW has been analysed by using a PAN analytical X'PERT PRO MPD X-ray diffraction machine, as represented in Figure 3.12, operating at 40 kV and 30 mA, which was used to obtain the diffraction profile at 2°C per min. Samples were scanned in step-scan mode with a 2 angle ranging from 5 to 70 using a monochromatic Cu-Kα radiation source (λ = 1.5406 Å).The crystallinity index, Crl, was determined by the Segal method using Equation 3.1, where I_{002} provides the maximum peak intensity value for the crystalline cellulose at about 2θ = 22.0°C to 24 and I_{am} gives the peak intensity of diffraction of the amorphous region at about 2θ = 18.0° for cellulose.

$$\text{Crl (\%)} = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \qquad \text{(Equation 3.1)}$$

While crystallite size can be calculated by using Scherer equation on Equation 3.2 , where K is a shape factor and is the Scherrer constant most adjusted to the nanocrystal shape, λ is the wavelength (1.5418 Å) and β correspond to the full width at half maximum intensity (FWHM) and θ is half the Bragg angle at peak maximum given in radian.

$$D = \frac{K\lambda}{\beta \cos\theta} \qquad \text{(Equation 3.2)}$$

Figure 3.12: PAN analytical X'PERT PRO MPD X-ray diffraction machine

Four samples were examine from different stages of chemical treatment of OPW which are: (i) untreated OPW, (ii) alkaline treatment (iii) acid hydrolysis can be measured according to the crystal peak that shown by XRD data analysis. To find the crystallite size of the nanocellulose from OPW, it will be determined by using the Scherer equation. From the calculation, it can be concluded that the higher the temperature, the smaller the crystallite size of the cellulose nanocrystals. This is due to the higher reaction temperature, which will lead to the effectiveness in obtaining shorter cellulose nanocrystals. From the result of the previous research review, it is expected that the XRD analysis will show that there will be differences associated with a phase transformation of cellulose as a result of chemical treatment and changes in the crystallinity of the samples

3.3.2 Fourier Transform Infrared (FTIR) analysis

FTIR Spectroscopy will be used to study the elemental chemical composition of different chemical stages. This analysis was also used to support the result of the XRD analysis in order to show whether the hemicellulose, lignin, and other impurities had been removed during the alkaline treatment by investigating their functional groups. The elemental chemical composition of three different OPW powders was analysed by using an FT-IR-6100 Spectrum GX-FT-IR spectrophotometer (Perkin Elmer, Germany) machine as shown in Figure 3.13 and carried out at room temperature.

Four samples will be examined from different stages of chemical treatment of orange peel waste which are: (i) untreated OPW (ii) alkaline treatment (iii) acid hydrolysis that have highest crystallinity index (Crl). FTIR spectral analysis was performed within the wave number range of 400 - 4000 cm^{-1}. According to FTIR analysis, lignin, hemicellulose, and other extractives have successfully removed by alkaline treatment. Based on the previous research review, it is expected that the FTIR spectroscopy analysis will show that the lignin and hemicellulose have been removed during cellulose isolation process through analysis of its functional groups.

Figure 3.13: FT-IR-6100 machine (Perkin Elmer,Germany).

3.4 Experimental Work Regarding Objective 3

This section explains the experimental characterization involved in Objective 3, which is the Scanning Electron Microscopy (SEM) Analysis.

3.4.1 Scanning Electron Microscopy (SEM) analysis

SEM analysis gives excellent imaging that is important in studying the shape of microscopic structures by scanning the ruptured surface of materials. The Scanning Electron Microscope is being used to examine this analysis. SEM was employed in this study to analyse the surface morphology of OPW at several phases of chemical treatment, including (i) untreated OPW, (ii) alkaline treatment, (iii) acid hydrolysis. Before the study, a small sample was coated with gold using a little sputter coater because the material utilised is fibre, which is hygroscopic and requires coating to avoid charge. Images of the samples were captured using an acceleration voltage of 2 kV, a resolution of around 10 nm, and magnifications ranging from 5 to 1,000,000X. Under SEM observation, it clearly seen that some of tissue surface of the fiber has been destroyed and their connections between the cells become less than the raw fiber. From the result of the previous research, it is expected that the SEM analysis will prevail the fiber has a cleaner surface but looks jagged and rougher upon the chemical treatment process.

Figure 3.14: Zeiss SEM type Evo 50 Series. (Zeiss, German)

CHAPTER 4
RESULT AND DISCUSSION

Chapter 4 describes the experimental result obtained from multiple analyses which were carried out during the experimental study period. The experiment was carried out according to the flowchart present in Figures 3.1. In this chapter several tests were performed to analyse the orange peel waste (OPW) after going through different type of acid, optimum acid concentration, constant hydrolysis time and temperature. On top of that, this chapter comprise the result and discussion regarding on the X- Ray Diffraction (XRD) analysis, Fourier Transform Infrared (FTIR) Spectroscopy analysis and Scanning Electron Microscopy (SEM) analysis.

4.1 INTRODUCTION

This chapter discussed the results of the experiments that were conducted for preparation and characterization of nanocellulose from OPW that involved a few processes. In order to study the surface morphology of the untreated OPW and treated OPW, the Scanning Electron Microscopy (SEM) was used. Meanwhile, the X- Ray Diffraction (XRD) analysis was used to identify the crystallinity of the nanocellulose in a different treatment condition. Lastly, Fourier Transform Infrared (FTIR) Spectroscopy was used to support the XRD analysis and determine the presence of elemental composition in OPW at a different treatment including untreated OPW, alkaline treated OPW and the nanocellulose OPW with the highest crystallinity. Further elaboration of the results that were obtained for this experiment was explained in detailed in this chapter.

4.2 Alkaline treatment of Orange Peel Waste

The OPW was used in the alkaline treatment process in order to extract nanocellulose from OPW. The primary purpose of the alkaline treatment is to create highly purified cellulose fibre by removing lignin, hemicellulose, and other impurities from OPW. This can be accomplished through removal of hemicellulose during alkaline treatment. According to Marín *et al.* (2007) the application of alkaline treatment alters the surface of the fibres by stripping away the lignin, hemicellulose, and wax to obtain highly purified nanocellulose fiber. Therefore, an XRD analysis was performed on the sample after the alkaline treatment has been completed. The purpose of this analysis is to compare the percentage of crystallinity and the crystallite size of orange peel waste before alkaline treatment and after alkaline treatment.

4.2.1 X-Ray Diffraction (XRD) Analysis

An experimental using X-ray diffraction (XRD) was conducted in order to determine the crystallinity index (CrI) and crystallite size of cellulose that had been isolated from OPW. Figure 4.1 shows the XRD of OPW and alkaline-treated OPW. The crystallinity index (Crl) of the fiber was calculated using Segal formula on Equation 4.1. Where I_{002} provides the maximum peak intensity value for the crystalline cellulose at about 2θ = 22.0°C to 24 and I_{am} gives the peak intensity of diffraction of the amorphous region at about 2θ = 18.0° for cellulose.

