UNIVERSITI PUTRA MALAYSIA

TRANSFORMATION OF OIL PALM MESOCARP FIBER TO CONVERTIBLE MATERIALS IN WATER AT SUBCRITICAL STATE

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TRANSFORMATION OF OIL PALM MESOCARP FIBER TO CONVERTIBLE MATERIALS IN WATER AT SUBCRITICAL STATE

By

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TRANSFORMATION OF OIL PALM MESOCARP FIBER TO CONVERTIBLE MATERIALS IN WATER AT SUBCRITICAL STATE

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Chair : Shamsul Izhar bin Siajam, PhD, Ir
Faculty : Engineering

In this research, water at subcritical state was studied as an alternative way to breakdown oil palm mesocarp fibre (OPMF) by analysing their structural and compositional changes at different reaction temperature. High ion products of water in subcritical conditions and the dielectric constant of water at temperature above 200°C makes water to behave as acid catalyst and environmentally friendly solvent. The decomposition of OPMF using subcritical water (Sub CW) method has been carried out at reaction temperature ranging from 200°C to 300°C and with constant reaction time of 5 min using a batch stainless-steel tube as reactor. The highest oil yield was obtained at 240°C with result values 219.6 mg g^{-1} dry OPMF due to the behaviour of water to act like ethanol solvent at 240°C (ε= 27.0). High-performance liquid chromatography (HPLC) analysis identified that the highest number of monosaccharides such as glucose and fructose presented at 240°C was due to optimum condition for hydrolysis process with sugar yield, 22.17mg g^{-1} dry OPMF. Moreover, morphological and particle size analyses proven that water at Sub critical state ease hydrolysis process occur as the Sub CW temperature increase to 240°C. When the reaction temperature gets higher than 240°C, cellulose of OPMF degrades through pyrolysis process. Increase in Sub CW reaction temperature decreases yield of solid from 0.286 g g^{-1} dry OPMF at 200°C to 0.037 g g^{-1} dry OPMF at 300°C. Thus, Sub CW at 240°C was able to extract optimum oil and sugar at high yield and reduces solid residue from mesocarp fibre with the absence of any chemical solvent.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Master Sains

TRANSFORMASI SERAT MESOCARP OIL PALM (OPMF) KEPADA BAHAN BOLEH UBAH MENGGUNAKAN AIR DALAM KEADAAN SUB-KRITIKAL

Oleh

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November 2020

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Dalam penyelidikan ini, air pada keadaan sub-kritical dikaji sebagai kaedah alternatif untuk memecahkan serat mesocarp kelapa sawit (OPMF) dengan menganalisis perubahan struktur dan komposisi OPMF pada suhu tindak balas yang berbeza. Produk ion tinggi air dalam keadaan sub-kritical dan pemalar dielektrik air pada suhu melebihi 200°C adalah serupa dengan pemalar dielektrik metanol pada suhu bilik, yang menjadikan air berperilaku sebagai pemangkin asid dan pelarut mesra alam. Penguraian OPMF di bawah menggunakan kaedah air sub-kritical telah dilakukan pada suhu tindak balas antara 200°C hingga 300°C dan dengan masa tindak balas berterusan 5 min menggunakan tiub keluli tahan karat kelompok sebagai reaktor. Hasil minyak tertinggi diperoleh pada suhu tindak balas 240°C (selama 5 minit) dengan nilai hasil 219.6 mg g-1 OPMF kering kerana tingkah laku air bertindak seperti pelarut etanol pada 240°C (ε = 27.0). Analisis HPLC mengenal pasti bahawa jumlah monosakarida tertinggi seperti glukosa dan fruktosa ditunjukkan pada suhu tindak balas 240°C kerana keadaan optimum untuk proses hidrolisis dengan hasil gula 22.17 mg g-1 kering OPMF. Lebih-lebih lagi, analisis morfologi dan ukuran zarah membuktikan bahawa air pada keadaan sub-kritical memudahkan proses hidrolisis berlaku ketika suhu reaksi meningkat hingga 240°C dan suhu reaksi lebih tinggi daripada 240°C, hasil selulosa OPMF berkurang melalui proses pirolisis. Peningkatan suhu tindak balas sub-kritical menurunkan hasil pepejal dari 0.286 g g-1 OPMF kering pada 200°C hingga 0.037 g g-1 OPMF kering pada 300°C. Oleh itu, air sub-kritical pada suhu reaksi 240°C dapat mengekstrak hasil minyak dan gula yang optimum dan mengurangkan sisa pepejal dari serat mesocarp dengan tidak menggunakan pelarut kimia.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Sciences. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

