# PRODUCTION OF XYLOSE FROM OIL PALM TRUNK (OPT)

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## SUPERVISOR'S DECLARATION

I hereby declare that I have checked this project and in my opinion, this project is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical & Natural Resources Engineering.

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## STUDENT'S DECLARATION

I hereby declare that the work in this project is my own except for quotations and summaries which have been duly acknowledged. The project has not been accepted for any degree and is not concurrently submitted for award of other degree.

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ID Number : KA09118

Date :

*Special dedication to my supervisors, my family members,*

*my friends, my fellow colleagues and all faculty members*

*for all your care, support and believe in me*

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**PRODUCTION OF XYLOSE FROM OIL PALM TRUNK**

## ABSTRACT

Oil palm trunk is an oil palm biomass which is the renewable source for the production of many useful products, such as xylose. The study provides efficient analysis on optimizing xylose concentration, in order to obtain higher productivity and yield of xylitol. Xylose is safe, healthy, toxin free substance that can allow people to enjoy the flavor of sugar without negative effects on the body. The objectives of this study are to produce valuable products (xylose and xylitol) from waste oil palm trunk, to identify the effects of sulfuric acid concentration and hydrolysis time on the production of xylose from oil palm trunk. The hydrolysis of oil palm trunk powder was carried out by contacting powder with 3%, 5% and 7% sulfuric acid at 60 °C for 60, 120 and 180 minutes. The acid hydrolysis is using sulfuric acid wood mass ratio of 8: 1 g/g. After hydrolysis reaction, solid part was removed by filtration and the filtrate was neutralized with $CaCO_3$ and then undergoes detoxification process by using activated carbon powdered with the mass ratio 10:1 of hydrolysate/charcoal powder. The percentage of xylose will be analyzed from the hydrolysate by using High Performance Liquid Chromatography machine. From the literature, the results obtained was the higher acid concentrations led to higher sugar recovery but longer hydrolysis's time caused lower amount in sugar recovery. For a conclusion, the high value products which are xylose can be produce from waste oil palm biomass.

# PENGHASILAN XYLOSE DARI BATANG KELAPA SAWIT

## ABSTRAK

Batang kelapa adalah biomass kelapa sawit merupakan sumber boleh diperbaharui bagi pengeluaran banyak produk yang berguna, seperti xylose. Kajian ini menyediakan analisis yang berkesan dalam mengoptimumkan kepekatan xylose, untuk mendapatkan produktiviti yang lebih tinggi dan hasil Xylitol. Xylose adalah selamat, menyihatkan, bahan bebas toksin yang membenarkan orang ramai untuk menikmati rasa gula tanpa kesan negatif pada badan. Objektif kajian ini adalah untuk menghasilkan produk berharga (xylose dan Xylitol) dari sisa batang kelapa sawit, untuk mengenal pasti kesan-kesan kepekatan asid sulfurik dan masa hidrolisis pada pengeluaran xylose daripada batang kelapa sawit. Hidrolisis serbuk batang kelapa sawit telah dijalankan dengan merendamkan serbuk dengan 3%, 5% dan 7% asid sulfurik pada suhu 60 ° C selama 60, 120 dan 180 minit. Hidrolisis asid menggunakan asid sulfurik kayu nisbah jisim 8: 1 g / g. Selepas tindak balas hidrolisis, sebahagian pepejal telah ditapis dan turasan telah dineutralkan dengan CaCO3dan seterusnya menjalani proses detoksifikasi dengan menggunakan serbuk arang dengan nisbah jisim 10:1 hidrolisat/arang. Peratusan xylose akan dianalisis dari Hidrolisat dengan menggunakan Kromatografi Cecair Prestasi Tinggi mesin. Daripada literatur, keputusan yang diperolehi adalah kepekatan asid yang tinggi membawa kepada penghasilan gula yang lebih tinggi tetapi masa hidrolisis lagi ini disebabkan jumlah yang lebih rendah dalam penghasilan gula. Kesimpulannya, produk bernilai tinggi seperti xylose boleh dihasilkan daripada sisa biomass kelapa sawit.

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## LIST OF SYMBOLS

| | |
|---|---|
| *%* | Percent |
| *g* | Gramme |
| $g^{-1}$ | Recipcrocal gramme |
| *°C* | Degree celcius |
| *ml* | milliliter |
| *nm* | nanometer |

## LIST OF ABBREVIATIONS

| | |
|---|---|
| HPLC | High Performance Liquid Chromatography |
| FTIR | Fourier Transform Infrared Spectroscopy |
| OPT | Oil Palm Trunk |
| EFB | Empty Fruit Bunch |
| OPF | Oil Palm Frond |
| Wt | Weight |
| IR | Infrared |
| UV | Ultraviolet |
| pH | Potential hydrogen |
| RPM | Rotation per minutes |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of Proposed Study

Malaysia produced an estimated 80 million tonnes/year dry weight of oil palm biomass, reflecting the importance and significant potential of biomass. This biomass includes empty fruit bunches, mesocarp fibres, oil palm shells, oil palm fronds and oil palm trunks. Much of the 70 million tonnes/year of oil palm trunk in 4.7million acre plantation produced is burnt or left to rot. Usually the trunks generated from replanting which causes some environment problems. There are reports on application of the oil palm trunk especially for veneer and plywood production being reported (Hamid *et al.*, 2008). The content of α-cellulose in this waste of oil palm trunk is slightly lower (41%) than wood (45–50%). However, it can play an important complementary role given the possibility of obtaining cellulosic pulp with an α-cellulose content, and therefore has possible applications in producing paper or pasteboard with different characteristics, especially in areas with

limited forest resources. Some attempts to characterize the palm trunk fibre as a raw material for paper making have been reported. Khoo et al.,reported that oil palm trunk fibre gave sulphate pulp with moderate yield and strength. The unbleached pulps would be suitable for medium grade wrapping paper.

A part from that, controlled hydrolysis of cellulose and hemicellulose from oil palm wood allows producing several useful chemical products such as xylose furfural, glucose and starch enhancing its economic feasibility (Chin *et al.*, 2010). This study actually is to produce xylose and xylitol from oil palm trunk. Xylose is the main building block for hemicellulose, which comprises about 30% of plant matter. Xylose is otherwise pervasive, being found in the embryos of most edible plants. It was first isolated from wood by Koch in 1881.

Bioconversion of lignicellulose to ethanol or xylitol requires the hydrolysis of carbohydrate polymers to their corresponding monomeric sugars prior to fermentation. Lignocellulose hydrolysis has been achieved either by acid hydrolysis or enzymatic hydrolysis. However, acid hydrolysis is the most commonly applied and the method can be used either as a pretreatment preceding the enzymatic hydrolysis or as a method of hydrolyzing lignocellulose for the sugars. As a consequence, the amount of sugars recovered from the raw material is dependent on the reaction time, temperature and acid concentration. However, acid concentration is the most important parameter affecting sugar yield. Apart from that, the sugar obtained from hemicellulose, other by-products, such as furfural, are also produced during the hydrolysis process.

Xylose is a great alternative to white sugar and has none of the negative side effects of sugar. Xylose is a natural sugar that is found in some woody materials besides palm oil biomass like empty fruit bunch and oil palm trunk it also found in straw, pecan shells, cottonseed hulls and corncobs. It is extracted through a scientific process. Xylose is not metabolized by humans. It is completely absorbed and secreted from the kidneys. In animal medicine, xylose is used to test for malabsorption by administration in water to the patient after fasting. If xylose is detected in blood and/or urine within the next few hours, it has been absorbed by the intestines. Reduction of xylose by catalytic hydrogenation produces the non-cariogenic sugar substitute xylitol.

## 1.2 Problem Statement

Replanting will cause environmental problems. After approximately 25 years of its economic life span, oil palm trunks are cut down so as to allow replanting, the trunks simply being left on the plantation and not been used productively. During replanting, a very large amount of waste trunks are exhausted and cut into pieces and burned simply to prevent the breeding of harmful insects but will cause local environmental pollution.

On the other hand, the demand and attraction for alternatively material from renewable resource had incredibly increased tremendously. Basically, the oil palm trunks contain lignin, holocellulose and quite significant amount of starch. This research is going to solve the problem of getting the best process for the production of cellulose, hemicelluloses and lignin from oil palm trunk. The production of xylose from oil palm trunk is an alternative to white sugar and as sugar-free sweetener. The high value economic conversion of oil palm wood to chemical products is the production of sugars-free sweetener.

## 1.3 Objectives

The purposes of this research are:

i. To produce a valuable products (xylose) from waste oil palm trunk
ii. To study the effect of sulfuric acid ($H_2SO_4$) concentration of the acid hydrolysis process.
iii. To study the hydrolysis time on the production of xylose from oil palm trunk (OPT).

## 1.4 Scopes of Research Work

i. The reaction hydrolysis time is selected at 60 to 180 min.
ii. The concentration of sulfuric acid ($H_2SO_4$) is used in range 3 to 7%.
iii. Acid hydrolysis temperature is constant at 60°C.
iv. The percentage of hemicellulose and cellulose need to be measured in the oil palm trunk.
v. The percentage of xylose compound need to be detected in hemicellulose fraction.
vi. The raw material used is oil palm trunk (OPT) powder.
vii. The chemicals used are sulfuric acid ($H_2SO_4$), calcium carbonate ($CaCO_3$), activated charcoal and acetonitrile HPLC grade.
viii. The equipments used are oven, orbital shaker, high performance liquid chromatography (HPLC) and analytical balance.

## 1.5 Rationale & Significance of study

**i.** Oil palm trunk seems to be a bio resource which can be entirely converted into valuable chemicals and products. (waste to wealth)

**ii.** The increasing interest on use of oil palm trunk for bioconversion to chemicals is justifiable as these materials are low cost, renewable and widespread sources of sugar-free sweetener.

**iii.** Xylose is a safe, healthy, toxin free substance that can allow people to enjoy the flavor of sugar without negative effects on the body.

**iv.** Xylose causes the growth of positive elements in the intestines. Through this, more absorption of all nutrients can take place. More absorption of nutrients equals a better overall immune system.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Oil Palm Biomass

The success of palm oil industry in Malaysia is from the confluence of government and private sector strategies and policies. In spite of the huge production, the oil consists of only about 10% of the total biomass produced in the plantation. The remainder consists of huge amount of oil palm wastes such as oil palm trunk and oil palm fronds from the field during replanting, palm shells, mesocarp fibers and empty fruit bunch which is from the mills.