Crystallinity index (%) : $\frac{I_{002}-I_{am}}{I_{002}} \times 100$ (Equation 4.1)

This formula is used to compute the percentage of crystallinity and to describe the relative amount of crystalline components in fibres. Next, Scherer equation is used to compute the crystallite size of nanocellulose crsytals obtained from XRD data. The formula for Scherrer equation on Equation 4.2. Where K is a shape factor, With K is the Scherrer constant most adjusted to the nanocrystal shape, λ is the wavelength (1.5418 Å) and β correspond to the full width at half maximum intensity (FWHM) and θ is half the Bragg angle at peak maximum given in radian.

$$D = \frac{K\lambda}{\beta \cos\theta} \qquad \text{(Equation 4.2)}$$

Figure 4.1 despicts the XRD graph pattern of untreated and treated OPW. Reffering to the graph pattern it shows that the peak intensity of treated OPW was sharper than untreated OPW. This is apparent when the intensity of treated OPW is 2238 while for untreated OPW is 2115 located at 2θ = 22° which indicates that treated OPW is more crsytalline than untreated OPW. While, on the peak 2θ = 15° had only small changes in broadening peak from 1821 to 2000, the intensity shows an increasing trend.

Therefore, cellulose extraction by alkaline treatment eliminates the residual amount of amorphous lignin and hemicellulose. While, at the peak 2θ = 15º between untreated OPW and treated OPW had only small changes in broadening peak. Due to the continuous removal of amorphous non cellulosic material, the treated OPW exhibited greater crystallinity than the untreated OPW. Thus, the XRD graph demonstrated that alkaline treatment occurs more effectively in significantly reducing amorphous region with attain higher peaks in crystalline region.

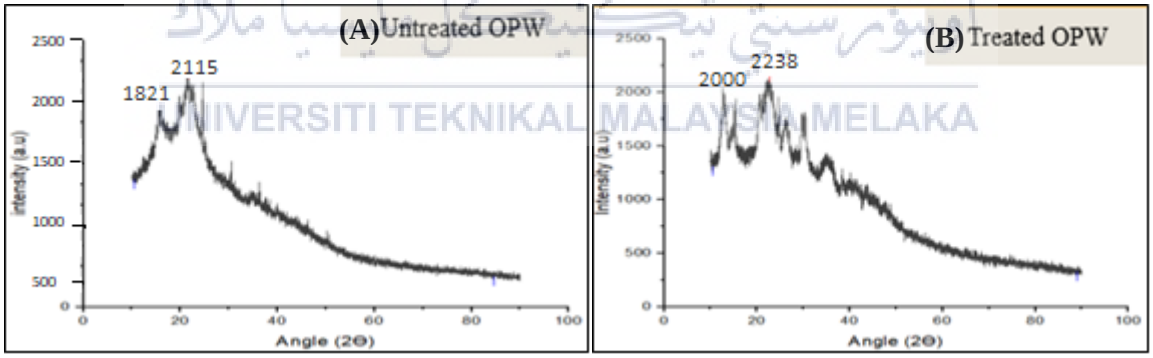

Figure 4.1: X-ray diffraction patterns of untreated OPW on (A) and alkaline treated OPW (B).

The results obtained was aligned with the result according to Chieng et al. (2017), 2θ = 22° is a maximum intensity value for crsytalline cellulose while 2θ = 15° represent the broad peak at peak minimum. Based on the XRD result on Figure 4.1 its shows that the peak of treated alkaline OPW is slightly higher compared to untreated OPW. In general, both untreated OPW and alkaline treated OPW samples possessed a high peak intensity of 2θ =

22°, which was attributed to their crystalline structure of cellulose, whereas the presence of broad peak at around 15º indicates the amourphous arrangement. Therefore, it indicate that cellulose obtained from untreated OPW can be said as having cellulose type I structure (Choi *et al.* 2018).

From this analysis, the crystallinity index (Crl) and crytallite size were calculated using Segal Equation and Scherer Equation, was supported by French *et al.* (2013) respectively, and the result are shown on the Table 4.1.

Table 4.1: Crystallinity Index (Crl) of untreated OPW and alkaline treated OPW

Sample	2θ (Amorphous)	2θ (Crystalline)	Crl %
	Intensity (I_{am})	Intensity (I_{002})	
Untreated OPW	1153	1508	23.54
Alkaline Treated OPW	853	4294	80.14

In Table 4.1, untreated OPW has the lowest percentage of crystallinity, at 23.54%, due to its high amorphous region content. However, the crystallinity index was determined, and it is shown that the percentage of Crl increases after alkaline treatment purification and after undergoing acid hydrolysis treatment. Crl may have increased as a result of the removal of amorphous which is lignin and hemicellulose during the alkaline treatment, or it may have increased as a result of the elimination of amorphous phase throughout the process and had successfully removed partially of non cellulosic materials in order to obtain highly purified nanocellulose (Phanthong *et al*. 2018).

It was observed that treated OPW exhibited a sharper diffraction peak than untreated OPW, with the intensity placed at a 2θ value of 22, while which presence of broad peak at 2θ value of around 15°. Therefore, non cellulosic material such as lignin and hemicellulose, on the other hand, must be clearly eliminated in order to produce highly purified nanocellulose.

Other researchers have proved that bleaching treatment is one method for removing residual hemicellulose and lignin in fibre. According to literature, by Shi *et al.* (2011) reaction of bleaching permanently removal of majority of non-cellulosic found in the fiber however from the result obtained, it can be concluded that several hemicellulose and other impurities were removed by the method of alkaline treatment solely, even though the bleaching process are not carried on. This is due to the technique and condition of alkaline treatment utilised in this study, which is NaOH, as suggested by Kyaw *et al.* (2017). In their study, lignin was removed in 1.5 wt.% of sodium hydroxide for 30 minutes at 121 °C. The findings indicated that higher solid loading may result in insufficient lignin removal during alkaline pre-treatment.

4.2.2 Fourier Transform Infrared (FTIR) Spectroscopy Analysis

The elemental chemical makeup of distinct chemical phases was studied using FTIR spectroscopy. This analysis was also utilised to support the results of the XRD analysis, demonstrating if hemicellulose, lignin, and other impurities were eliminated during the process. Alkaline treatment by looking into their functional group Table 4.2 summarised the common the presence of a functional group in the FTIR spectra of untreated OPW and alkaline treated OPW.

Table 4.2: Typical functional group presence in sample of untreated OPW and alkaline treated OPW.