C	Carbon
FFA	Free Fatty Acid
FTIR	Fourier Transform Infra-Red
HPLC	High Performance Liquid Chromatography
kg	Kilogram
K_w	Ion Product of Water
L	Litre
MC	Moisture Content
mg	Milli Gram
min	Minute
ml	Milli Litre
mol	Molecule
Mpa	MegaPascals
MPOB	Malaysian Palm Oil Board
OPEFB	Oil Palm Empty Fruit Brunch
OPF	Oil Palm Frond
OPMF	Oil Palm Mesocarp Fibre
OPT	Oil Palm Truck
P	Pressure
PKS	Palm Kernel Shell
POME	Palm Oil Mill Effluent
ppm	Parts Per Million

PSA	Particle Size Analysis
rpm	Rotations Per Minute
s	Second
SEM	Scanning Electron Microscopy
Sub CW	Sub Critical Water
T	Temperature
UV	Ultraviolet
µm	Micro Meter

CHAPTER 1

INTRODUCTION

1.1 Research Background

Oil palm mesocarp fibre (OPMF) is known as oil palm fruit lignocellulose fibre that can be obtained after extraction process of palm oil (Iberahim *et al*, 2013). Cellulose, hemicelluloses and lignin are the common components found in mesocarp fibres of oil palms.

Hemicelluloses, according to Yang *et al.,* (2007), loses its weight at temperature between of 220°C to 315°C while cellulose loses its weight at temperature between of 315°C to 400°C. Nevertheless, at temperature between of 160°C to 900°C, lignin loses its weight which makes it difficult to decompose. Hemicellulose has high tendency to degrade compared to lignin and cellulose due to its lesser thermal stability. The complex structure of the oil palm cell makes it difficult to break down the cellulose and lignin using a method of physical industrial scale (Ariffin *et al.,* 2008).

Hydrothermal degrade of cellulose involves chemical reaction such as hydrolysis, pyrolysis, rearrangement reaction and splitting of aldol (Kruse, 2008). Because of its ability to extract high quality product in less time without harming the environment, subcritical water technology is much desirable to be used for the palm oil extraction process instead of solvent as extraction tool. Organic solvents like hexane and ether are usually used in commercial extraction processes. The properties of water in a subcritical state are identical to organic solvent. Such water properties make subcritical water (Sub-CW) as an alternative method of extraction of palm oil, thus reducing the consumption of toxic solvent in industries. Cellulose hydrolysis to fermentable sugars is effective in subcritical water than water at supercritical state (Olanrewaju, 2012).

The water pressure increases with the increasing water temperature to maintain the liquid state of water in the process. At high temperature, water behaves like acid and base catalyst. As a consequence, the order magnitude of H_3O^+ and OH^- ion concentrations in Sub-CW is higher at a temperature level greater than 200°C and the production of ions goes up to $1x10^{-11}$ mol^2/L^2 at 250°C. Higher ion production facilitates a strong hydrolysis reaction (Omid, 2010). However, as the temperature increases above 250°C, pyrolysis reaction takes place and reduces the ion production drastically (Omid, 2010). The dielectric constant of methanol, ethanol and water are 32.5, 27.0 and 79.9 respectively at room temperature. Dielectric constant value of water decreases from 79.9 at room temperature to 17 at 312°C (Pourali *et al.,* 2009). This shows that water at subcritical state can behave as organic solvent like methanol and ethanol. These properties of water

are beneficial for use in treatment of OPMF and transform OPMF into value added products.