Oil palm tree normally passed their economic age, on an average after 25 years, and due to replanting, the bole length of felled palm trunk is in the range 7m to 13m, with a diameter of 45cm to 65cm, measured at breast height. About 53.87% (dry weight) of fiber bundles can be extracted from a trunk, with the remaining parts are the bark and parenchyma tissues which contribute to 14.45% and 31.68% of the dry weight of the trunk respectively.

Oil palm biomass contains quite significant amount of organic nutrient, which contributes to its fertilizer values. Basically, the oil palm biomass contains about 18 – 21% of lignin, and 65-80% of holocellulose (a-cellulose and hemicellulose), which are more or less comparable with that of other wood or lignocellulosic materials shown in TABLE 1. This makes the oil palm biomass is also suitable as a raw material for the production of pulp and paper, composites, carbon products and chemicals extraction. Oil Palm Trunk (OPT) is a bioresource because it can entirely converted into valuable chemicals and products. The most economic conversion of OPT to chemical product is production of sugars (Rahman*et al*., 2006; Kasugi*et al*., 2010). According to Chin *et al.*, 2010, controlled hydrolysis of cellulose and hemicellulose from OPT allows producing several useful chemical products such as xylose furfural, glucose and starch.

Table2.1: Proximate analysis of biomass of oil palm biomass (%, dry weight)

| | Oil palm trunk | Oil palm fronds | Empty fruit bunch |
|---|---|---|---|
| lignin | 18.1 | 18.3 | 21.2 |
| Hemicellulose | 25.3 | 33.9 | 24.0 |
| a-cellulose | 45.9 | 46.6 | 41.0 |
| Holocellulose | 76.3 | 80.5 | 65.5 |
| Ash | 1.1 | 2.5 | 3.5 |
| Alcohol-benzene solubility | 1.8 | 5.0 | 4.1 |

## 2.2 Natural Occurrence of Xylose

Xylose is a sugar isolated from wood and it is contains five carbon atoms and includes an aldehyde functional group and that is why it is classified as a monosaccharide of aldopentose type. A part from that, it is precursor to hemicellulose, one of the main constituents of biomass. Like most sugars, it can adopt several structures depending on conditions. With its free carbonyl group, it is a reducing sugar. According to Silva et al., xylose is the major component of the hemicellulosic fraction from vegetable biomass, is a pentose sugar that can be converted by biotechnological methods into several chemical products of high value. Same with Abd Rahman et al., 2004, approximately 24% of xylan which is a polymer made of pentose sugar xylose is recovered from the lignocellulosic waste from oil palm empty fruit bunch fiber. The conversion of xylan polymer into monomeric sugar xylose is by reduction of xylan with the presence of catalyst by hydrolyzing acid such as sulfuric acid, hydrochloric acid or hydrofluoric acid.

The natural occurrence of xylose, it is the main building block for hemicellulose, which comprises about 30% of plant matter. Xylose is otherwise pervasive, being found in the embryos of most edible plants. It was first isolated from wood by Koch in 1881. Xylose is also the first saccharide added to the serine or threonine in the proteoglycan type O-glycosylation, and, so, it is the first saccharide in biosynthetic pathways of most anionic polysaccharides such as heparan sulfate and chondroitin sulfate. Dominguez et al., 1997 ; Silva et al., 1998 ; Roberto et al., 2008 said that, the xylose recovered from lignocellulosic waste is by using the acid hydrolysis process. While, according to Kumoro et al., 2008 the lignocellulosic hydrolysis has been achieved by enzymatic hydrolysis. The most commonly method applied is acid hydrolysis and the method can be used either as a pretreatment preceding the enzymatic hydrolysis or as a method of hydrolysing lignocellulose for the sugars. As a consequence, the amount of sugars recovered from the raw material is dependent on the reaction time, temperature and acid concentration (Taherzadeh and Karimi, 2007). However, Roberto et al., 2003 mentioned that the acid concentration is the most important parameter affecting sugar yield.

Xylose is the carbon source for the growth of microorganism for the production of xylitol according to Mohamad et al., 2011. Similarly with Humairah et al., 2004, said the starting material for xylitol production is xylose. During hydrolysis process other sugars such as arabinose, galactose, glucose and some unwanted components such as acetic acid and furfural are also released. For optimum production production of xylitol it is necessary that byproducts like acetic acid and

furfural concentrations in the hydrolysate are to be kept minimum for efficient bioconversion of xylose to xylitol.

Xylose is safe for use in foods. It is antibacterial and antifungal and contains natural healing agents. For this reason, it's become popular in many arenas. Xylose is a great alternative to white sugar and has none of the negative side effects of sugar. Xylose is a natural sugar that is found in some woody materials such as straw, pecan shells, cottonseed hulls, and corncobs. It is also found in berries, spinach, broccoli, and pears. It is extracted through a scientific process. In addition to this, your body actually produces a small amount of Xylose on its own.

## 2.3 Preparation for Samples and Extraction Method for Xylose

According to Wong *et al*. (2011), the 20-year old palm trunks were debarked chopped into chips size (20x20x20 $mm^3$) and grinded into powder which was sieve to obtained size of 20, 40, and 60 mesh and oven-dried at 103°C to 5% moisture content prior to futher work-up. Chemical composition of oil palm wood was determined by using TAPPI standards which are T222-74 for lignin TS os-73 for extractives, and T203 os-61 for cellulose while for hollocellulose was determined using procedure proposed by Wise and Addieco (1946). Wong *et al.* (2011), have used steeping method in order to extract the starch and sugars. They are using two steeping methods with or without 0.5% lactic acid for extracting starch. In the first method, 3.0 g of powdered palm wood which are 20, 40, and 60 mesh size were soaked with 0.2% sodium metabisulphite ($Na_2S_2O_5$) solution at room temperature

26±2°C for 24, 36, 48 or 60 h. Whereas, for the second method, powdered palm were soaked into 0.2% sodium metabisulphite ($Na_2S_2O_5$) and 0.5% of lactic acid solutions in ratio 1:5 w/w. Then, the samples were steeped at room temperature 26±2°C for 24, 36, 48 or 60 h. After that, the steeping medium was then filtered off and the residue was washed with 150mL of distilled water. At last, starch content in the filtrate was determined by VIS spectrophotometry by using iodine solution as indicator at wavelength of 650nm (Humphreys and Kelly, 1961).

Astimar*et al.* (2002) stated that, for the samples preparation, the samples was washed with water at first and dried in an oven at 50°C - 60°C until the weight is constant before grinding in a shredder and sifting into fractions of <0.3mm, 0.3 – 0.4mm, and 0.4mm. They have prepared three types of particles, the first is untreated samples which the samples were used as ground and sieve. The second is holocellulose, this was the residual comprising only hemicellulose and cellulose after the lignin was removed by bleaching using Wise and Daddieco (1946). After that, the third sample is an alkali-treated samples which were prepared from all different-siezed of samples. One portion in grams of samples was mixed with 20 portions in mL of 1% (w/v) NaOH and heated in a boiling water bath for 2hr. Then, the treated samples was then sieved out and pressed. The samples were oven dried at 50°C - 60°C until its achieved constant weight.

A part from that, similarly with Wong *et al.* (2011), Yoichi tomimura (1992) also used slightly the same technique for extracting the starch and sugars form oil palm trunk. According to Yoichi tomimura (1992), the wood samples from 25-year-old palm trunks were debarked and cut into small chips which were then crashed in order to separate vascular bundles from parenchyma. Less than 200 mesh size of

wood meal was prepared for the determination of starch content. After that, for the preparation of milled wood lignin, the extractive free wood meal was milled for 100hours by using a vibratory ball mill. Then, the sample extracted and purified according to the standard method of Bjorkman (1956).Nitrobenzene for 0.24 mL and 2N potassium hydroxide for 4 mL were added to the lignin sample (10mg) in a stainless micro tube and the mixture was heated for 2 hours at 160°C with continuous vigorous shaking. After cooling, the solution was then extracted with ethyl ether to remove the residual nitrobenzene. The solution was then acidified with hydrocloric acid and extracted again with ethyl ether. Then, the extract was dried over anhydrous sodium sulfate and evaporated to dryness. The resultant phenols were converted to trimethylsilyl derivatives and analyzed by gas chromatograph using acetovanillon as an internal standard. GC was performed using Shimadzu GC-14 A with a capillary column HiCAP-CBPI.

## 2.4 Acid Hydrolysis of Xylose

Acid hydrolysis is used to recover the xylose from oil palm trunk. Wong *et al*. (2011) pointed that for acid hydrolysis process, powdered palm wood samples were treated with 2, 4, 6% sulphuric acid at the powder/acid weigth ratio of 1:5. The mixture was then hydrolysed in autoclave at temperature of 115°C for 30 or 60min. After that, the solution is diluted with distilled water and the insoluble solids are filtered by using filtration method. Before the xylose being brought for determination by using HPLC, the hydrolysate was then neutralized to pH 7. Using Varian PL-Hi Plex column and refractive index indicator for analyze the xylose content. The

mobile phase used is distilled water with flow rate of 0.4mL/min and oven temperature is 80°C. San *et al.,* 2008 stated that, the two steps procedure was described elsewhere. The first stage hydrolysis, the samples were treated with different acid concentrations of 60, 65, 70, 75% at two different reaction times of 60 or 120 min at 60°C. Then, for the second stage, 10 or 30% of sulfuric acid for 60 or 120 min at 80°C.

Referring to Najahpaur*et al.,* 2007, acid hydrolysis is a direct hydrolysis method used for biomass conversion and single stage acid hydrolysis is actually a single and simple reaction vessel. It is better to use this single method with diluted acid which less than 5% of acid concentration rather than use the concentrated acid. In order to achieve a maximum conversion with a short residence time, high temperature is needed.However, high temperature used will give contamination with the presence of soluble derivatives, furfural and hydroxyl-methyl furfural, which is generated in the presence of acids from sugars. In acid hydrolysis of cellulose, it was believed that the mechanisms were leading to a scission of glycosidic bonds which initially catalyzed by the action of proton ($H^+$) existing in the aqueous medium. Hydrolysis reaction is mostly effected by the acid concentration and temperature. In the kinetic modeling studies of acid hydrolysis, it has been observed that the rate of generation reaction of monomeric sugar from polysaccharides was influenced by temperature and acid concentration.

