THE EFFECTS OF AMOUNT OF POWDERED ACTIVATED CHARCOAL (PAC) ON PURIFICATION OF RAW SAGO SUGARS

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Bachelor of Science with Honours
(Resource Biotechnology)
2015

DECLARATION

I hereby declare that this thesis entitled "The Effect of Amount of Powdered Activated Charcoal (PAC) on Purification of Raw Sago Sugars" is my own work and all sources have been quoted and referred to have been acknowledged by means of complete references. It has been submitted and shall not submit to other university or institute of higher learning.

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ACKNOWLEDGEMENT

First of all, I would like to express the deepest appreciation to my Supervisor, Professor Kopli Bin Buajng and my co-supervisor Madam Dayang Salwani Binti Awang Adeni, who have the attitude and convincingly conveyed a spirit of adventure in regard to research and excitement in regard to teaching. Their enthusiasm, encouragement, and faith in me throughout have been extremely helpful. Without their guidance and persistent help this research project would not have been successful.

I would like to thank my post-graduate students in Biochemistry laboratory, Miss Nadia Dayane Sikem, Miss Sharifah Binti Mohammad, Mr. Zakaria and Mr. Muhammad Norhelmi Bin Ahmad, who demonstrated to me that concern for the progress in this research and provide a quest for our times.

I thank this opportunity to record my sincere thanks to all my friends for helping me survive all the stress from this year, their encouragement, not letting me give up all the time and thus supporting me both on and off the water. I also thank to my parents for their unceasing encouragement and support.

Finally, I also place on record, my sense of gratitude to one and all who, directly or indirectly, have lent their helping hand in this research.

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LIST OF ABBREVIATIONS

Abbreviation	Description
%	Percentage
°C	Celsius (temperature)
w/v	Weigh per volume
g/L	Gram per liters
B. C.	Before century
H_2SO_4	Sulphuric acid
NaOH	Sodium hydroxide
HCl	Hydrochloric acid
AMG	Amyloglucodidase enzyme
LSS	Liquid sago sugar
PSS	Purified sago sugar
HSS	Hydrolyzed sago sugar
PAC	Powdered activated charcoal
GAC	Granular activated charcoal
DE	Dextrose Equivalent
Kg	Kilogram
RM	Ringgit Malaysia
Tons/ha	tons per hectra
HPLC	High Performance Liquid Chromatography
g/L	gram per litre
ml	mililitre per minute
ABS	Absorbance
BSS	Brown sago sugar

The Effect of Amount of Powdered Activated Charcoal (PAC) on Purification of Raw Sago Sugar

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ABSTRACT

Sago starch, *Metroxylon sagu,* is used for the production of glucose which is potentially viable as the new source of commercial sugar through enzymatic hydrolysis of sago starch into sugars. The production of sugar was performed in 1L lab scale vessel from sago starch. The starch was enzymatically hydrolysed for 26 hours (2 hours for liquefaction and 24 hours for saccharification). The hydrolysate was purified by filtration using different amount of powdered activated charcoal (PAC) of 1g, 3 g, 5 g, 8 g, and 10 g while the sugar concentration were measured based on dextrose equivalent (DE). For filtration under gravity, the highest sugar recovery was obtained by using 5 g of PAC which yield 228.01 g/L lead to 71% of DE. The lowest sugar recovery obtained is filtration by 10 g of PAC lead to 89.78 g/L and 28% of DE. Instead of that, filtration by pump shows filtration by 1 g of PAC yield higher sugar recovery of 173.88 g/L (87%) but least sugar recovery by 10 g of PAC (23% DE). This experiment shows that filtration by pump is more economical in shorten filtration time which can produce 1 tonne of sugar per day in sugar industrial. Therefore, the production of sago sugar is more economical than sugar cane as it provide anti-flavonoid properties.

Keywords: enzymatic hydrolysis, *Metroxylon sagu,* PAC, dextrose equivalent (DE).