References	Wavenumber (cm^{-1})	Involves functional group
Nik et al., (2019)	870	C-O stretching mode
	2918-3340	C-H stretching vibration
	3340	O-H stretching vibration
Wang et al., (2016)	3300-3500	O-H stretching vibration
	2900	C-H stretching vibration
	1040	C-O stretching mode
Lu et al., (2010)	3000-3650	O-H stretching vibration
	2900	C-H stretching vibration
Sgriccia et al., (2008)	3300-3500	O-H stretching vibration
	1230-1240	C-O stretching mode
	1734	C=O stretching vibration

Figure 4.2 depicts the FTIR spectra of untreated and alkaline-treated OPW. According to the FTIR spectrum, treated OPW has a slightly different pattern of spectra compared to untreated orange peel waste. This was related to the alkaline treatment used to remove hemicellulose and lignin before chemical treatment which is acid hydrolysis. For untreated OPW, the vibration peak at 3280 cm^{-1} region indicate region indicate the hydrogen bond O – H were bending of absorbed water of cellulose while for alkaline treated vibration peak at 3298 cm^{-1} for hydrogen bond O – H. and the peaks around 815.7 cm^{-1} correspond to C–H stretching for untreated orange peel waste while 802.4 cm^{-1} for alkaline treated OPW. Other than that, it was found that the peaks around 1014 cm^{-1} in the spectrum of alkaline treated corresponds to the C-O stretching peak of alkaline treatment was slightly lower than untreated OPW which 1014.4 cm^{-1} indicates that most of the hemicellulose and lignin were removed.

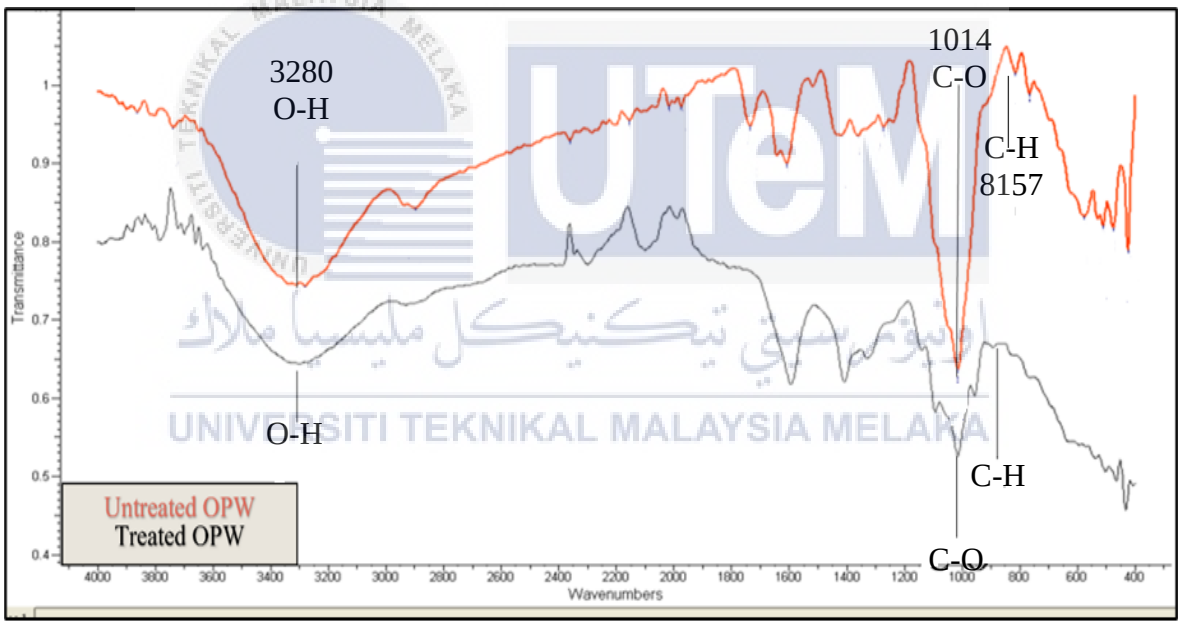

Figure 4.2 : FTIR for untreated OPW and alkaline treated OPW.

In addition, the FTIR spectrum revealed a smooth peak between 1100 cm^{-1} and 1600 cm^{-1}, showing that the alkaline treatment removed the majority of the hemicellulose and lignin. It will demonstrate that there are decreasing amount of lignin in alkaline treated OPW compared to untreated OPW.

Thus, it can be concluded that the FTIR spectra confirm that the hemicellulose and lignin present in the sample were successfully eliminated by the alkaline treatment. According to study by Hassan et al., (2014) with a clearer surface, NaOH can suggest the elimination of hemicellulose and lignin and alkaline treatment helped to reduce water absorption of fiber.

4.2.3 Scanning Electron Microscopy (SEM)

The surface morphology of orange peel waste (OPW) before and after alkaline treatment was obtained following the completion of the alkaline treatment of the fibre. Using a Carl Zeiss Model 1450VP variable pressure scanning electron microscope (SEM), the surface is analysed.

The OPW undergoes the alkaline treatment by being immersed in a Sodium Hydroxide (NaOH) solution with a 5 wt% concentration at room temperature for 24 hours. The fibres were then filtered and washed completely with distilled water until the pH was neutralised. The fibre is then dried in an oven at 40°C for 24 hours. As depicted in Figure 4.3, there are modest variations in the fiber's colour and texture before and after alkaline treatment. The sample continues to undergo SEM analysis in order to assess the surface morphology before and after alkaline treatment.

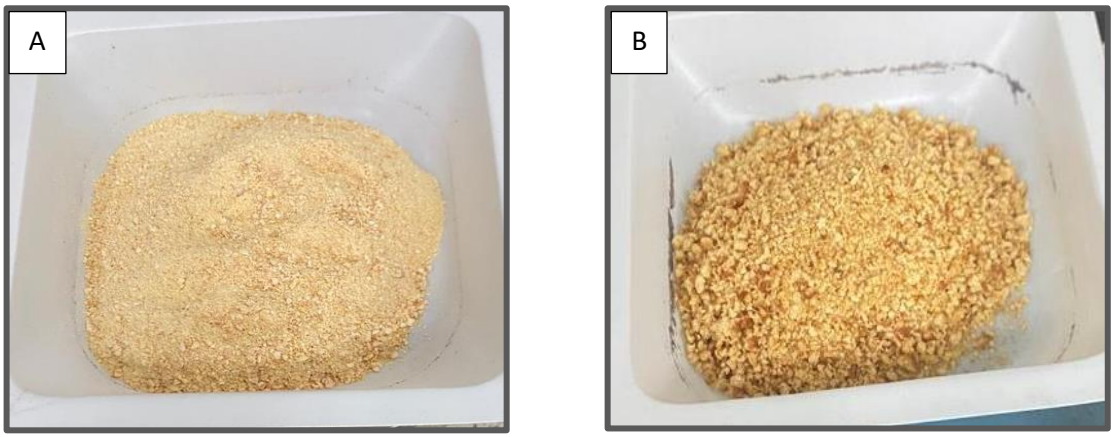

Figure 4.3: Image of orange peel waste fiber (a) before alkaline treatment and (b) after alkaline treatment