1.2 Problem Statement

In 2017, the crude palm oil production rose by 15% to 19.92 million tonnes with respect to 17.32 million tonnes in 2016, due to an increase in the yield of fresh fruit bunches by 5.3 percent (Kushairi *et al.*, 2018). Only 10% of palm oil can be extracted from the total biomass harvested creates the problem of disposal and degradation of environment (Kurnia *et al.*, 2016). The type of biomass waste produced from oil palm industry includes oil palm empty fruit bunches (OPEFB), oil palm trunks (OPT), oil palm mesocarp fibre (OPMF), palm kernel shell (PKS), oil palm fronds (OPF) and palm oil mill effluent (POME) (Abdullah & Sulaiman, 2013). According to Noorshamsiana *et. al* (2017), 6.95 million tons of OPMF is being produced in 2016 after palm oil extraction from 453 palm oil mills in Malaysia. The amount of OPMF produced is more than needed by the oil palm industry to utilize as a source of useful gases production, thus OPMF is used for mulching which can harm the environment (Iberahim *et al.*, 2013).

Cellulose, hemicelluloses and lignin are the common components that can be found in OPMF in the percentage of 42%, 32% and 22% respectively (Nordin *et al.*, 2013). The complex structure of palm oil cell causes difficulty in breaking down the cellulose using the physical method used at current industrial such as press digester, screw press, bead mill and etc. (Ariffin *et al.*, 2008). Cellulose is classified as recalcitrant substance due to its molecules arrangement and hydrogen bonds that make the degradation process difficult (Sasaki et. al, 2004; Olanrewaju, 2012). Arrangement of molecules in cylindrical conformation structure with crystallites form and hydrogen bonds between microfibre's layers makes the cellulose formation stable (Yunos *et al*., 2012). Cellulose decomposes into water-soluble substance in the absence of catalyst between 200°C to 400°C which proves that hydrothermal reaction is able to hydrolyse cellulose (Nordin *et al.*, 2013). Pyrolysis reaction occurs at a higher temperature in the absence of oxygen to release carbon dioxide, carbon monoxide and a significant amount of char. According to Tolonen, (2016), slow pyrolysis and fast pyrolysis reactions take place below 300°C and above 300°C respectively.

The commercial method which is the Soxhlet extraction currently uses organic solvents such as hexane and ether in the extraction process of the oil (Azmir *et al.*, 2013; Mohammad and Fereshteh, 2007). Subcritical water technology is much preferable to be used for palm oil extraction process instead of organic solvent as extraction medium due to its capability to extract high-quality product in a lesser amount of time without harming the environment (Abdelmoez *et al.*, 2014). Value of dielectric constant of water above 200°C is similar as the dielectric constant of methanol at room temperature, which makes Sub-CW to be a suitable solvent for hydrophobic organic (Amashukeli *et al.*, 2007 and Singh and Saldana, 2011). At subcritical state, water behaves as acid and base catalyst (Carr *et al.*, 2011). This technology gets attention due to its behaviour of producing high ion and low dielectric constant at an increased temperature (Adachi, 2009).

Subcritical water reaction is capable of facilitating structural conversion of OPMF to sugar as 240°C and alternative oil at higher than 240°C without any chemical solvent being used. Hydrolysis process occurs at temperature below 240°C by breakdown hydrogen bond present in cell wall of OPMF and release lignocellulose material. As the temperature gets higher, dehydration occur in process of pyrolysis which alter sugar content into alternative oil. According to Agensi Inovasi Malaysia (2013), the highest biomass from palm oil plantation is from Oil Palm Frond (OPF). However, OPMF was used to identify alteration after treat with Sub CW due to it is known as sustainable resources that able to utilise for various of valuable processes and products (Iberahim *et al.,* 2013).