Abstrak

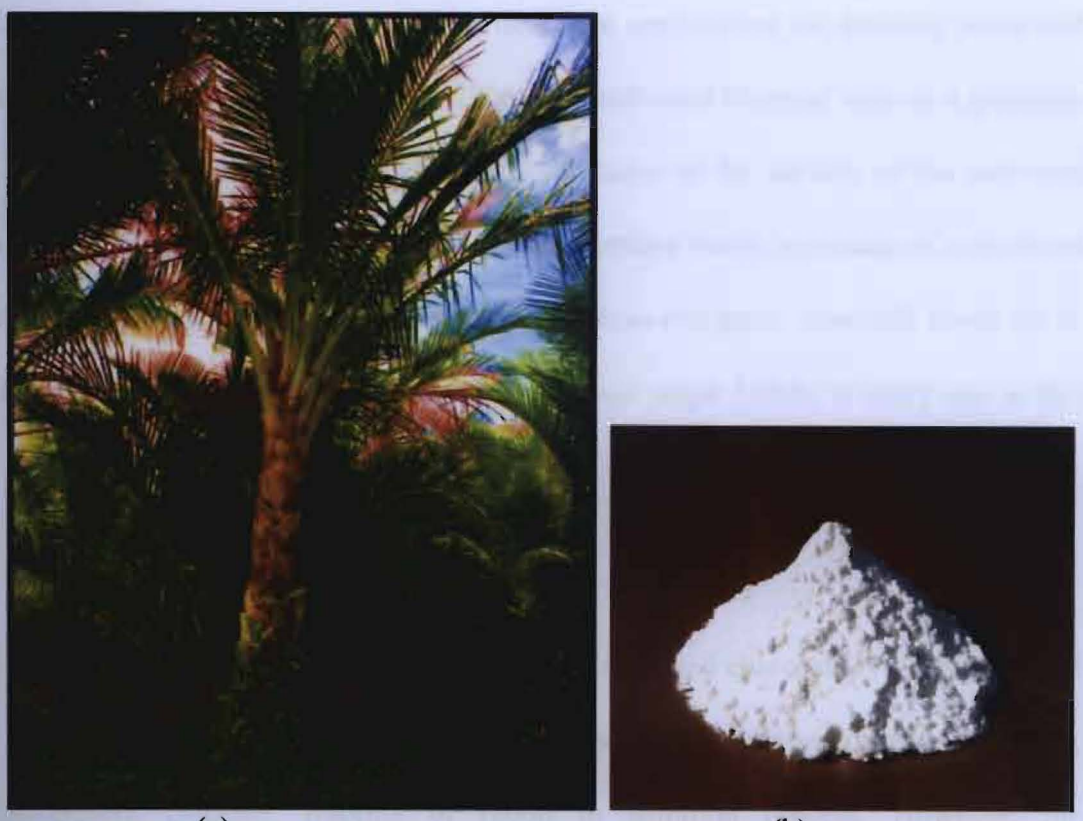
Kanji sagu, sagu *Metroxylon* telah digunakan dalam penghasilan glukos di mana ia berpotensi sebagai sumber baru dalam mengkomersialkan gula melalui hydrolisis enzim daripada kanji sagu kepada gula. Penghasilan gula dijalankan di dalam 1L vessel berskala makmal daripada kanji sago. Kanji telah dihidrolisis selama 26 jam (2 jam untuk pencairan dan 24 jam untuk pensarafikasian). Hidrolisat ditapis menggunakan serbuk arang aktif (PAC) dalam kuantiti yang berbeza iaitu 1 g, 3 g, 5 g, 8 g, dan 10 g sertakepekatan gula diukur bedasarkan Kesamaan Dextrosa (DE). Untuk penapisan bagi graviti, penemuan gula tertinggi diperolehi dengan menggunakan 5 g PAC di mana ia menghasilkan 228.01 g/L menyumbang kepada 71% DE. Penemuan gula yang terendah diperolehi daripada penapisan menggunakan 10 g PAC iaitu 89.78 g/L dan 28% DE. Selain itu, penapisan menggunakan pam menunjukkan penapisan menggunakan 1 g PAC menghasilkan penemuan gula paling tinggi dengan 173.88 g/L (87%) tetapi penemuan gula paling rendah adalah 10 g iaitu 23% DE. Experiemnt ini menunjukkan bahawa penapisan menggunakan pam sangat menguntungkan dengan mengurangkan masa yang diambil untuk penapisan di mana ia dapat menghasilkan 1 ton gula sagu dalam sehari dalam industri gula. Namun begitu, penghasilan gula sagu sangat menguntungkan daripada penghasilan gula tebu kerana ia menyediakan ciri-ciri anti flavonoid.

Kata kunci: Hidrolisis enzim, glukos, sagu *Metroxylon,*serbuk aktif arang (PAC), kesamaan dextrose (DE).