Based on the observation on Figure 4.4 for untreated OPW and alkaline treated OPW. The surface morphology of the untreated OPW fiber on Figure 4.4 (A) was irregular and covered with the impurities such as hemicellulose, lignin, wax and other extractives substances. While, the surface morphology of the treated OPW fiber changed after the alkaline treatment on Figure 4.4 (B). On the surface of alkaline treated fiber, the surface looks a bit cleaned but with a bit rough surface if compared to the untreated ones. According to Wan *et al.* (2010) ,the majority of lignin may be removed from the cellulose during the extraction process by using an alkaline solution. On the other hand, the acid hydrolysis used to produce eliminates the residual amount of the amorphous lignin.

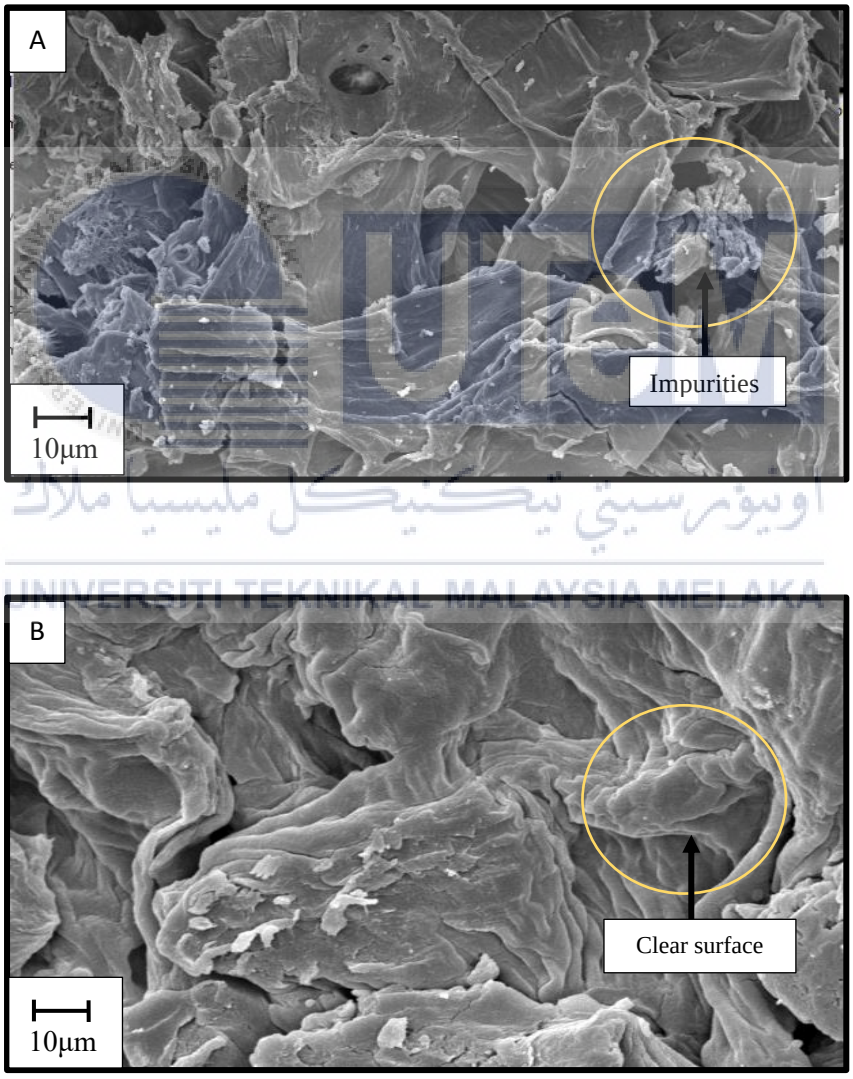

Figure 4.4: SEM Micrograph of (a) untreated OPW and (b) alkaline treated OPW

The results obtained was aligned with the result based on the Figure 4.5 which shows SEM microscope of raw sugarcane bagasse for raw fiber and alkaline treated fiber (Mzimela *et al.* 2018). According to Mzimela *et al.* (2018) numerous non-fibrous substances, such as waxes and pectin, are dispersed across the surface on Figure 4.5 (A). Figure 4.5 (B) shows that the surfaces of all of the cellulosic materials are cleaner and contain a minor increase in roughness, in contrast to the surfaces of raw bagasse, which are shown in Figure 4.5 (A).

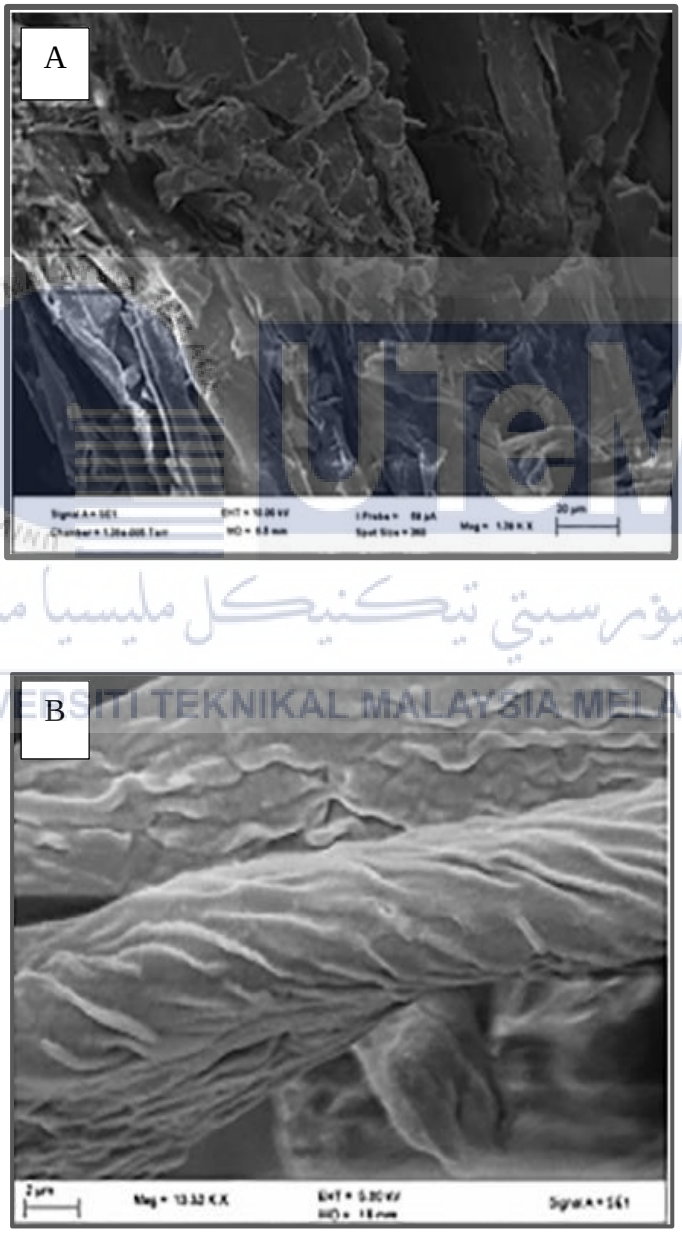

Figure 4.5: SEM micrographs of (a) raw sugarcane bagasse and (b) treated sugarcane bagasse (Mzimela *et al.* 2018)