Thus, OPMF treated in Sub-CW at a 240°C reaction temperature provides optimum sugar yield with less alternative oil output by ease cell disruption of OPMF. Structural transformation and conversion of lignocellulosic biomass such as OPMF after treat with Sub CW was not studied. The relationship between the structural and compositional alteration of OPMF and the substance formed at water in sub-critical state needed to be identifying to maximize Sub CW reaction effect towards conversion of OPMF.

1.3 Research Objective

The main objective of this research is to study on the transformation of mesocarp fibre composition in water at different temperature in subcritical state. The specific objectives of the studies are as following:

1. To determine optimum Sub-CW reaction temperature in breaking down of fibre structure for optimum biomass conversion into desired product through hydrolysis and pyrolysis reactions.

2. To examine the structural changes of oil palm mesocarp fibre after Sub-CW reaction at different reaction temperature

3. To identify chemical composition of oil palm mesocarp fibre after undergoes Sub-CW reaction.

1.4 Scopes of Study

This study was carried out mainly to analyse the structural and compositional transformation of mesocarp fibre treated in water at different temperature of subcritical state. The OPMF samples used in this research was collected from Gomali Mill, Gemas before and after press digester. Moisture content of sample was determined by dry the samples in oven at 80°C. The samples were viewed under a light microscope and a Scanning Electronic Microscope (SEM) before and after the samples were treated by Sub-CW to study the breakdown and structural changes of OPMF's cellulose at different temperatures. Reaction temperatures were between 200°C and 300°C to obtain the

optimum temperature to produce desirable product from OPMF. Compositional changes of OPMF were identified using HPLC, UV Spectroscopy and IR spectroscopy.

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APPENDICES

Appendix 1: Raw data for moisture content based on dry basis and wet basis

Equation (1) used to obtain moisture content of average sample for 1 hour duration based on dry basis:

$$\text{MC (Dry Basis)} = \frac{\text{initial weight sample} - \text{final weight sample}}{\text{initial weight sample}} \text{ X } 100 \qquad (1)$$

$$= \frac{1.0489\text{g} - 0.7971\text{g}}{1.0489\text{g}} \text{ X } 100$$

$$= 23.7\ \%$$

Table A.1: Weight and MC of wet sample over series of drying hours

		Sample 1 (g)	Sample 2 (g)	Sample 3 (g)	Average (g)	Dry Basis (%)
Dish Weight		0.5495	0.602	0.5055	0.5523	
Sample Weight		0.4994	0.4991	0.5168	0.5051	
Total Weight		1.0489	1.1011	1.0223	1.0574	
Duration (hr)	1	0.7971	0.8489	0.7737	0.8066	23.7%
	2	0.7925	0.8400	0.7699	0.8008	24.3%
	3	0.7876	0.8335	0.7693	0.7968	24.6%
	4	0.7853	0.8330	0.7688	0.7957	24.8%
	5	0.7845	0.8327	0.7683	0.7952	24.8%
	24	0.7843	0.8327	0.7680	0.7950	24.8%

Appendix 2: Raw Data of dry sample weight used in different reaction temperature in Sub-CW reaction (wet basis and dry basis)

Equation (6) was used to obtain the dry weight of sample used in temperature 200°C in 5 minutes' reaction time:

$$\text{Weight of dry sample} = 1 - \frac{MC}{100} \text{ X weight of wet sample} \quad (6)$$

$$= (1 - \frac{24.8}{100}) \text{X } 0.5072 \text{ g dry sample}$$

$$= 0.3814 \text{ g dry sample}$$

Table A.2: Dry weight of sample based on dry basis MC

Temperature (°C)	Sample Weight (g)	Dry Basis (g)
200	0.5072	0.3814
210	0.5066	0.3810
220	0.5016	0.3772
230	0.5041	0.3791
240	0.5122	0.3852
250	0.5001	0.3761
260	0.5140	0.3865
300	0.5089	0.3827