Yoichi Tomimura has used different method of hydrolysis which is alkaline hydrolysis compared to others authors which use acid hydrolysis. According to him, the sample was dissolved in 1N sodium hydroxide solution in 50 mL with stirring under $N_2$ and left for 24hours at room temperature. Then, the solution was acidified

with hydrochloric acid and the resultant precipitate was recovered by filtration and washed with hot water. The filtrate and washings were combined and extracted with ethyl ether. The ether fraction was then dried over anhydrous sodium sulfate and evaporated to dryness and the resultant phenolic acids were analyzed by gas chromatography-mass spectrometry (GC-MS) as TMS derivatives.

### 2.5 Analytical Methods Analyzing Xylose

Xylose and furfural usually was determined by using High Performance Liquid Chromatography (HPLC). According to Mohamed et al, xylose and furfural were analyzed by using High Performance Liquid Chromatography (HPLC) equipped with UV Visible and Refractive index detector (Shimadzu, Japan). Xylose was determined by Inertsil $NH_2$ column at 40°C. The mobile phase used was Deionized water and acetonitrile at ratio 25:75 at flow rate 0.5 ml/min. While, furfural was analyzed by Zorbax Eclipse XDB-C18 column at 60°C with the same flow rate and mobile phase for xylose detection. Concentration of xylose was determined by RI detector and furfural was identified by UV detector at 210 nm.

Similarly with Abd Rahman et al., High Performance Liquid Chromatography (HPLC) also used in order to determine the component of xylose in hyrdolysate. At given reaction times, neutralized hydrolysate, charcoal treated hydrolysate and samples from the fermentation media were taken and then filtered through 0.45 µm membranes and were analyzed using HPLC method for xylose,

glucose and xylitol component with LC-$NH_2$ column with oven temperature, 50°C, mobile phase is 75% acetonitrile and flow rate is 1.5 ml/min using RI detector.

According to Silva et al., xylose, xylitol and glucose was determined by high performance (HPLC) under the following condition which is using a column Bio-Rad Aminex HPX 87H (300 x 600 mm), H2SO4 as a mobile phase at a flow rate 0.6 ml/min and refractive index detector type 16X.

Analytical method for determine the xylose content can be concluded by using high performance liquid chromatography with different mobile phase, flow rate and detector use.

## 2.6 Application and Uses of Xylose

Xylose is a natural sugar that is found in some woody materials such as straw, pecan shells, cottonseed hulls and comcobs and also found berries, broccoli and pears. It is extracted through a scientific process. A part from that, the body of human also actually produces a small amount of xylose on its own. Xylose is safe for use in foods because it is an antibacterial and antifungal and contains natural healing agents.

Many physicians and health care professionals recommend xylitol as a substitute for sugar. Unlike cane sugar, xylitol does not cause tooth decay. In fact, xylitol can be found in many products that can fight tooth decay. According to Humairah et al., (2004), xylitol is a sugar that can be used as sugar substitute for

diabetic patients and also used to prevent tooth decay and against acute otitis of children (Bon et al., 1995). Therefore, it is safely to be used in tooth paste, mouth wash and sugar-free gum. According to studies, 75% of adults suffer from some kind of gum disease. Xylitol can actually improve some of the decay and aid in overall dental as it decreases the ability of bacteria to cling the teeth.

Xylitol is suitable for the diabetic patients as an alternative sugar. When white sugar is introduced into the body it raises the body's insulin levels. After years of this, the body can no longer handle the sugar and type 2 diabetes is often the result. In contrast, when Xylitol is introduced into the body it does not raise insulin levels as white sugar does. This makes it a great alternative for people with diabetes, or a likelihood of getting the disease. S.s. silva et al (1995) said that xylitol is also utilized as a sugar substitute in the case of diabetis or for people with problems of obesity.

# CHAPTER 3

# METHODOLOGY

## 3.1 Introduction

Xylose is a pentose sugar which is used in the production of xylitol and other sweetening additives for foods. It is prepared by the acid hydrolysis of ligno-cellulosic materials containing hemicelluloses having proportion of xylose units or xylans in their molecules. In addition to hemicellulosees, ligno-cellulosic materials contain lignin, cellulose and other carbohydrates. Furthermore, hemicellulose molecules contain in addition to xylans, units of other sugars.

Acid hydrolysis is the most commonly applied and the method can be used either as a pretreatment or as a hydrolysing lignocellulose for the sugars. As a consequence, the amount of sugars recovered from the raw material is dependent on the reaction time, temperature and acid concentration. For this study, the reaction time used for the hydrolysis is 60 to 180 minutes. The acid concentration for the

hydrolysis is 3%, 5% and 7% of $H_2SO_4$. While for the temperature is constant at 60°C.

A part from the sugar obtained from the hemicellulose, other by-products such as furfural are also prduced during the hydrolysis process. It is known that furfural can be reduced by contacting the hydrolysate from the hydrolysis process with activated carbon in ratio 1:10 g of cabon:hyrdolysate. Then, the solution was analysed for sugar (xylose) and determination by using High Performance Liquid Chromatography (HPLC).

## 3.2 Materials

1. Oil palm trunk (OPT) powder.

## 3.3 Chemical

1. Sulfuric acid, $H_2SO_4$
2. Activated carbon
3. Acetonitrile (HPLC grade)
4. Xylose, $C_5H_{10}O_5$ (standard solution)

## 3.4 Equipment

1. Analytical balance ( to weight the oil palm trunk powder and activated charcoal)
2. Orbital shaker ( to shake the solution of hydrolysate and charcoal)
3. HPLC ( to identify the amount of xylose and furfural)
4. Oven (to give heat for the sample)

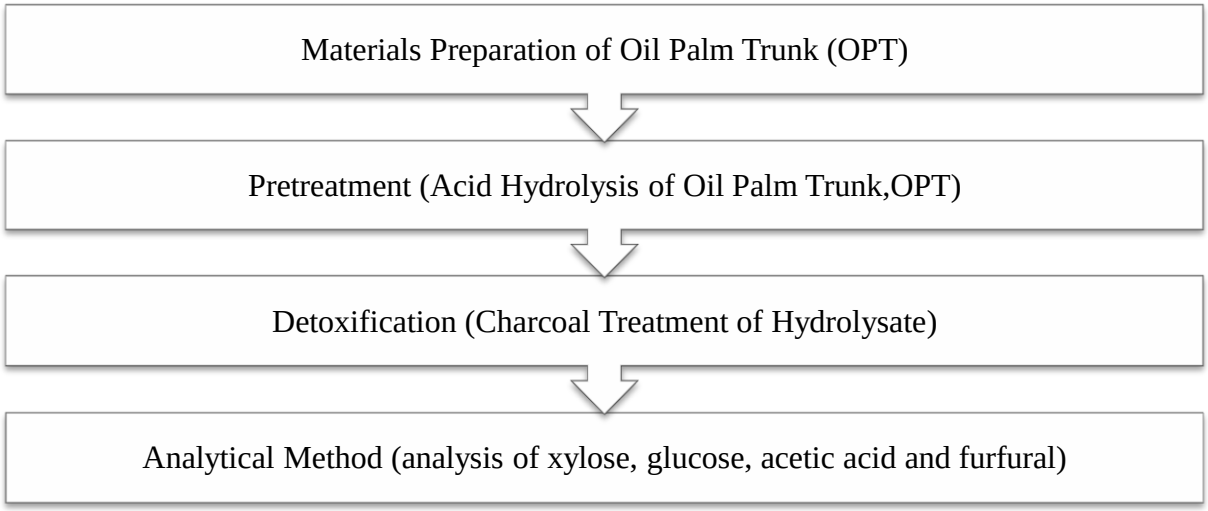
**Figure 3.1: Analytical Balance**

**Figure 3.2: Orbital shaker**

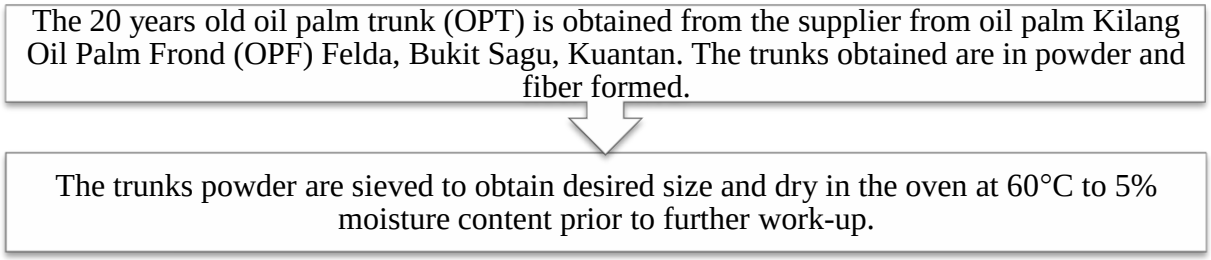
**Figure 3.3: HPLC**

**Figure 3.4: oven**

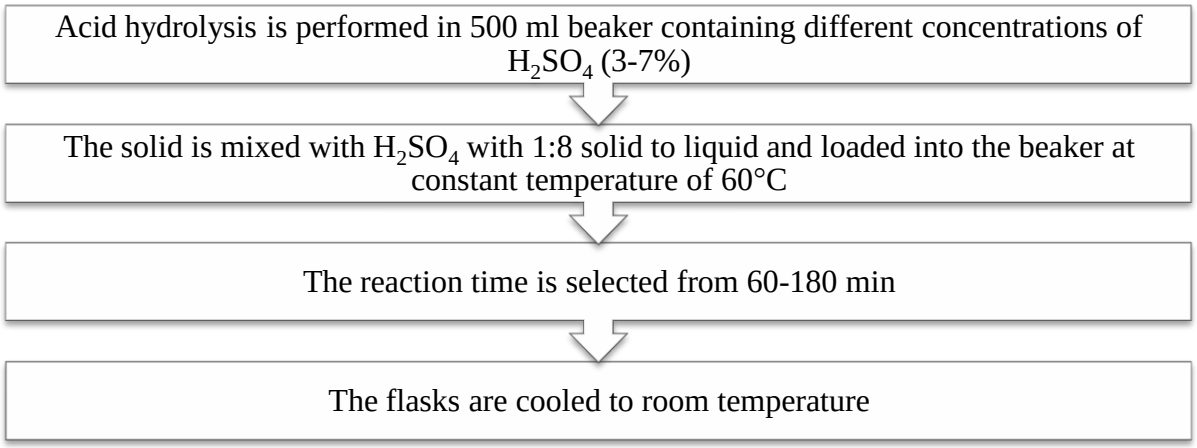
## 3.5 Experimental Procedure




### 3.51 Materials Preparation of Oil Palm Trunk (OPT)




### 3.5.2 Acid Hydrolysis of Oil Palm Trunk (OPT)



Then, the solid is removed from liquid solution using a vacuum pump with filter paper