4.3 Acid Hydrolysis of Orange Peel Waste

One of the main processes in order to isolate the nanocellulose from cellulosic materials was by acid hydrolysis method, this method is easy and fast to produce nanocellulose that had a better properties and most widely used method. This method was conducted based on the designed parameter in Chapter 3. According to the study by Mekala *et al.* (2014) nanocellulose can be obtained by acid hydrolysis. This process also resulted in the removal of the amorphous component of the raw materials, which allowed for the extraction of nanocellulose that had a high degree of crystallinity. In order to obtain highly purified nanocellulose it is necessary to entirely removed both the hemicellulose and the lignin. Therefore, acid hydrolysis with different type of acid used that is sulphuric acid (H_2SO_4) and hydrocloric acid (HCl).

4.3.1 X-Ray Diffraction Analysis (XRD)

After undergoing the acid hydrolysis treatment with A is sulphuric acid (H_2SO_4) and B is hydrocloric acid (HCl), on Figure 4.6. The XRD patterns were analyzed in order to study the effect of the parameters that were used.

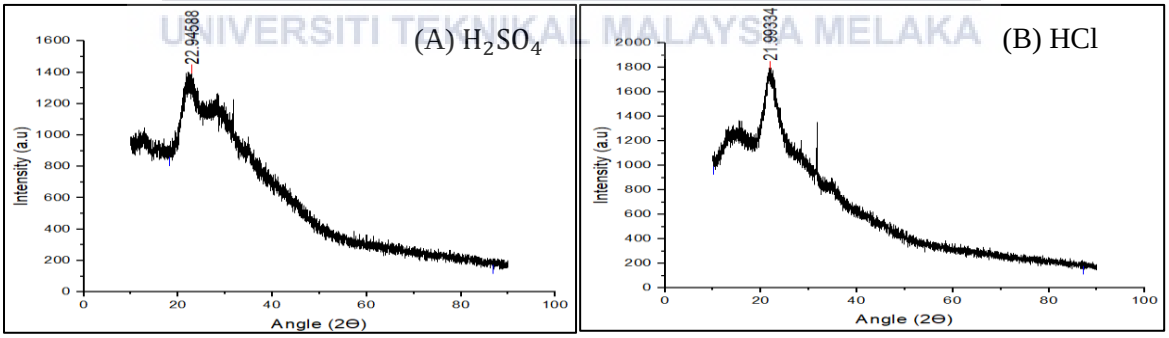

Figure 4.6: XRD analysis of (A) H_2SO_4 and (B) HCl

Based on Figure 4.6, it was determined that a comparison of two samples by different types of acids for acid hydrolysis that is H_2SO_4 and HCl with concentration of 30 wt% and constant time (120 min) and fixed temperature (45°). From the XRD graph, it can be concluded that the crystallinity peak increases following alkaline purification and acid

hydrolysis. Based on the diffraction pattern, the presence of broad peak at around 15˚ hence, can be said as having cellulose type I structure on both acids. (Choi *et al.*, 2018). From the result it shows nanocellulose have three diffraction peak they are 2θ = 15° ,22° and 35°.

The crsytallinity index for isolated nanocellulose from OPW its can be seen on Table 4.3. H_2SO_4 had crsytallinity index 87.69% while HCl is 78.00%. this difference due to the type of acid used and due to the strong acid HCl it will result lower on crystallinity index indicates the amorphous and crystalline region could be highly significantly degraded. It suggests that the addition of acid with a strong acid not only destroys the amorphous portion of the cellulose, but also damages its crystalline structure. Study by Poletto *et al*. (2014) stated that as crystallinity index increased, the crystallite size also increase because the crystallite surface corresponding to the reducing of amorphous cellulose regions.

The acid hydrolysis by using H_2SO_4 and HCl will break the amourphous region of cellulose to produce nanocellulose with high crsyatallinity index but when strongest acid HCl is used with 30wt% of acid concentration, the crystalline part can be damaged during acid hydrolysis process, and the crystallinity index is decreased. Therefore due to the strongest acid HCl and H_2SO_4 and higher concentration for 40wt% it will cause the fiber to burn during the acid hydrolysis process. As a result, the orange peel waste turns black and dissolves with the acid. These situation cause the characterization cannot be conducted for the analysis.

Table 4.3: Cellulose intensity peak and the respective CrI

Sample	**2θ (Amorphous)**		**2θ (Crystalline)**		**Crystallinity Index %**
	Degree	Intensity (I_{am})	Degree	Intensity (I_{002})	
Untreated OPW	15.82	1153	21.67	1508	23.54
Alkaline Treated OPW	15.68	853	22.73	4294	80.14
30wt% (H_2SO_4)	12.75	993	22.94	8064	87.69
40wt% (H_2SO_4)	-	-	-	-	-
30wt% (HCl)	12.99	321	21.99	1459	78.00
40wt% (HCl)	-	-	-	-	-

Table 4.4: Crystallite size of nanocellulose OPW at different parameter.

Sample	Position (°2Th.)	FHWM (°2Th.)	Crystallite Size (nm)
30wt% (H_2SO_4)	21.8879	2.6765	3.19
40wt% (H_2SO_4)	-	-	-
30wt% (HCl)	22.2901	1.6059	5.34
40wt% (HCl)	-	-	-

Besides that, based on the calculation of crystallite size from Table 4.4 HCl provides higher crystallite size compared to H_2SO_4. This was due to the severe erosion of the granule surface. According to, Wulandari et al. (2016), the nanocellulose obtained from the acid hydrolysis also has a smaller size. These are the reasons that acid hydrolysis method is selected to obtained nanocellulose. Therefore, it can be concluded that slightly change in crystallinity index and size are directly related to the acid strength since hydrolysis time were constant. It has been shown that, at high acid concentrations of 40wt%, sulfuric acid and hydrochloric acid have been demonstrated to be capable of breaking hydrogen bonds, allowing penetration into amorphous and crystalline cellulose areas and causing the sample to burn and dissolve in solution.