1.0 INTRODUCTION

1.1 Background of Proposed Study

Metroxylon sagu (sago palm) as in **Figure 1** has long been considered as one of the oldest sources of food for humans because of the presence of huge amounts of starch in its trunk. Sago palm is an important food source in Papua New Guinea, Malaysia and Indonesia (Goyal, 2012). Sago palm (*Metroxylan sagu*) is an agronomically important which contribute to the economy of Malaysia with an export about 65, 000 tons of sago starch to Peninsular Malaysia, Hong Kong, Taiwan, Singapore and other countries in 2012 (DoS, 2013). Sago palms are those species of the genus *Metroxylon* belonging to the Palmae family and is of extreme importance to over a million people who use the palms as their primary dietary starch source (Adeni *et al.*, 2010).



(a) (b)

Figure 1: (a) The true sago palm or *Metroxylan* sago, (b) sago starch.
(Source: http://eatingasia.typepad.com/eatingasia/2008/03/the-tree-of-lif.html)

From the previous study by Bujang *et al.,* (2006), sago starch has the potential to replace the sugar cane in the sugar industry through enzymatic hydrolysis of the starch into sugars or hydrolysed sago starch (HSS) and purified using powdered activated carbon (PAC). Lab-scale crystallization of the liquid sugar has been developed at UNIMAS by utilizing different approaches and process. Sago starch and its by product are obtained from the trunk (pith) when the tree is about 12 to 15 years old.

Activated charcoal in its broadcast sense is a term that includes a wide range of amorphous carbonaceous materials that exhibit a high degree of porosity and an extended interparticulate surface area. Active charcoal are unique and versatile adsorbents, and they are used extensively for the removal of undesirable odour, colour, taste, and other organic and inorganic impurities from domestic, and industrial waste water; in the purification of many chemicals, pharmaceuticals and food products.

Historically, the use of activated charcoal in the form of powder and granular have limited to filtration system in food industry, treatment applications for drinking water and does so in pharmaceuticals (Hung *et al.,* 2006). All activated charcoal acts on a principle called adsorption, which is an adherence of a substance to the surface of the activated carbon. Activated charcoal is prepared by high-temperature steam activation of carbonized samples of powdered cellulose and composite cellulose-inorganic materials based on it. The filtration method of PAC toward hydrolysed sago sugar (HSS) is carry out in this study.

1.2 Problem Statement

Purification of sago sugar by using powdered activated charcoal (PAC) is preferred in this study it is efficient to remove certain organics (such as unwanted taste and odour, micro-pollutants), chlorine, fluorine or radon in filtration process. However, the purification process which functions to eliminate other impurities will inadvertently also

adsorbs some sugars albeit at small concentration. It also is not effective for microbial contaminants, metals, nitrates and other inorganic contaminants and thus the contaminant are separated from water but not destroyed. Besides that, the filter has to be replaced regularly.

1.3 Research objectives:

The objectives of the study are:

1. To determine the best amount of powdered activated charcoal (PAC) in purifying the hydrolysed sago starch (HSS) to produce purified sago sugars (PSS) by filtration methods.
2. To analyse the optimum glucose concentration in sago sugar by using DNS method and high performance liquid chromatography (HPLC).

1.4 Rational and Significant Study

The rational and significant of this study is to replace sugar cane with sago starch in the sugar industry through enzymatic hydrolysis of the starch into sugars since the production of sugar cane is more expensive even though the sugar recovery from sugar cane is lower than sago starch. Sago sugar is very low in protein but is exceptionally high in carbohydrates rather than sugar cane which containing about 50 percent of carbohydrates in total sugar. Furthermore, production of sugar from sago starch is an alternative way to replace sugar cane as it yield 100% recovery of sugar and contains anti flavonoids properties such as anti-cancer, anti-aging and anti-oxidant which is useful for human health. Corresponds to sugar cane, it is only yield 7% recovery of sugar.

2.0 LITERATURE REVIEW

2.1 Sago Starch and Its Production

Sago starch is known as *Metroxylon rumphii* species and is collected locally in Sarawak from Herdson Sago Mill at Pusa and transported to UNIMAS Laboratory for the purpose of study.