The fitered solution which contained xylan is then neutralized with $CaCO_3$

Again, the neutralized solution is filtered to obtain the clear solution

The solution for sugar (xylose) and by-product (furfural) are analyzed

Hydrolysis Sample:

Figure 3.5: Acid Hydrolysis

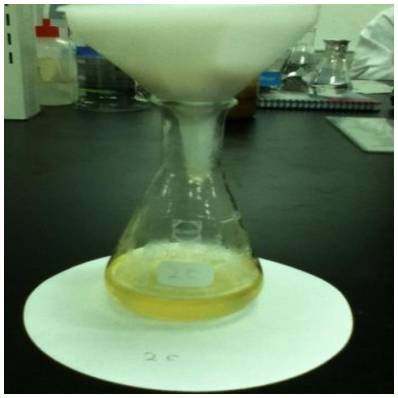


Figure 3.6: Acid Hydrolysis Filtration

Figure 3.7: Sample 1a

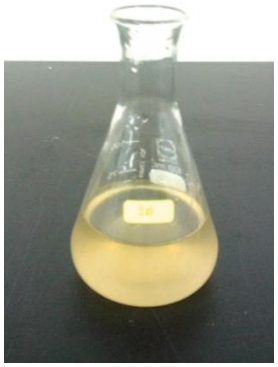


Figure 3.8: Sample 1b

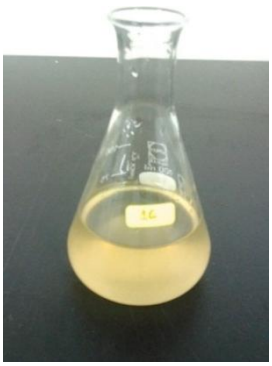


Figure 3.9: Sample 1c

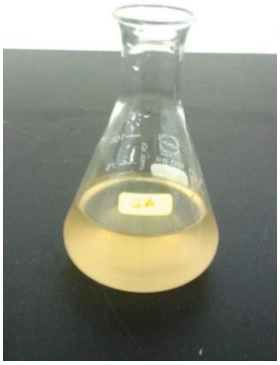


Figure 3.10: Sample 2a

Figure 3.11: Sample 2b

Figure 3.12: Sample 2c

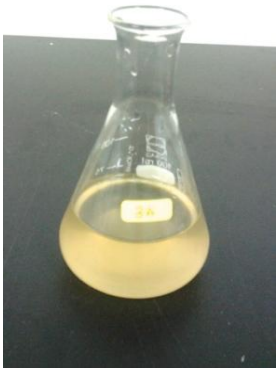


Figure 3.13: Sample 3a

Figure 3.14: Sample 3b

Figure 3.15: Sample 3c

Neutrasation Process:

Figure 3.16: Sample 1a

Figure 3.17: Sample 1b

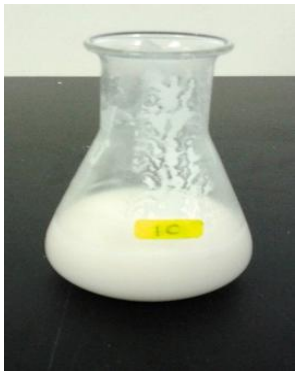


Figure 3.18: Sample 1c

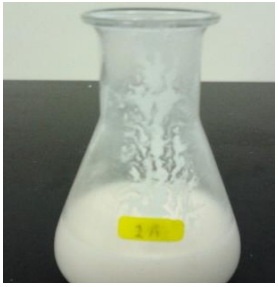


Figure 3.19: Sample 2a

Figure 3.20: Sample 2b

Figure 3.21: Sample 2c

Figure 3.22: Sample 3a

Figure 3.23: Sample 3b

Figure 3.24: Sample 3c

### 3.5.3 Charcoal Treatment of Hydrolysate

The hydrolysate is contacted with activated powdered charcoal using the mass ratio 10 g/g at room temperature

Then, it is stirred in an orbital shaker for two hour

The liquid phase is centrifuged with rotation speed 150 per minutes

The hydrolysate is filtered


Figure 3.25: Charcoal Treatment

Figure 3.26: Charcoal Treatment Filtration

### 3.5.4 Analytical Method

Analysis for sample is done by using high performance liquid chromatography (HPLC) .

At given reaction times, neutralized hydrolysate and charcoal treated hydrolysate was taken, centrifuged, filtered through 0.45 ìn membranes.

All samples was analyzed using HPLC method for xylose component with LC-$NH_2$ column (oventemperature: 50°C, mobile phase: 75 % acetonitrile, flow rate: 1.5mL/min) using UV detector with the wavelength 210nm, 254nm and 280nm.

The corresponding biomass concentrations were calculated from calibration against dry weight determinations in fermentation media made from standard xylose solutions.

Figure 3.27: Automatic HPLC

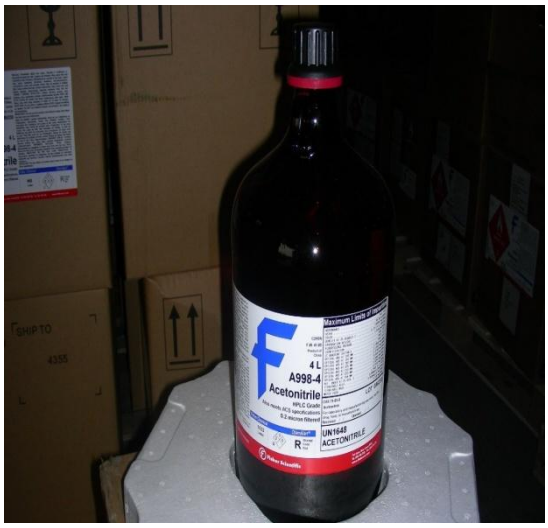



Figure 3.28: Acetonitrile (Mobile Phase)

# CHAPTER 4

# RESULT AND DISCUSSION

## 4.1 Introduction

The experimental works analysis the results of the concentration of xylose recovery from the acid hydrolysis treatment to oil palm trunk. The parameters involved are the concentrations of sulphuric acid ($H_2SO_4$) and hydrolysis times. The analysis was done by using high performance liquid chromatography (HPLC).

## 4.2 pH value every sample before and after neutralization process with $CaCO_3$

After the acid hydrolysis treatment, the hydrolysate is neutralized with $CaCO_3$. The pH values before and after neutralization process are shown in Table 4.1.

Table 4.1: pH value for every sample before and after neutralization process with $CaCO_3$

| Sample | $H_2SO_4$ concentration (%) | Time (min) | Temperature (°C) | pH before | pH after |
|---|---|---|---|---|---|
| 1A | 3 | 60 | 60 | 1.18 | 7.34 |
| 1B | 3 | 120 | 60 | 2.02 | 7.20 |
| 1C | 3 | 180 | 60 | 2.00 | 7.40 |
| 2A | 5 | 60 | 60 | 1.00 | 6.70 |
| 2B | 5 | 120 | 60 | 1.70 | 6.70 |
| 2C | 5 | 180 | 60 | 1.00 | 6.70 |
| 3A | 7 | 60 | 60 | 3.57 | 6.70 |
| 3B | 7 | 120 | 60 | 2.57 | 6.90 |
| 3C | 7 | 180 | 60 | 2.00 | 7.30 |

### 4.2.1 Discussion

The table shows the pH values for samples are neutral within the range from 6.70 until 7.40.

## 4.3 Composition of oil palm trunk (OPT)

Table 4.2: Composition of oil palm trunk (OPT)

| Composition | % |
|---|---|
| Cellulose | 47.5 |
| Holocellulose | 78.5 |
| Hemicelluloses | 25.3 |
| a-cellulose | 45.9 |
| Lignin | 18.4 |

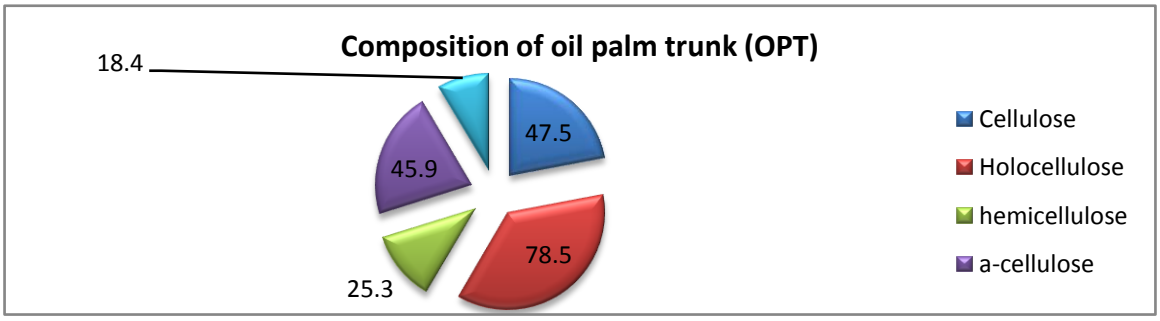



Figure 4.1: Composition of oil palm trunk (OPT)

### 4.3.1 Discussion

Figure 4.1 represents the oil palm trunk (OPT) contains quite significant amount of organic nutrient. Basically, the oil palm trunk contains about 18 – 21% of lignin, and 65-80% of holocellulose by dry weight according to Wong et al. (2011). A part from that, the chemical composition of wood subjected to experiments was as follows which have 18.4% lignin, 47.5% cellulose, 78.5% holocellulose, 25.3% hemicelluloses and 45.9% a-cellulose by dry weight % state by San et al. (2008).