4.3.2 Fourier Transform Infrared Spectroscopy Analysis

Figure 4.7 shows the FTIR spectra of H_2SO_4 and HCl for acid hydrolysis. The spectra of nanocellulose obtained under various type of acid used in acid hydrolysis. Based on the observation, nanocellulose were produced in the form of type I structure and it was showed by the transmittance signal at 1144 cm^{-1}

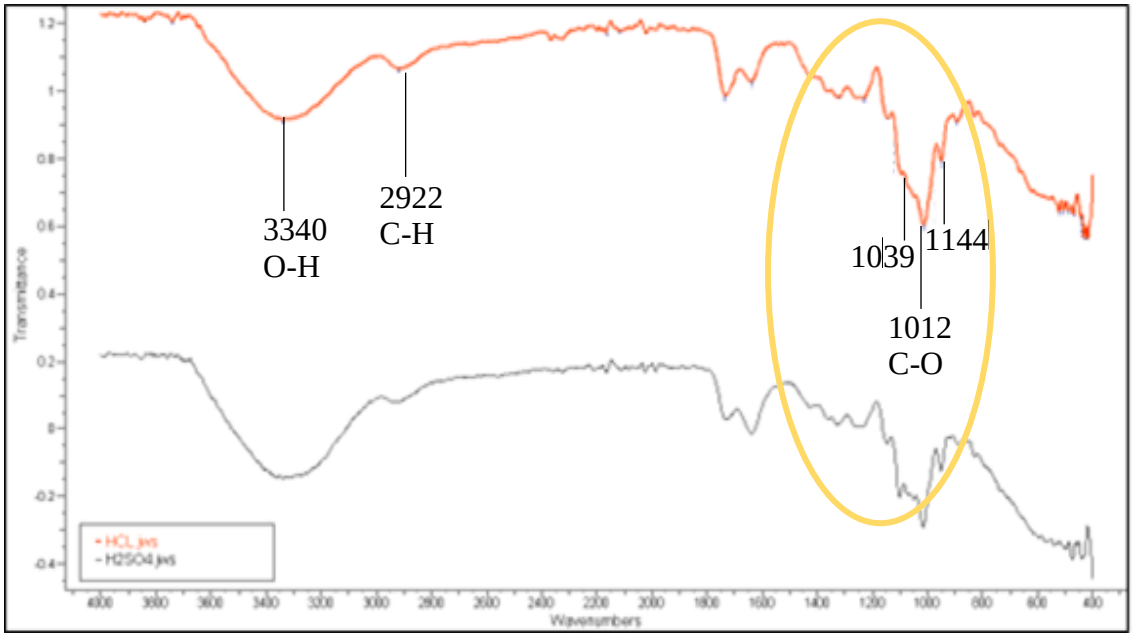

Figure 4.7: FTIR Spectra of H_2SO_4 and HCl of acid hydrolysis

The peak at 1012 cm^{-1} refers to the carbon-oxygen single bond which related to the C-O stretching mode. It was observed that at two band 1012 and 1039 cm^{-1} that correspond to the symmetric CO stretching mode and O-C-O bending mode of calcite respectively, there were a produced of calcium carbonate (Lani, *et al.* 2014). However, the intensity of the band at 1012 cm^{-1} was absent undergo acid hydrolysis treatment. This was resulted in extraction of highly purified nanocellulose as the calcite were successfully removed by the treatment.

Furthermore, the absorption peaks in the 3300-3450 cm^{-1} range are attributed to the stretching and bending vibrations of the OH groups of cellulose that absorbed water (Moran *et al.* 2008). The presence of broader peak that correspond to the O-H stretching vibration at peak 3340 cm^{-1} showed that there was an increasing in removal water absorption upon the treatment. Hence, it can be concluded that the difference of FTIR spectrum between $H_2SO_4$and HCl and was indicated to the succeed in isolation the nanocellulose from the OPW by the acid hydrolysis method. Therefore, it is possible to conclude that the nanocellulose synthesised in this work was nearly pure, containing only small amount of lignin and other noncellulosic components.

4.3.3 Scanning Electron Microscopy (SEM)

After alkaline treatment undergoes to acid hydrolysis by being immersed in a H_2SO_4 and HCl solution with a 30 wt% concentration. The fibres were then filtered and washed completely with distilled water until the pH was neutralised. The fibre is then dried in an oven at 40°C for 24 hours. As depicted in Figure 4.8, there are modest variations in the fiber's colour on H_2SO_4 result the white colour of OPW nanocellulose powder. While HCl result the dark grey colour of OPW nanocellulose powder. The sample continues to undergo SEM analysis in order to assess the surface morphology of acid hydrolysis.

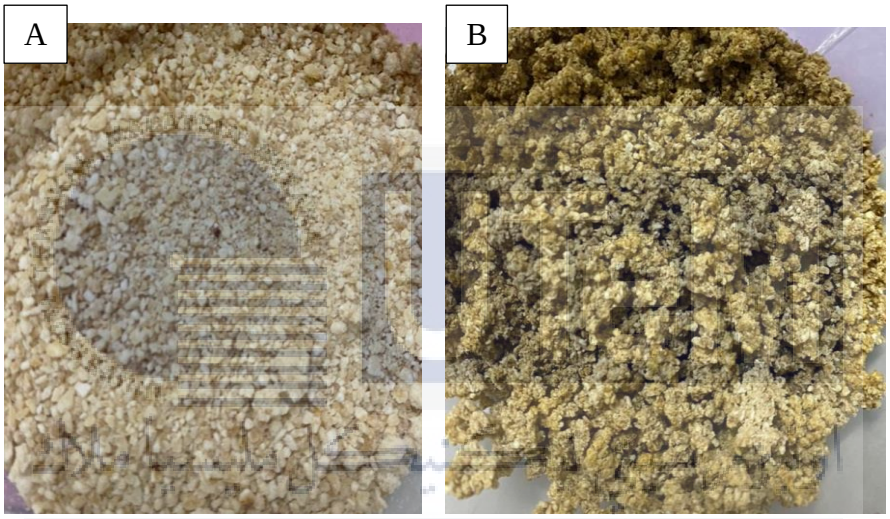

Figure 4.8: Image of acid hydrolysis of orange peel waste (a) H_2SO_4 and (b) HCl