The principle growing areas where there exists also moderate commercial sago starch production are Sarawak (where there are modern refineries at Sibu, Sabah, and Brunei) and New Guinea. It is used for the production of glucose which is potentially viable as the new source of commercial sugar through enzymatic hydrolysis of sago starch into sugars (96% glucose), achieved after about 6 hours. Bujang *et al.,* (2000) and Bujang & Jobli (2002) had reported that enzymatic hydrolysis was performed using Novo enzymes such as Termamyl-120L (a thermostable α-amylase from *Bacillus licheniformis*/120 KNU/g) for liquefaction (0.5 µl/g of starch) and incubated at 90 °C for 2 hours. 1 KNU/g is defined as the amount of the enzyme which breaks down 5.26 g starch per hour at 37°C at pH of 5.6 (Heiz R. et al, 2004). This was followed by amyloglucosidase from *Aspergillus niger* for saccharification (0.6 µl/g of starch), and incubated at 60 °C for another 24 hours in producing large volumes of hydrolysis (Bujang & Law, 2006).

In separate study, it was concluded that 20% (w/v) sago starch was the optimum starch concentration for enzymatic hydrolysis producing sago sugars at over 100% recovery (Bujang *et al.,* 2000; Bujang *et al.,* 2004; Booty & Bujang, 2009). pH of 4.5 and 6.5 has been confirmed earlier by the enzyme supplier (Novo) to be optimum pH for general starch hydrolysis but this has not been established for sago starch.

2.2 Global Market of Sago Starch and Sugar Cane

In Malaysia, more than 90% of all sago-planting area is found in the state of Sarawak in East Malaysia. The annual export of pure sago starch from Sarawak fluctuates between 40,000 - 50,000 tons of sago in 2013 procuring incomes between US$4.5 million to US$10.8 million as in **Table 1** below. About 100,000 tons of sago starch is used annually in Malaysia for various applications, mainly in the production of glucose (15,600t), MSG (15,000t), and noodle (13,200t) while other household used account for 36,000t (Bujang, 2006).

6.3 EKSPORT HASIL RUMBIA 2004 – 2013
Export of Sago Products 2004 – 2013

Tahun *Year*	Tepung & Bijian Rumbia *Sago Flour and Meal*		Kanji Rumbia *Sago Starch*		Empelur Rumbia *Sago Pith*		Jumlah *Total*	
	tan metrik *tonne*	RM	tan metrik *tonne*	RM	tan metrik *tonne*	RM	tan metrik *tonne*	RM
2004	4,104.90	3,088,119	42,899.03	33,953,479	0.00	0	47,003.93	37,019,598
2005	198.00	183,150	45,332.43	40,432,542	0.00	0	45,530.43	40,615,692
2006	0.00	0	42,870.90	42,955,166	0.00	0	42,870.90	42,955,166
2007	0.00	0	44,766.88	51,407,110	0.00	0	44,766.88	51,407,110
2008	792.00	686,381	44,424.75	57,067,424	0.00	0	45,216.75	57,753,805
2009	2,034.00	1,788,352	41,484.64	60,403,459	0.00	0	43,518.64	62,191,811
2010	0.00	0	44,448.83	62,831,429	1.50	1,065	44,450.33	62,832,494
2011	0.00	0	50,726.78	90,949,230	0.00	0	50,726.78	90,949,230
2012	162.00	145,285	47,687.26	86,199,739	0.00	0	47,849.26	86,345,004
2013 (p)	0.00	0	47,946.37	80,979,108	0.00	0	47,946.37	80,979,108

(p) : Permulaan
Sumber : Jabatan Perangkaan

(p) : Preliminary
Source : Department of Statistics

6.4 IMPORT HASIL RUMBIA 2004 – 2013
Import of Sago Products 2004 – 2013

Tahun *Year*	Tepung & Bijian Rumbia *Sago Flour and Meal*		Kanji Rumbia *Sago Starch*		Empelur Rumbia *Sago Pith*		Jumlah *Total*	
	tan metrik *tonne*	RM	tan metrik *tonne*	RM	tan metrik *tonne*	RM	tan metrik *tonne*	RM
2004	0.00	0	10.25	13,390	31.50	43,499	41.75	56,889
2005	38.29	68,990	0.00	0	19.92	34,060	58.21	101,050
2006	0.00	0	87.67	107,378	32.22	63,808	99.79	171,186
2007	0.00	0	53.44	109,306	13.53	27,315	66.97	136,621
2008	0.00	0	151.72	261,196	15.59	34,948	167.31	296,144
2009	0.00	0	77.41	138,867	29.85	59,292	107.26	198,159
2010	6.25	20,000	126.58	233,407	34.69	72,940	169.52	326,347
2011	26.55	92,642	75.97	203,304	0.48	2,520	103.00	298,466
2012	11.05	48,956	120.31	330,527	1.02	6,044	132.38	383,527
2013 (p)	10.25	21,020	69.14	197,243	5.25	13,680	84.64	231,943

(p) : Permulaan
Sumber : Jabatan Perangkaan

(p) : Preliminary
Source : Department of Statistics

Table 1: Export and import of sago products from 2004 - 2013
(Source: Department of Statistics Malaysia, Sarawak Branch, 2013).