## 4.4 Sugar compound detected by HPLC in the oil palm trunk (OPT) hydrolysate

Table 4.3: Sugar compound detected by HPLC in the oil palm trunk (OPT) hydrolysate

| Hemicelluloses | Sugar recoveries (%) |
|---|---|
| Xylose | 19.71 |
| Glucose | 76 |
| Arabinose | Not detected |
| Mannose | Not detected |
| Galactose | Not detected |

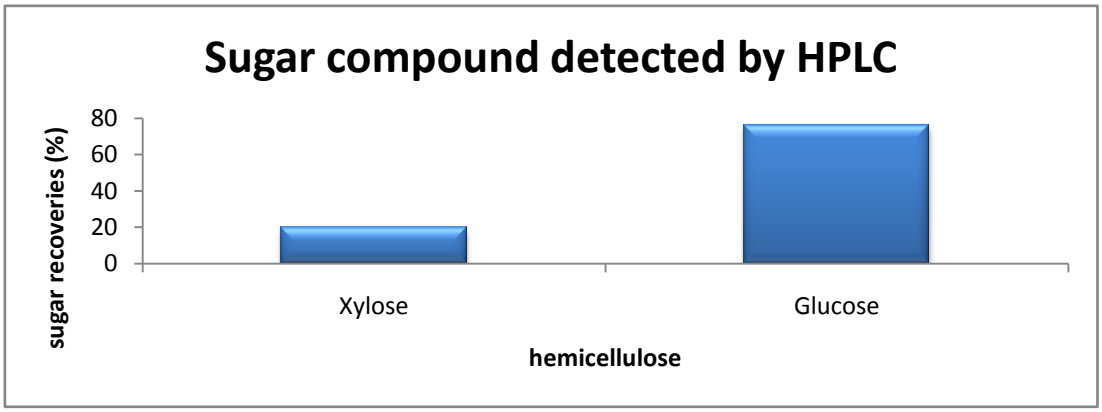



Figure 4.2: Sugar compound detected by HPLC in the oil palm trunk (OPT) hydrolysate

### 4.4.1 Discussion

Table 4.3 shows the results from the HPLC analysis of sugar content of the oil palm trunk hemicelluloses hydrolysate. The analysis of sugar content revealed that the oil palm trunk contained primarily xylose and glucose. Arabinose, galactose and mannose were not detected in this substrate. From 25.3% of hemicelluloses portion of the oil palm trunk, xylose yielded 19.71% of the total sugar. In another research, according to Rahman et al. (2006), 90.35% of xylose yield was successful obtained from 24.01% of hemicelluloses from oil palm empty fruit bunch. While, rice straw contained 25% of hemicelluloses and Roberto et al. (2003) was able to obtain the xylose yield of 88%. It can be conclude that different materials will give different compositions of hemicelluloses and its sugar compounds.

## 4.5 Standard xylose concentration based on peak area

Table 4.4: Standard xylose concentration based on peak area

| Peak area (mAU*s) | Xylose concentration (%) |
|---|---|
| 10 | 0.5 |
| 40 | 1.5 |
| 60 | 2.5 |
| 90 | 3.5 |
| 110 | 4.5 |
| 130 | 5.5 |
| 290 | 12 |

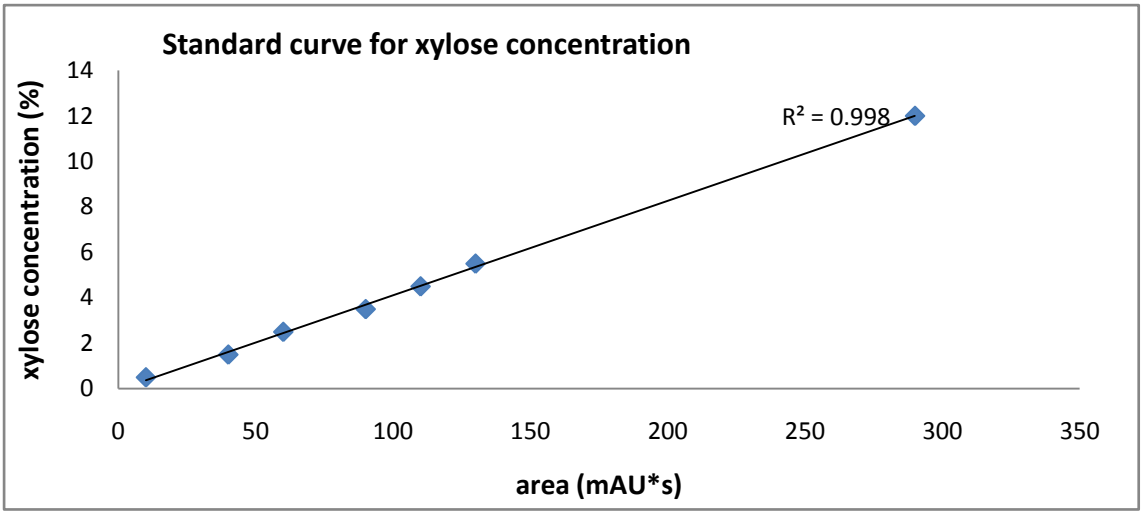



Figure 4.3: standard curve for xylose concentration

### 4.5.1 Discussion

Figure 4.3 shows the standard curve for xylose concentration based on the area of the chromatogram of HPLC. From this standard curve, the concentration of xylose recovery from the acid hydrolysis treatment for oil palm trunk (OPT) can be determined. The concentrations of xylose of each sample are determined by comparing the peak area from the area percent report of the HPLC analysis to the standard curve.

## 4.6 Xylose concentration recovery for acid hydrolysis of 3% $H_2SO_4$

Table 4.5: Xylose concentration recovery for acid hydrolysis of 3% $H_2SO_4$

| Sample | Hydrolysis time (min) | Peak area (mAU*s) | Xylose Concentration (%) |
|---|---|---|---|
| 1A | 60 | 15 | 0.5 |
| 1B | 120 | 44 | 1.5 |
| 1C | 180 | 37 | 1.8 |

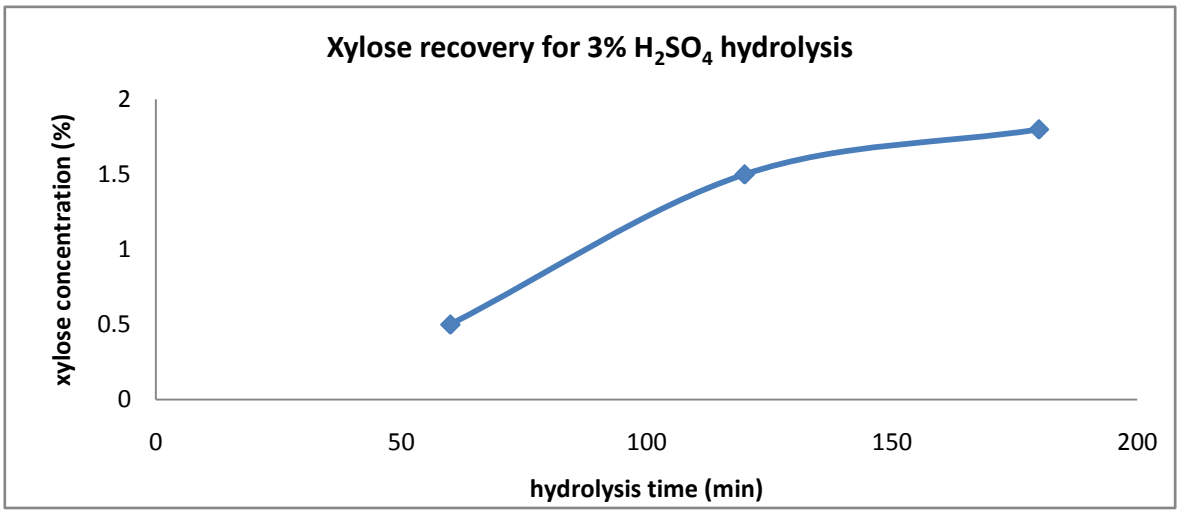



Figure 4.4: Xylose recovery for 3% $H_2SO_4$ hydrolysis

### 4.6.1 Discussion

Figure 4.4 presents the xylose recovery for acid concentration of 3%. For sample 1A which undergo 60 minutes hydrolysis times, the concentration of xylose is 0.5%. Then, for the sample 1B which undergoes 120 minutes of hydrolysis times, the concentration of xylose is 1.5% and the concentration of xylose recovery from the sample 1C is 1.8%. The graph shows the xylose recovery for 3% acid concentration is proportional to the hydrolysis time.

## 4.7 Xylose concentration recovery for acid hydrolysis of 5% $H_2SO_4$

Table 4.6: Xylose concentration recovery for acid hydrolysis of 5% $H_2SO_4$

| Sample | Hydrolysis time (min) | Peak area (mAU*s) | Xylose Concentration (%) |
|---|---|---|---|
| 2A | 60 | 20 | 0.8 |
| 2B | 120 | 52 | 2.2 |
| 2C | 180 | 41 | 2.0 |

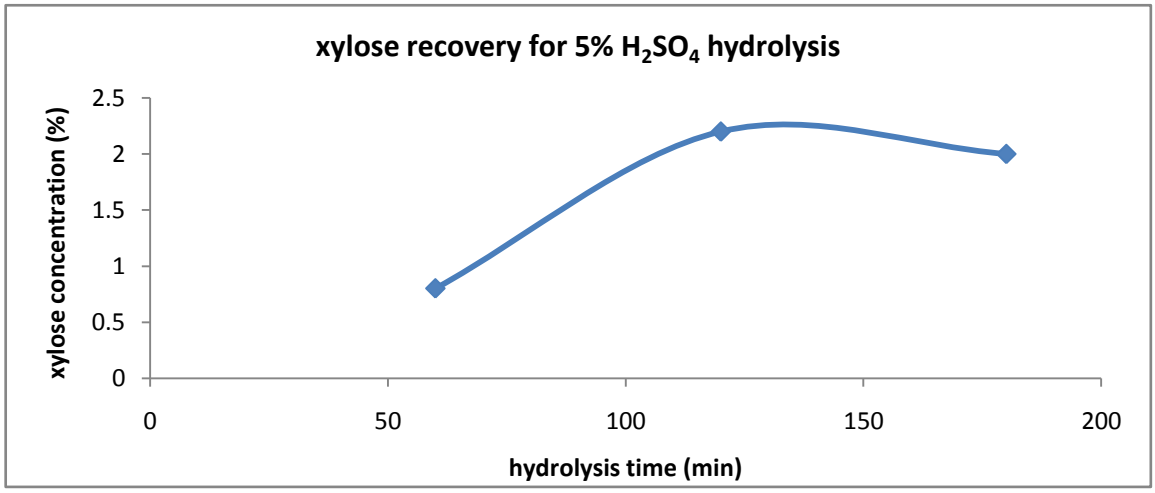



Figure 4.5: Xylose recovery for 5% $H_2SO_4$ hydrolysis

### 4.7.1 Discussion

Figure 4.5 shows the xylose recovery for acid concentration of 5%. For sample 2A which undergo 60 minutes hydrolysis times, the conentration of xylose is 0.8%. Then, for the sample 2B which undergoes 120 minutes of hydrolysis times, the concentration of xylose is 2.2% and the concentration of xylose recovery from the sample 2C is 2.0%. The graph shows the xylose recovery for 5% acid concentration is increase from 60 minutes to 120 minutes of hydrolysis time while decrease at 180 minutes of hydrolysis time.