Appendix 3: Raw data of yield of oil obtained from different reaction temperature in Sub-CW reaction

Equation (2) was used to obtain yield of oil at reaction temperature 200°C in 5 minutes' reaction time:

$$\text{Oil phase yield (g oil/g dry OPMF)} = \frac{\text{dry oil phase weight}}{\text{charged dry sample weight based on wet basis}} \quad (2)$$

$$= \frac{0.0080\ (\text{g})}{0.3814\ (\text{g dry sample})}$$

= 0.0210 g/g dry sample

Table A.3: Total Oil Collected at Different Sub-CW Temperature Reaction

Temperature (°C)	Vial Weight (g)	Vial + Oil Weight (g)	Total Oil Collected (g)
200	15.1350	15.1430	0.0080
210	19.2132	19.2276	0.0144
220	15.0456	15.0644	0.0188
230	19.2731	19.2972	0.0241
240	19.0460	19.0814	0.0354
250	14.9743	14.9903	0.0160
260	19.4392	19.4480	0.0088
300	19.0963	19.0971	0.0008

Table A.4: Yield of Oil at Different Sub-CW Temperature Reaction

Temperature (°C)	Yield of Oil (g/g-dry sample)	Yield of Oil (mg/g-dry sample)
200	0.0210	20.97
210	0.0378	37.80
220	0.0498	49.84
230	0.0636	63.57
240	0.0919	91.91
250	0.0425	42.54
260	0.0228	22.77
300	0.0021	2.09

Appendix 4: Raw data of yield of sugar obtained from different reaction temperature in Sub-CW reaction

Table A.5: Standard Curve of Total Sugar

Glucose (ppm)	Absorbance		Average
	A1	A2	
0	0.2210	0.2500	0.2355
10	0.3110	0.3530	0.3320
25	0.4730	0.5520	0.5125
50	0.7226	0.7310	0.7268
100	1.2776	1.3200	1.2988

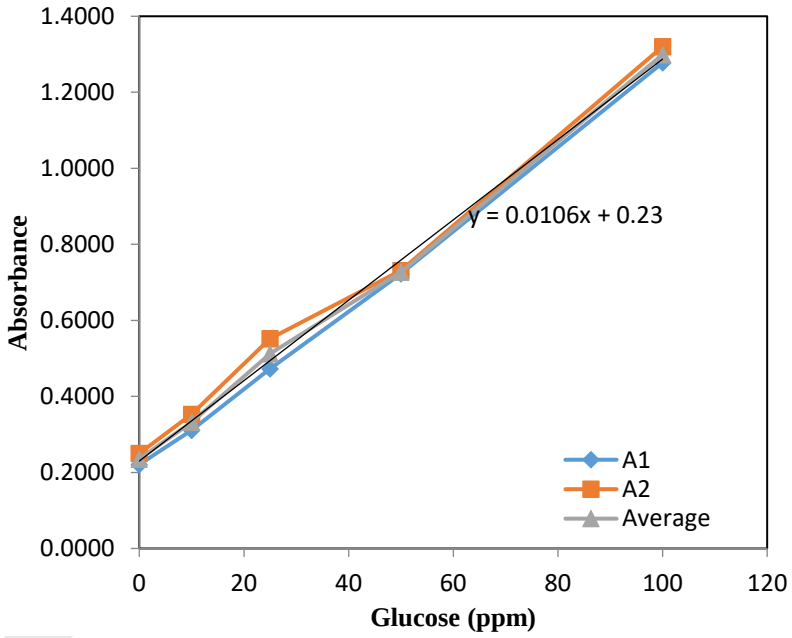


Figure A.1: Graph of Sugar Substance Standard Curve

Table A.6: Total Sugar obtained at Different Sub-CW Temperature Reaction

Temperature (°C)	Absorbance Reading	Concentration of Sugar (g/ml)	Total Sugar (g)
200	0.324	8.868	0.0022
210	0.358	12.075	0.0030
220	0.422	18.113	0.0045
230	0.474	23.019	0.0058
240	0.592	34.151	0.0085
250	0.516	26.981	0.0067
260	0.432	19.057	0.0048
300	0.271	3.868	0.0010

Absorbance value and concentration of sugar of reacted sample at different reaction temperature identified using standard curve of total sugar.