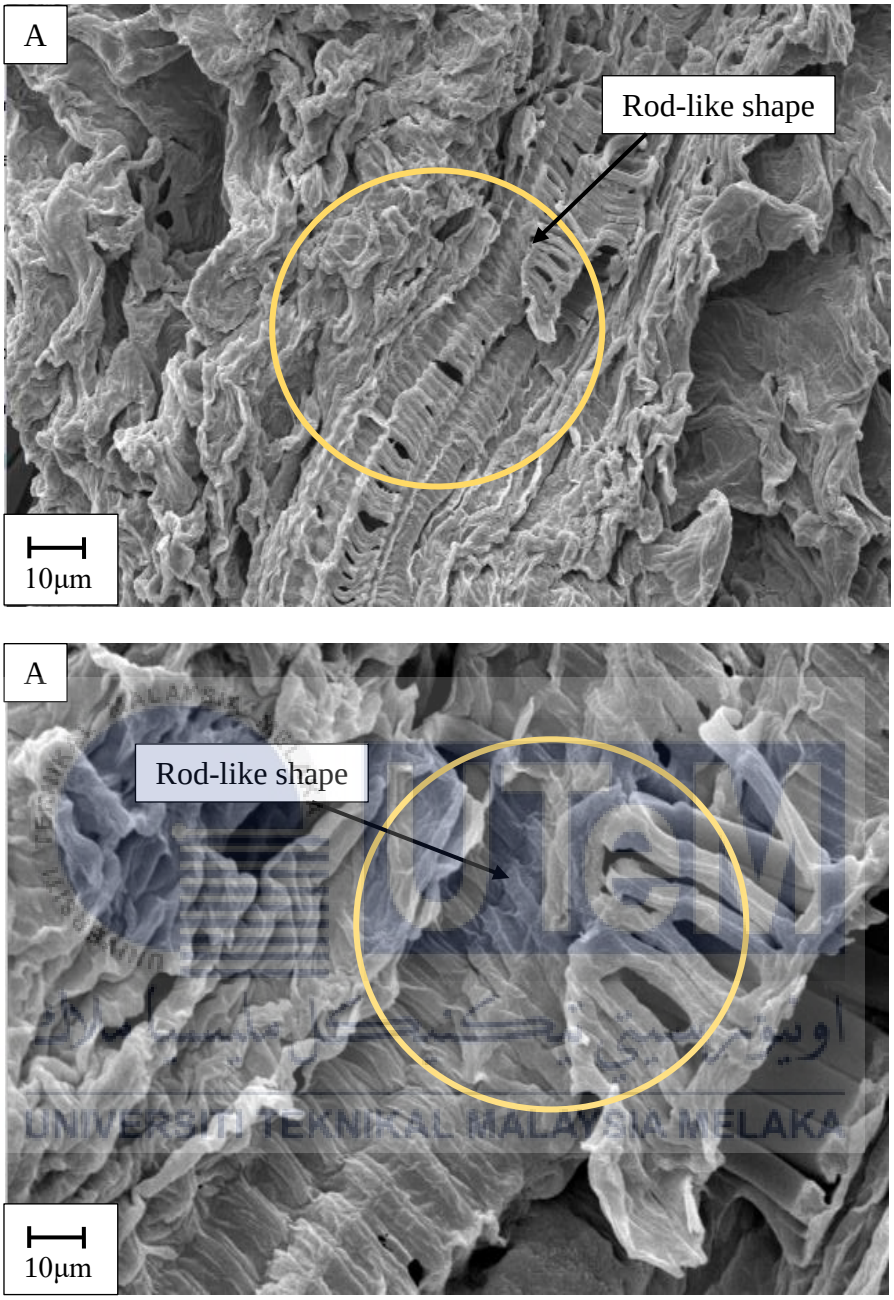

Figure 4.9: Surface morphology for H_2SO_4 under the magnification of 100x and 500x

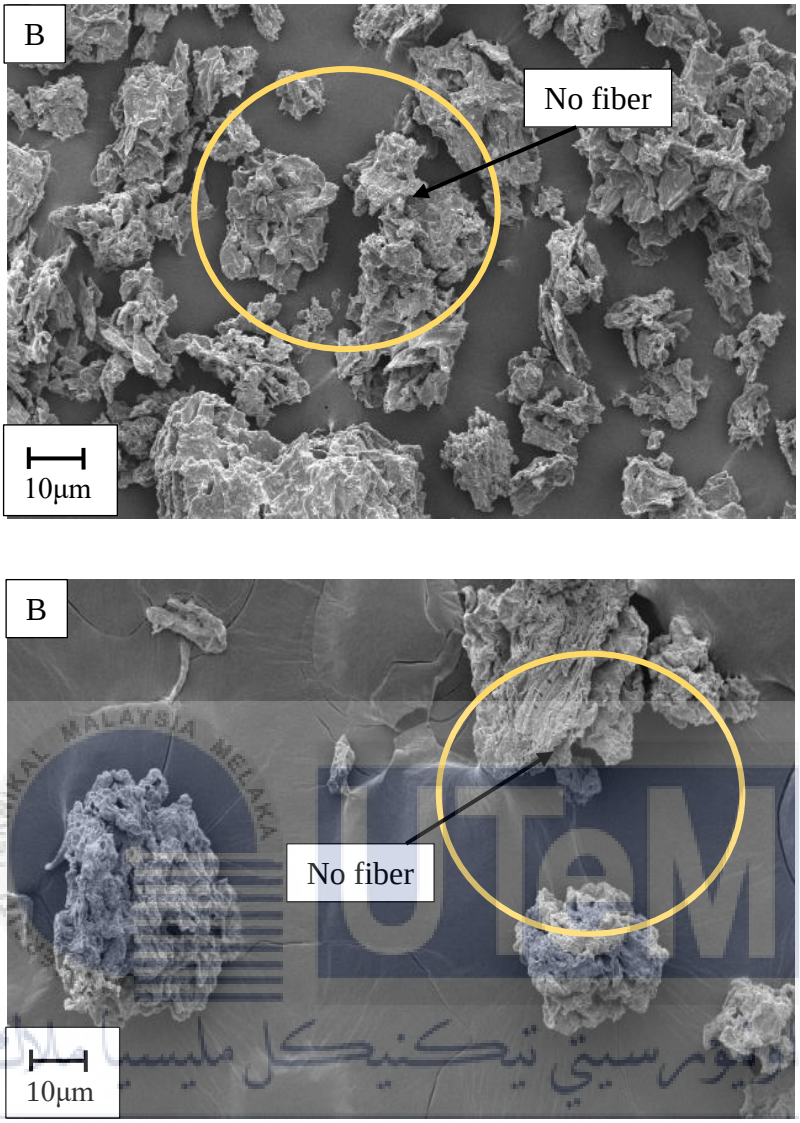

Figure 4.10: Surface morphology for HCl under the magnification of 100x and 500x

Figure 4.9 showed the morphology surface are rod-like shape with might be due to degradation primarily occurs in the amorphous cellulose. H_2SO_4 recognized most widely used for acid hydrolysis because process is simple and result with highly crystalline, stiff and effective on elimination of amorphous. Figure 4.10 depicts the surface morphology of HCl under these conditions, where no cellulose fibres are produced and only large-scale aggregation is detected (Huntley *et al*. 2015). HCl has a higher corrosion resistance, and its nanocellulose fibres are well-defined and primarily aggregate on a large scale. Compared to H_2SO_4 because of strong acid and make the OPW dissolves with the acid. Next, H_2SO_4 is the best method for acid hydrolysis needed to generate well-dispersed crystalline cellulose with minimal aggregation affect this proved by Levis *et al.* (2001).