## 4.8 Xylose concentration recovery for acid hydrolysis of 7% $H_2SO_4$

Table 4.7: Xylose concentration recovery for acid hydrolysis of 7% $H_2SO_4$

| Sample | Hydrolysis time (min) | Peak area (mAU*s) | Xylose Concentration (%) |
|---|---|---|---|
| 3A | 60 | 87 | 3.8 |
| 3B | 120 | 67 | 3.0 |
| 3C | 180 | 35 | 1.6 |

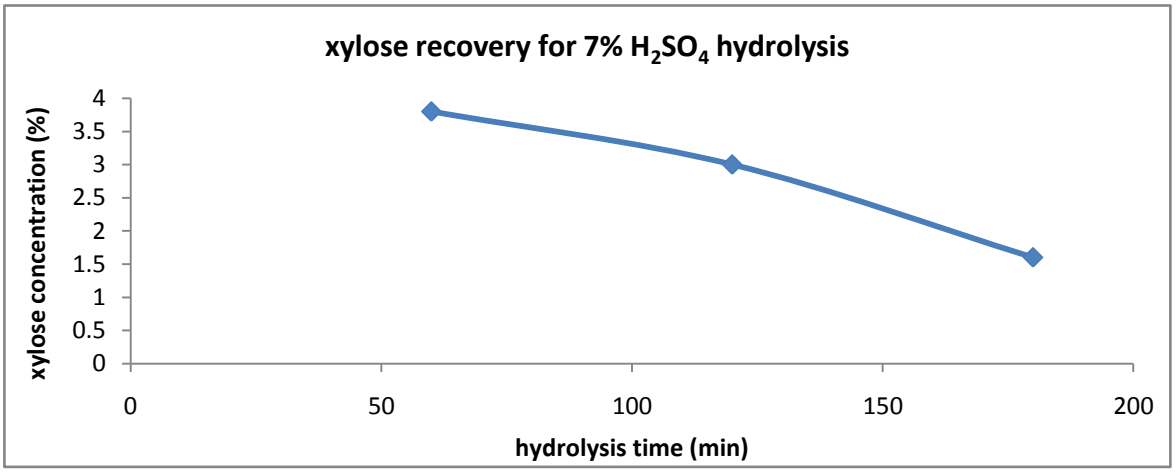



Figure 4.6: Xylose recovery for 7% $H_2SO_4$ hydrolysis

### 4.8.1 Discussion

Based on Figure 4.6, it shows the xylose recovery for acid concentration of 7%. For sample 3A which undergo 60 minutes hydrolysis times, the conentration of xylose is 3.8%. Then, for the sample 3B which undergoes 120 minutes of hydrolysis times, the concentration of xylose is 3.0% and the concentration of xylose recovery from the sample 2C is 1.6%. The graph shows the xylose recovery for 7% acid concentration is decrease with increasing the hydrolysis time.

## 4.9 The xylose recovery after the treatment with acid at different concentrations for different hydrolysis times

Table 4.8: The xylose recovery with the treatment of acid at different concentrations for different hydrolysis times

| Time(min) | Cancentration of $H_2SO_4$ (%) | | |
|---|---|---|---|
| | 3 | 5 | 7 |
| 60 | 0.5 | 0.8 | 3.8 |
| 120 | 1.5 | 2.2 | 3.0 |
| 180 | 1.8 | 2.0 | 1.6 |

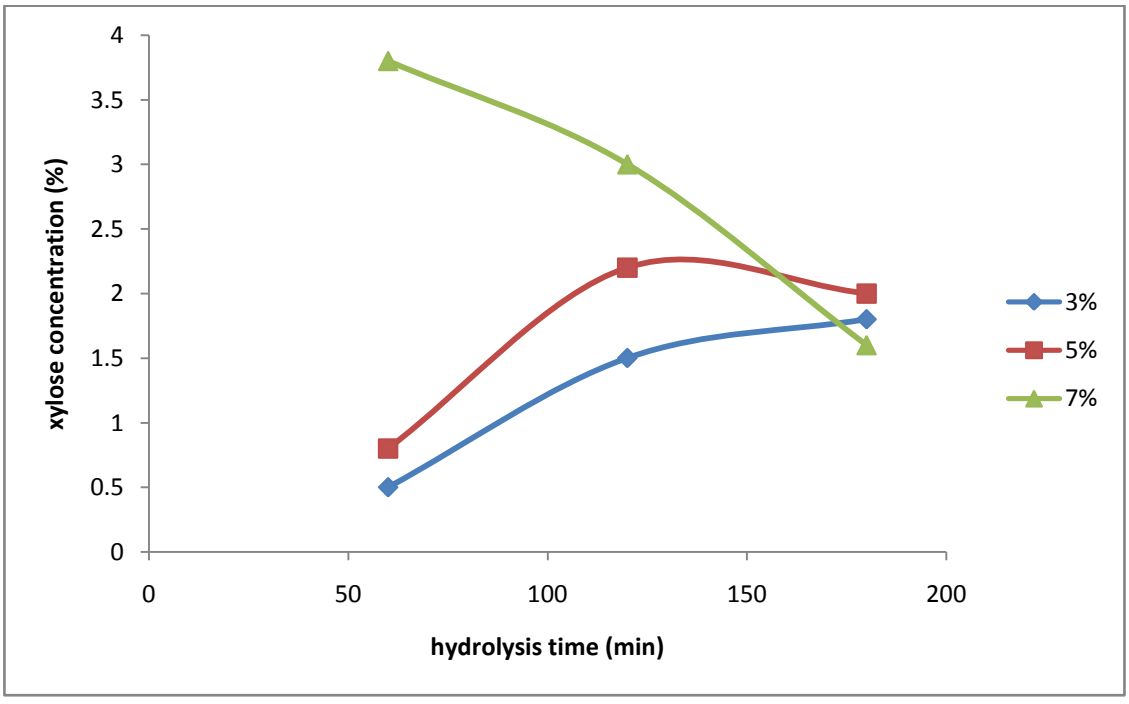



Figure 4.7: The xylose recovery after the treatment with acid at different concentrations for different hydrolysis times

### 4.9.1 Discussion

Based on Figure 4.7, the xylose concentration recovery for acid concentration of 3% and 5% were slightly same. A part from that, it was remarked that 8% of acid concentration gave higher xylose yield. Mohamad et al. (2011) state that, 8% of acid concentration and at 60 minutes hydrolysis times gave highest xylose yield. Mohamad et al (2011) had approved that higher acid concentration led to higher sugar recovery but longer hydrolysis time caused lower amount of sugar recovery and higher furfural formation. However, the result obtained from this research was quite different. The xylose recovery for 3% and 5% acid concentration were increase when the hydrolysis times increase, while, for 8% acid concentration was decrease when the hydrolysis times increase.

# CHAPTER 5

# CONCLUSION AND RECOMMENDATION

## 5.1 Conclusion

The experimental results gave the chemical composition of oil palm trunk (OPT) subjected to experiments are as follows of which 18.4% lignin, 47.5% cellulose, 78.5% holocellulose, 25.3% hemicelluloses and 45.9% a-cellulose. From 25.3% of hemicelluloses portion of the oil palm trunk, xylose yielded 19.71% of the total sugar.

The results obtained also shows that, 7% of acid concentration gave higher xylose yield with 3.8% of xylose at 60 minutes hydrolysis time. In can be conclude that higher acid concentration led to higher sugar recovery but longer hydrolysis time caused lower amount of sugar recovery.

Moreover, by this research, it shown that waste oil palm trunk, when properly treated, can be a renewable resource with high value of xylose and other sugar which

can be recovered by acid hydrolysis of wood. The production of xylose has been commercial nowadays.

## 5.2 Recommendation

The waste of oil palm trunk (OPT) is definitely can be converted into more valuable products. It can be treated to produce high value of product especially in producing sugar such as xylose. As for recommendation, there are many factors that can be further study in order to get the highest concentration and high value of xylose, such as:

i. Used other method for producing xylose by using enzymatic hydrolysis instead of acid hydrolysis.

ii. The production of xylose also can be produced from other raw materials such as empty fruit bunch, cornstalk, sago trunk and other suitable wood.

iii. Other parameters that can be studied such as more acid concentration, reaction times, temperature, size of raw material etc.

iv. Analyze the xylose concentration using the response surface methodology (RSM) to optimize the hydrolysis reaction parameters.

v. Investigate other products that can be produce from oil palm trunk (OPT) such as xylitol and etc.