2.3 PRODUCTION OF SUGAR CANE

Sugar cane is a member of the *graminea* family. It is grown in both tropicl and subtropical climates. Its main constituents are fibre, sugar and water. The raw sugar production process is summarizing as below (Antonio an Carlos, 2001):

- Harvest and transport the cane to sugar factory
- Juice extraction
- Juice purification by ion-exchange resin
- Evaporation of water
- Crystallization of sucrose and the production of massecuite
- Storage of sugar and molasses

Figure 2 shows the process involved in manufacturing sugar cane.

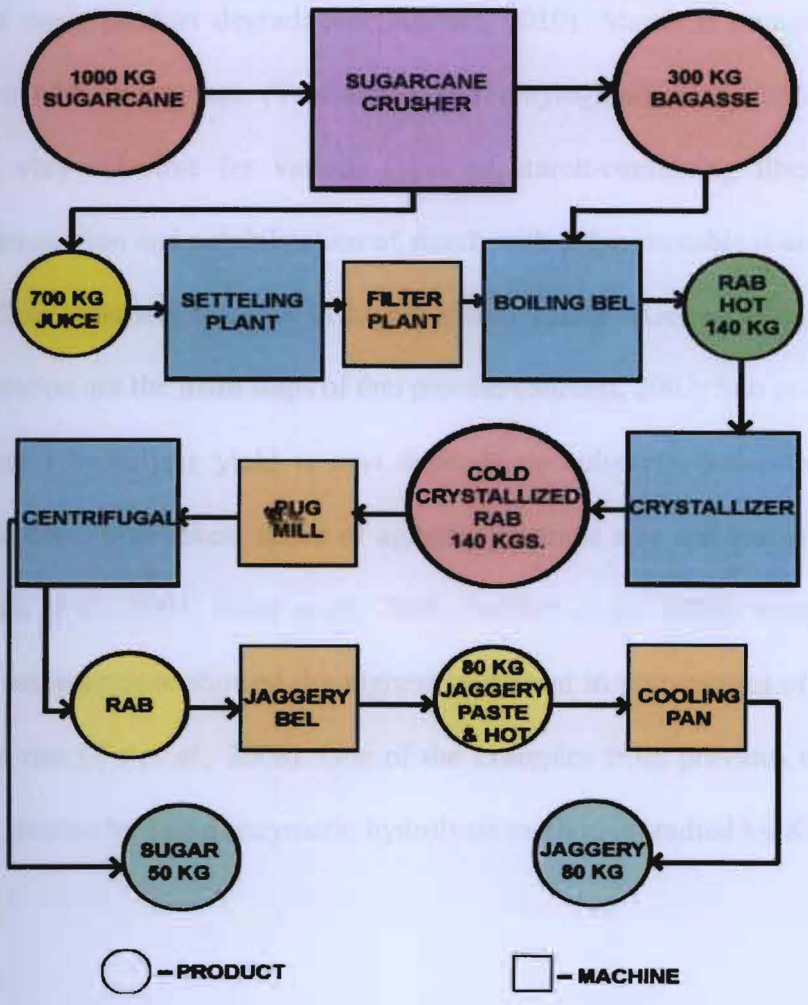


Figure 2: The process involved in manufacturing sugar cane.

Based on **Figure 2,** 1 ton of raw sugar cane only yields 70% of sugar cane from its total production. This loss occurs at the crystallization stage of raw sugar production due to poor recovery of sucrose from the final molasses. Poor post-harvest treatment of the cane, as well a industrial factors contributes to this to this poor recovery. With respect to post-harvest treatment, a long time lapse between cutting and grinding of the cane results in an increase in both polysaccharides and oligosaccharide content, thus increasing the viscosity of the molasses and decreasing diffusion of sucrose from the mother liquor to the crystal (Antonio and Carlos, 2001).