Equation (3) was used to obtain yield of total sugar at reaction temperature 200°C in 5 minutes' reaction time:

$$\text{Total sugar yield (g/g-dry sample)} = \frac{\text{weight of total sugar (g)}}{\text{weight of charged sample(g-dry sample)}} \quad (3)$$

$$= \frac{0.002\ \text{g}}{0.3814\ (\text{g-dry sample})}$$

$$= 0.0065\ \text{g/g-dry sample}$$

Table A.7: Yield of Total Sugar at Different Sub-CW Temperature Reaction

Temperature (°C)	Yield of Sugar (g/g-dry sample)	Yield of Total Sugar (mg/g-dry sample)
200	0.0058	5.831
210	0.0079	7.924
220	0.0120	12.005
230	0.0152	15.181
240	0.0222	22.166
250	0.0179	17.936
260	0.0123	12.326
300	0.0025	2.527

Appendix 5: Raw data of solid residue obtained from different reaction temperature in Sub-CW reaction

Table A.8: Total Solid Collected at Different Sub-CW Temperature Reaction

Temperature (°C)	Solid Collected from Centrifuge Tube (g)	Solid Collected from Filter Paper (g)	Total Solid Collected (g)
200	0.0371	0.2494	0.2865
210	0.0322	0.2150	0.2472
220	0.0321	0.2124	0.2445
230	0.0310	0.1512	0.1822
240	0.0244	0.1368	0.1612
250	0.0226	0.1106	0.1332
260	0.0212	0.1096	0.1308
300	0.0120	0.0246	0.0366

Equation (4) was used to obtain yield of oil at reaction temperature 200°C in 5 minutes' reaction time:

$$\text{Solid phase residue yield} = \frac{\text{weight of solid phase residue(g)}}{\text{weight of charged sample (g−dry sample)}} \quad (4)$$

$$= \frac{0.2865\ \text{g}}{0.3814\ \text{(g−dry sample)}}$$

$$= 0.7512\ \text{g/g-dry sample}$$

Table A.9: Yield of Solid Collected at Different Sub-CW Temperature Reaction

Temperature (°C)	Yield of Solid (g/g-dry sample)	Yield of Solid (mg/g-dry sample)
200	0.7512	751.1515
210	0.6489	648.8816
220	0.6482	648.1917
230	0.4806	480.6333
240	0.4185	418.5117
250	0.3542	354.1845
260	0.3384	338.3972
300	0.0956	95.6381

Appendix 6: Raw data of tar obtained from different reaction temperature in Sub-CW reaction

Equation (5) was used to obtain yield of tar at reaction temperature 200°C in 5 minutes' reaction time:

$$\text{Yield of Tar} = \frac{\text{weight of tar(g)}}{\text{weight of charged sample (g−dry sample)}} \quad (5)$$