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APPENDIX A

Chemstation Data and Area Percent Report HPLC for sample 1a

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                                                Inj Volume : 10.0 µl
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Last changed    : 12/26/2012 10:54:56 AM by Syee
Analysis Method : C:\CHEM32\1\METHODS\NAFSIAH FKKSA.M
Last changed    : 12/26/2012 10:54:56 AM by Syee
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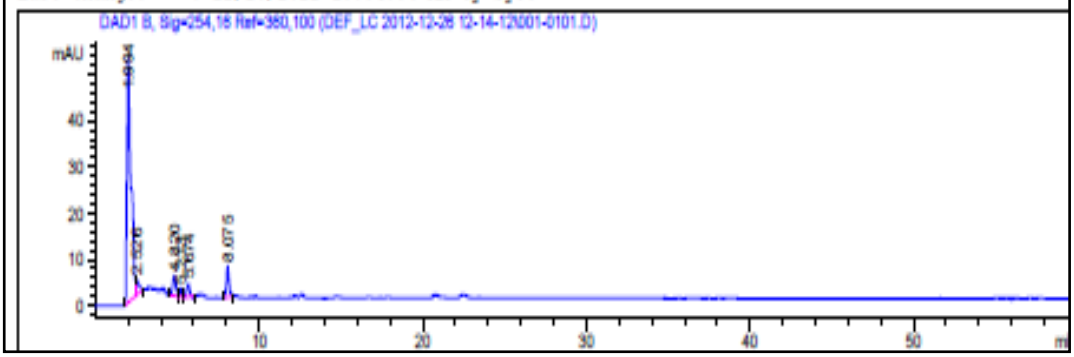


```
ample Name: 1A

                              Area Percent Report

Sorted By             :       Signal
Multiplier:                    :       1.0000
Dilution:                      :       1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 B, Sig=254,16 Ref=360,100

Peak RetTime Type  Width     Area       Height      Area
  #   [min]        [min]    [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   1.994 BV    0.2343  909.07355   53.48901  79.5540
   2   2.526 VB    0.1768   42.87355    3.19013   3.7519
   3   4.820 BV    0.2284   63.45801    4.29423   5.5533
   4   5.213 VV    0.1324   15.31285    1.81549   1.3400
   5   5.674 VB    0.2157   35.77013    2.75118   3.1303
   6   8.075 BB    0.1755   76.22441    6.56070   6.6705

Totals :                   1142.71250   72.10073
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APPENDIX B

Chemstation Data and Area Percent Report HPLC for sample 1b

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Inj Volume : 10.0 µl
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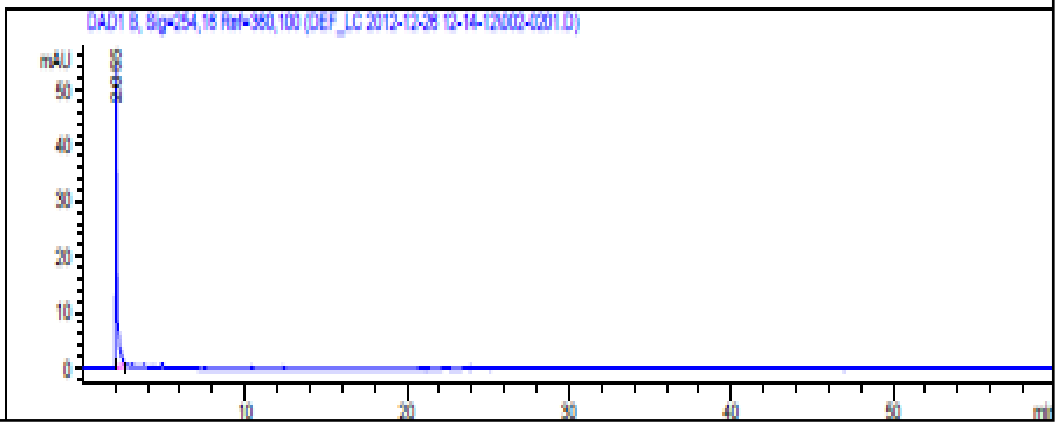
Last changed : 12/26/2012 10:54:56 AM by Syaa
Analysis Method : C:\CHEM32\1\METHODS\NAFSIAH FKKSA.M
Last changed : 12/26/2012 10:54:56 AM by Syaa




Data File D:\CHEMSTATION DATA\DEF_LC 2012-12-26 12-14-12\002-0201.D
Sample Name: 1B

Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 B, Sig=254,16 Ref=360,100

| Peak # | RetTime [min] | Type | Width [min] | Area [mAU*s] | Height [mAU] | Area % |
|---|---|---|---|---|---|---|
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| Totals : | | | | 344.68271 | 55.07607 | |

APPENDIX C

Chemstation Data and Area Percent Report HPLC for sample 1c

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Inj Volume : 10.0 µl
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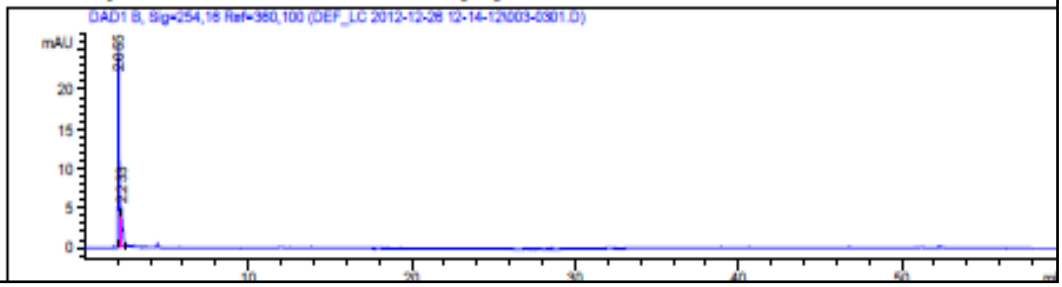
Analysis Method : C:\CHEM32\1\METHODS\NAFSIAH FKKSA.M
Last changed : 12/26/2012 10:54:56 AM by Syee

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Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 B, Sig=254,16 Ref=360,100

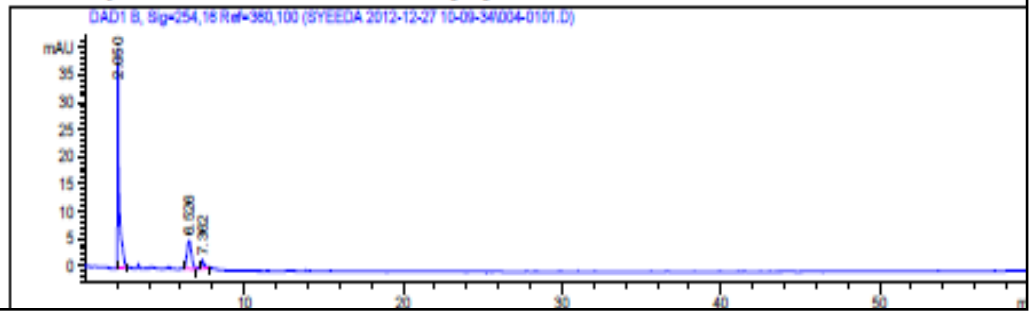
| Peak # | RetTime [min] | Type | Width [min] | Area [mAU*s] | Height [mAU] | Area % |
|---|---|---|---|---|---|---|
| 1 | 2.055 | BV | 0.0693 | 121.70550 | 25.69293 | 76.6806 |
| 2 | 2.233 | VB | 0.1129 | 37.01189 | 4.75010 | 23.3194 |
| Totals : | | | | 158.71739 | 30.44304 | |

APPENDIX D

Chemstation Data and Area Percent Report of HPLC for sample 2a

```
Data File D:\CHEMSTATION DATA\SYEEDA 2012-12-27 10-09-34\004-0101.D
Sample Name: 2A

================================================================
Acq. Operator   : Salma                      Seq. Line :  1
Acq. Instrument : Instrument 1                Location : Vial 4
Injection Date  : 12/27/2012 10:09:52 AM           Inj :  1
                                             Inj Volume : 10.0 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 20.0 µl
Acq. Method     : D:\CHEMSTATION DATA\SYEEDA 2012-12-27 10-09-34\NAFSIAH FKKSA.M
Last changed    : 12/26/2012 10:54:56 AM by Syee
Analysis Method : C:\CHEM32\1\METHODS\NAFSIAH FKKSA.M
Last changed    : 12/26/2012 10:54:56 AM by Syee
```




```
Data File D:\CHEMSTATION DATA\SYEEDA 2012-12-27 10-09-34\004-0101.D
Sample Name: 2A

================================================================
                      Area Percent Report
================================================================

Sorted By             :      Signal
Multiplier:                  :      1.0000
Dilution:                    :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 B, Sig=254,16 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   2.050 BB    0.0921  277.33466   40.41190  69.2152
   2   6.526 BV    0.3079  103.78191    5.17603  25.9012
   3   7.362 BB    0.1717   19.56780    1.44753   4.8836

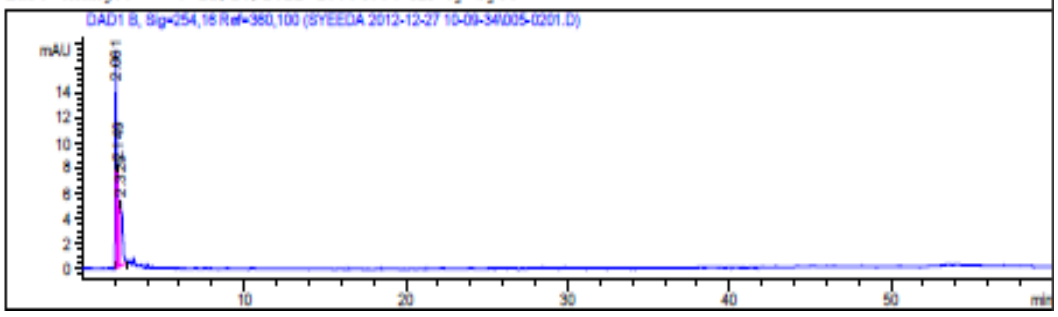
Totals :                   400.68436   47.03546
```

APPENDIX E

Chemstation Data and Area Percent Report of HPLC for sample 2b

```
Data File D:\CHEMSTATION DATA\SYEEDA 2012-12-27 10-09-34\005-0201.D
Sample Name: 2B

------------------------------------------------------------------
Acq. Operator   : Salma                          Seq. Line :   2
Acq. Instrument : Instrument 1                    Location : Vial 5
Injection Date  : 12/27/2012 11:11:11 AM             Inj :   1
                                                 Inj Volume : 10.0 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 20.0 µl
Acq. Method     : D:\CHEMSTATION DATA\SYEEDA 2012-12-27 10-09-34\NAFSIAH FKKSA.M
Last changed    : 12/26/2012 10:54:56 AM by Syee
Analysis Method : C:\CHEM32\1\METHODS\NAFSIAH FKKSA.M
Last changed    : 12/26/2012 10:54:56 AM by Syee
```