CHAPTER 5
CONCLUSION AND RECOMMENDATION

5.1 Conclusion

As the conclusion, it can be concluded that the nanocellulose derived from orange peel waste (OPW) has been successfully prepared based on the selected parameter with the different type of acid at optimum acid concentration with constant temperature and time which is comply with the first objective of this study. It was found that the optimum acid concentration to isolate nanocellulose was at 30 wt% and with the best acid used are sulphuric acid, constant hydrolysis time 120 min and temperature 45°C. The nanocellulose obtained was prepared by two stages of treatment which are alkaline treatment by using sodium hydroxide (NaOH) with the concentration of 5 wt% before subjected to the acid hydrolysis method by using sulphuric acid (H_2SO_4) and hydrochloric acid (HCl) as a selected parameter. Nanocellulose has been successfully isolated from orange peel waste via acid hydrolysis method.

The second objective was to determine the crystallinity and crystallite size of nanocellulose from OPW by X-Ray Diffraction (XRD) analysis and Fourier Transform Infrared (FTIR) Spectroscopy. The crystallinity were calculated based on the Segal Equation after being analyzed from the XRD graph and data. After that, followed by the calculation of crystallite size by using Scherer Equation in order to confirm the nanocellulose that is extract from OPW. FTIR was used in order to study the elemental chemical composition for different chemical stages. Based on the result of XRD analysis, the optimum condition for the waste office paper to achieve the highest crystallinity and crystallite size was at 30 wt% concentration with the hydrolysis time 120 min and temperature 45°C. Meanwhile, FTIR result was proved that the partially hemicellulose, lignin and other impurities was successfully removed by the alkaline treatment.

The third objective was to study the surface morphology and structure of untreated OPW and alkaline treated OPW by using Scanning Electron Microscopy (SEM). Based on the result, it is obvious showed that the impurities such as hemicellulose, lignin and other non-cellulosic materials were successfully removed by alkaline treatment and this subjected to the achievement of the third objective.

5.2 Recommendation

Based on the findings and discussions of this study project, there are more areas to investigate in order to improve the findings in future studies. In this study, there were many questions about further investigation in order to observe the best technique for using acid hydrolysis method. For the recommendation, further research is used to obtained nanocellulose, such as acid hydrolysis, ultrasonic technique, and enzymatic hydrolysis. Next, in order to achieve the greatest results for the elimination of lignin and hemicellulose during alkaline treatment, additional research should be conducted with Transmission Electron Microscopy (TEM) as a recommendation.

5.3 Sustainable Design and Development

A crucial component in this study was sustainable design and development. This is due to the utilisation of waste that can be used as raw materials to make other materials that can be used as reinforcement. The utilisation of green resources as raw materials has gotten a lot of attention recently. Without a doubt, the cellulose structure in OPW has a significant potential for utilisation as a nanomaterial. As a result, this research can assist reduce the possibility for environmental problems while also using waste as a reinforcement. This was in accordance with the definition of sustainable design, which aims to lessen the negative influence of the environment on human health and the waste can be used and is abundant in chemicals.

5.4 Complexity

To carry out this experiment, several tasks including complex technical challenges must be approached cautiously and sensibly. In order to get the required concentration for this experiment, for instance, the sulphuric acid and hydrochloric acid required for the acid hydrolysis procedure must be titrated. Complexity arose during the acid titration because the procedure required meticulous execution and an exact calculation prior to beginning the titration. A fume hood is required for the acid titration to make sure the safety when conducting the chemical. The acid titration procedure must also adhere to the standard operating procedure developed by lab assistants to prevent of any incident happened when conducting acid titration. In addition, calculations requiring expertise must be performed to derive the crystallinity index and crystallite size.

Thus, the complicated issue solving, complex engineering activities, and knowledge profile have been employed in this study. For example, the implementation of different acid concentrations of sulphuric acid and hydrochloric acid which required correct calculation regarding on wt% and the complexity performance handling the chemicals regarding on containers of chemical are not labelling correctly and for using the chemical need to asked for permission before used.

5.5 Life Long Learning and Basic Entrepreneurship

The commercialization of nanocellulose extracted from used OPW has the potential to occur. As an example, the element fullerites has a high degree of crystallinity and the waste is transformed into bio sorbents for removal of heavy metals and dyes. In light of these findings, it was concluded that OPW might be used as a raw material for the production of nanoparticles and could be employed in business. Bioelectricity and carbon nanodots for bioimaging can be generated from the waste. Finally, waste is converted into fibres, textiles, and 3D-printed materials.

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APPENDICES

Appendix A

Gantt Chart of PSM 1

No	Task	Week																
		Semester 1 (October-March)																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	PSM Title Registration								Mid-Semester Break							Study Week	Final Exam	
2	Briefing of Title Selection																	
3	Find Journals and Reference Materials on the PSM Title																	
4	Define Objectives and Problem Statement																	
5	Identify Background and Scope of Study																	
6	Carry Out for Literature Review																	
7	Conduct on Findings Methodology of Overall Process, Methods, and Instruments																	
8	Submission of Log Book to Supervisor																	
9	Preparation of Presentation PSM																	
10	Presentation for PSM on Online Video																	
11	Final Report PSM Checked by Supervisor																	
12	Submission of PSM Report to Supervisor and Examiner																	

	Plan
	Mid-Semester Break, Study Week, and Final Exam

Appendix B

Gantt Chart of PSM 2

No	Project Activities	February				March				April				May				June		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	Correction report PSM 1								Mid Semester Break								Revision Week	Final Examination		
2	General Briefing PSM 2																			
3	Discussion with supervisor																			
4	Preparation of material and equipment for experiment																			
5	Preparation of sample : Cleaning process of raw material																			
6	Preparation of sample : Grinding process of raw material																			
7	Alkali treatment																			
8	SEM analysis																			
9	Preparation of acid hydrolysis: dilution of acid																			
10	Acid hydrolysis																			
11	XRD and FTIR analysis																			
12	Submission of General Conduct Form and log book to SV																			
13	Report Writing on Chapter 4 (Result and Discussion)																			
14	Report Writing on Chapter 5 (Conclusion and Recommendation)																			
15	Preparation on poster presentation																			
16	FYP Presentation																			
17	Submission of draft report to SV and examiner																			
18	Submission of Final Report to SV																			
19	Hardbound submission to Faculty																			