$$= \frac{0.0021\ \text{g}}{0.3814\ \text{(g−dry sample)}}$$

$$= 0.0055\ \text{g/g-dry sample}$$

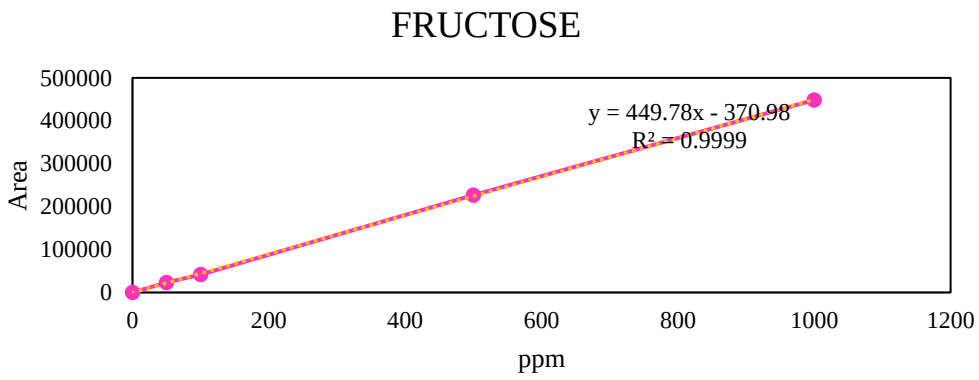
Table A.10: Yield of Tar Collected at Different Sub-CW Temperature Reaction

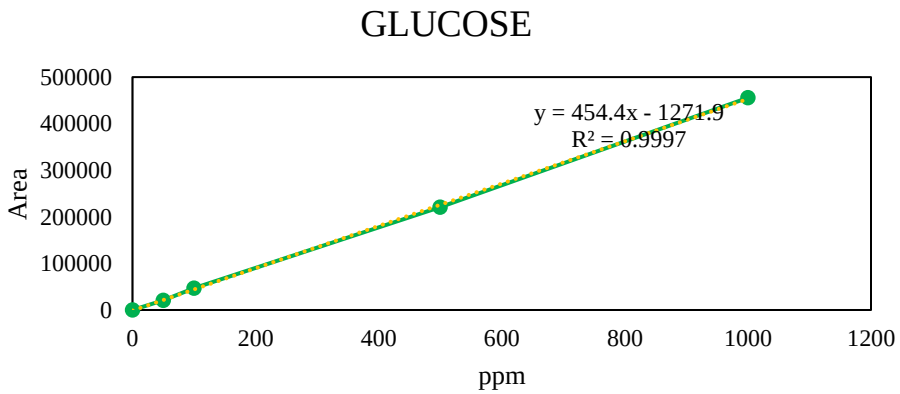
Temperature (°C)	Weight of tar (g)	Yield of Tar (g/g-dry sample)	Yield of Tar (mg/g-dry sample)
200	0.0021	0.0055	5.5058
210	0.0057	0.0150	14.9621
220	0.0069	0.0183	18.2925
230	0.0101	0.0266	26.6432
240	0.0231	0.0600	59.9728
250	0.0443	0.1178	117.7956
260	0.0632	0.1635	163.5069
300	0.0718	0.1876	187.6178

Appendix 7: Raw data of HPLC analysis on aqueous phase obtained from different reaction temperature in Sub-CW reaction

Table A.11: Sugar Component Standard Equations

Component	Calibration curve equation	R^2
Cellobiose	y = 442.26x - 81.288	R^2 = 0.999
Sucrose	y = 493.49x - 7066.9	R^2 = 0.9942
Glucose	y = 454.4x - 1271.9	R^2 = 0.9997
Fructose	y = 444.24x + 955.97	R^2 = 0.9998





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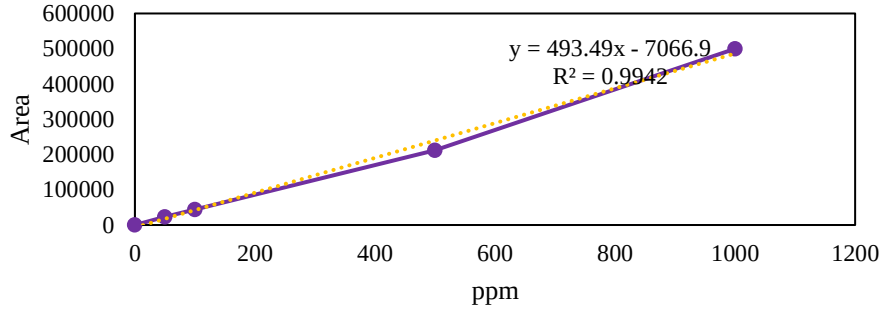