```
------------------------------------------------------------------------
                        Area Percent Report
------------------------------------------------------------------------

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 B, Sig=254,16 Ref=360,100

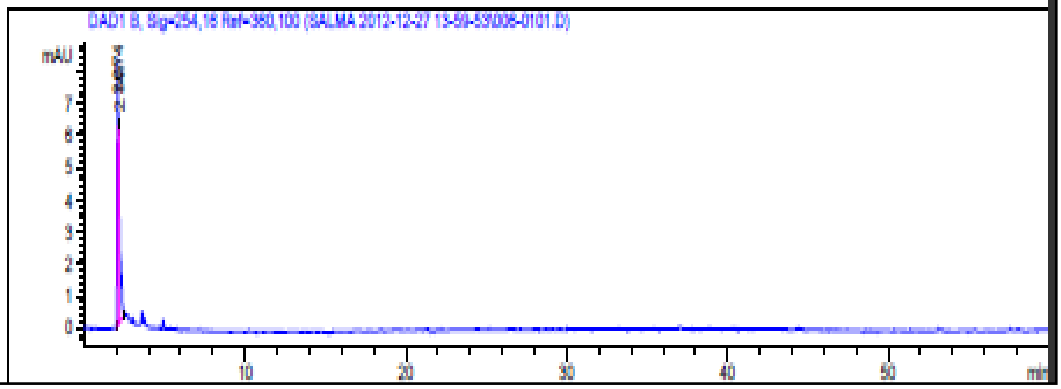
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]    [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   2.061 BV    0.0656   77.13094   17.42999  39.1649
   2   2.149 VV    0.0931   52.34560    7.91595  26.5796
   3   2.325 VB    0.1684   67.46232    5.16561  34.2555

Totals :                    196.93887   30.51155
```

APPENDIX F

Chemstation Data Area and Percent Report of HPLC for sample 2c

```
Acq. Operator   : Salma                          Seq. Line :  1
Acq. Instrument : Instrument 1                   Location : Vial 6
Injection Date  : 12/27/2012 2:00:11 PM              Inj :  1
                                                 Inj Volume : 10.0 µl
Acq. Method     : D:\CHEMSTATION DATA\SALMA 2012-12-27 13-59-53\NAFSIAH FKKSA.M
Last changed    : 12/26/2012 10:54:56 AM by Syse
Analysis Method : C:\CHEM32\1\METHODS\NAFSIAH FKKSA.M
Last changed    : 12/26/2012 10:54:56 AM by Syse
```




```
                        Area Percent Report

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 B, Sig=254,16 Ref=360,100

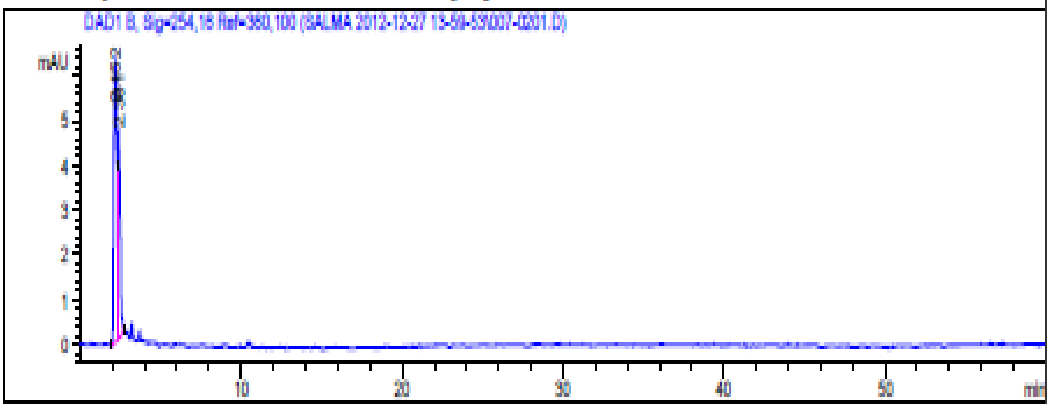
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   2.074 BV    0.0810   43.61199    8.34198  51.1905
   2   2.147 VB    0.0876   41.58343    6.41500  48.8095

Totals :                    85.19542   14.75698
```

APPENDIX G

Chemstation Data Area and Percent Report of HPLC for sample 3a

```
Acq. Operator   : Salma                          Seq. Line :  2
Acq. Instrument : Instrument 1                   Location : Vial 7
Injection Date  : 12/27/2012 3:01:35 PM              Inj :  1
                                                 Inj Volume : 10.0 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 20.0 µl
Acq. Method     : D:\CHEMSTATION DATA\SALMA 2012-12-27 13-59-53\NAFSIAH FKKSA.M
Last changed    : 12/26/2012 10:54:56 AM by Syee
Analysis Method : C:\CHEM32\1\METHODS\NAFSIAH FKKSA.M
Last changed    : 12/26/2012 10:54:56 AM by Syee
```




```
                        Area Percent Report

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 B, Sig=254,16 Ref=360,100

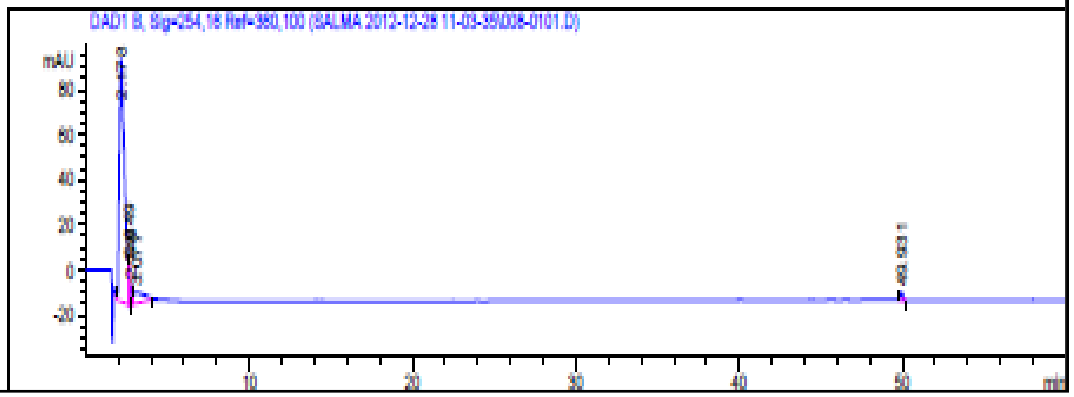
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   2.152 BV    0.1824   87.23669    6.26660  64.4955
   2   2.381 VB    0.1366   48.02341    4.56957  35.5045

Totals :                   135.26010   10.83617
```

APPENDIX H

Chemstation Data Area and Percent Report of HPLC for sample 3b

```
Acq. Operator   : Salma                          Seq. Line :  1
Acq. Instrument : Instrument 1                   Location : Vial 8
Injection Date  : 12/28/2012 11:03:52 AM             Inj :  1
                                                 Inj Volume : 10.0 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 20.0 µl
Acq. Method     : D:\CHEMSTATION DATA\SALMA 2012-12-28 11-03-35\NAFSIAH FKKSA.M
Last changed    : 12/26/2012 10:54:56 AM by Syee
Analysis Method : C:\CHEM32\1\METHODS\NAFSIAH FKKSA.M
Last changed    : 12/26/2012 10:54:56 AM by Syee
```




```
                        Area Percent Report

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 B, Sig=254,16 Ref=360,100

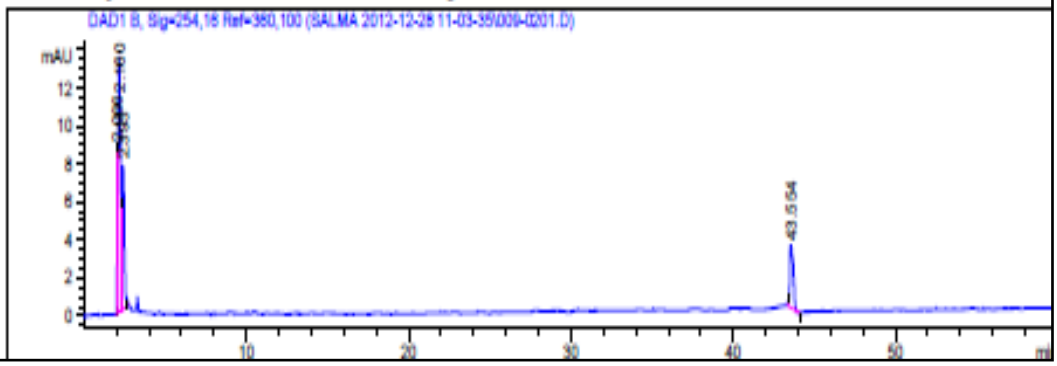
Peak RetTime Type  Width     Area       Height      Area
  #   [min]          [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   2.178 VV     0.2924 2390.54150  107.08558  86.8721
   2   2.649 VV     0.0416   51.76037   19.08659   1.8810
   3   2.743 VV     0.1102   67.32753    8.16519   2.4467
   4   3.077 VB     0.4947  209.30956    5.05935   7.6063
   5  49.931 BB     0.1608   32.85563    3.45841   1.1940

Totals :                   2751.79459  142.85512
```

APPENDIX I

Chemstation Data Area and Percent Report of HPLC for sample 3c

```
Acq. Operator   : Salma                          Seq. Line :   2
Acq. Instrument : Instrument 1                    Location : Vial 9
Injection Date  : 12/28/2012 12:05:11 PM             Inj :   1
                                                 Inj Volume : 10.0 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 20.0 µl
Acq. Method     : D:\CHEMSTATION DATA\SALMA 2012-12-28 11-03-35\NAFSIAH FKKSA.M
Last changed    : 12/26/2012 10:54:56 AM by Syee
Analysis Method : C:\CHEM32\1\METHODS\KHAN SAMPLE.M
Last changed    : 12/31/2012 9:27:41 PM by Salma
```




```
                        Area Percent Report

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 B, Sig=254,16 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   2.066 BV    0.0630   34.93349    8.67468  12.3797
   2   2.160 VV    0.1235  123.99788   13.45991  43.9424
   3   2.393 VB    0.1456   74.98026    7.56976  26.5715
   4  43.554 BB    0.2550   48.27116    3.25608  17.1063

Totals :                   282.18278   32.96044
```