CELLOBIOSE

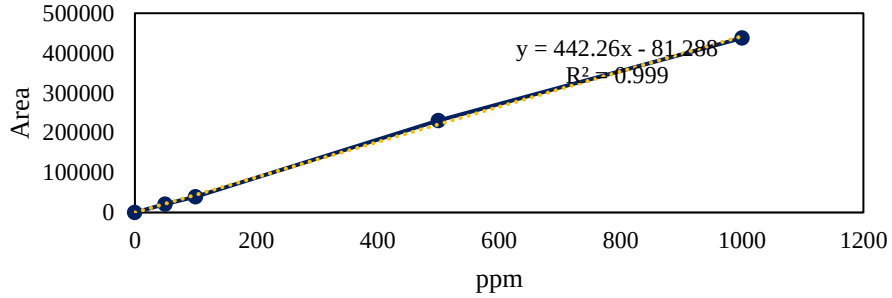

Table A.12: Summary of HPLC Analysis and Sugar Content (mg) Obtained at Different Sub-CW Temperature Reaction

Temperature (°C)	tR	Type of Sugar	Area	ppm	Sugar Content (mg/ml)	Sugar Content (mg)
200	17.267	Cellobiose	13511	30.733	76.834	1.536
	19.258	Glucose	33343	81.886	204.714	4.094
220	17.942	Cellobiose	21191	48.099	120.247	2.405
	19.075	Glucose	44461	104.415	261.038	5.220
240	17.442	Cellobiose	4151	9.569	23.924	0.478
	18.492	Glucose	34836	84.911	212.278	4.245
	22.017	Galactose	3100	9.621	24.053	0.481
	22.875	Mannose	30700	69.080	172.701	3.454
260	17.033	Cellobiose	326	0.920	2.302	0.046
	18.392	Glucose	28190	71.444	178.61	3.572
	23.183	Mannose	25410	57.319	143.297	2.866
	22.100	Galactose	1006	5.013	12.532	0.250

Appendix 8: Raw Data of Sub-CW Reaction Product and Ion Production

Table A.13: Ion Production and Yield of Total Sugar at Different Sub-CW Temperature Reaction

Temperature (°C)	Ion Product Constant in base 10 log (mol/kg)2	Yield of Total Sugar (mg/g-dry sample)
200	-11.16	5.81
250	-11.01	22.17
300	-11.14	2.53

Appendix 9: Raw Data of Sub-CW Reaction Product and Ion Production

Table A.14: Dielectric Constant (ε) and Yield of Tar at Different Sub-CW Temperature Reaction

Temperature (°C)	Dielectric Const. (ε)	Yield of Tar (mg/g-dry sample)
200	34.742	5.506
210	33.091	14.962
220	31.496	18.293
230	29.952	26.643
240	28.455	59.973
250	26.999	117.796
260	25.579	163.507
300	20.135	187.618

BIODATA OF STUDENT

Sanggithapriya Mahandran was born on 19th October 1994 in Negeri Sembilan. Her primary school at Sekolah Jenis Kebangsaan (tamil) Ladang Senama. She proceeds her secondary school at Sekolah Menengah Kebangsaan Batu Kikir (Model Khas). Then, she continued her study in matriculation level at Perak Matriculation College in 2012. She furthered her degree with Bachelor of Chemical Engineering at Universiti Putra Malaysia in 2013. She graduated her degree in November 2017 and enrolled for the master study in September 2018. She struggles to finish her master study in December 2020 and looking forward any opportunity to further for PhD study.

LIST OF PUBLICATIONS

Mahandran, S., Sabli, N., Ismail, M. H. S., Yoshida, H., & Izhar, S. (2020). Structural transformation of oil palm mesocarp fibre (OPMF) in water at subcritical state. Journal of Oil Palm Research, 32(4), 590-598.

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