2.4 Enzymatic Hydrolysis of Sago Starch

Enzymatic hydrolysis provides a method to convert cellulose to glucose at high yields without sugar product degradation (Ahmad, 2010). Starch is removed by boiling with a thermostable α-amylase (Termamyl) and amyloglucosidase. This enzyme has proved to be very effective for various types of starch-containing fiber sources and products. Gelatinization and solubilization of starch with a thermostable α-amylase prior to amyloglucosidase treatment resulted in higher starch values. (Gene, 2001). Liquefaction and saccharification are the main steps of this process (Suraini, 2002; Sun *et al.,* 2006).

Enzymatic hydrolysis yield is also depends on substrate concentration, type of starch, enzyme dose, time taken, speed of agitation, granule size and viscosity of the raw starch (Madihah *et al.,* 2001; Balat *et al.,* 2008; Zulfikri *et al.,* 2008). Longer hydrolysis time and high enzyme dose showed the highest increment in percentages of glucose yield as temperature rise (Yu *et al.,* 2008). One of the examples from previous research about production of glucose by using enzymatic hydrolysis method is studied by Kunamneni and Singh (2005).

2.4.1 Liquefaction of starch

Based on previous studies, gelatinization and the amount of reducing sugar produces from sago starch was 0.464 g/Lh. Bujang *et al.,* (2000) and Bujang & Jobli (2002) reported that enzymatic hydrolysis was performed using Novo enzymes such as Termamyl-120L (a thermostable α-amylase from *Bacillus licheniformis*, 120 KNU/g) for liquefaction (0.5 µl/ gram of starch) and incubated at 90 °C for 2 hours. In general liquefaction is carried out at pH of 6.0-6.5.

2.2.2 Saccharification of starch

Saccharification step is important to further hydrolyse the liquefied starch. This was followed by amyloglucosidase (AMG) from *Aspergillus niger* for saccharification (0.6 µl/g of starch), and incubated at 60°C for another 24 hours in producing large volumes of hydrolysis. The optimum saccharification has been conducted at 60°C and pH 4.5. In separate study, Aggarwal *et al.* (2001) has found that at high temperature, the rate of saccharification reduced substantially at the optimum condition for saccharification were at 45 °C and pH 5. Saccharification leads to about 96% yield of glucose and about 4% by product. (Bujang & Law, 2006).

2.5 Purification of Raw Sago Sugar

The manufacturing of white sugar by decolourization of sugar solutions by adsorption on wood charcoal was first reported from London refinery. The activated charcoal treatment is the last stage of the purification process before the sugar juices are boiled to produce white mother liquor from which white sugar can be obtained (Roop & Meenakshi, 2005).

Within the range of pH value met with in the sugar manufacturing process, lowering of the pH value usually improves decolourization. A pH of 4.5 is optimum for decolourization. The treatment of sugar solutions with active carbons only slightly increases the purity of the solution, usually 0.1 percent at most, but gives the solution better optical appearance. It also markedly enhances the processing properties (Roop & Meenakshi, 2005).

Bujang *et al.*, (2011) has reported that purification of hydrolysed sago sugar (HSS) at 200 g/L glucose had performed on 5 g of powdered activated charcoal (PAC) packed on glass wool in a glass tube (2.5 cm diameter, and 2 cm filtration height). Filtration of HSS to produce purified sago sugars (PSS) under gravity (2.5 ml/min) show that higher (85%) recovery of sugars was obtained at lower amount of PAC (5 g) compared to 10 g of PAC (75%).

Our analysis confirmed that PSS contains glucose and maltose at 94% and 3% respectively (Bujang *et al.*, 2011). Further analyses revealed that the recovery of glucose increased by over 70% (from 101.3 g/L to 153.7 g/L) after 7 cycles upon filtration on the same batch of PAC.

Ang *et al.* (2006) reported that adsorption between PAC is lower towards glucose and lactate but higher towards protein and colour. However, the purification process to eliminate other impurities will inadvertently also adsorbs some sugars albeit at small concentration. This was amplified when purification was performed at higher amount of PAC (10 g) where the yield of sugars was lower. Similar results were reported earlier when using PAC in the purification of L-lactic acid (Bujang *et al.*, 2005).

2.6 Powdered Activated Charcoal (PAC)

2.6.1 Discovery and usage

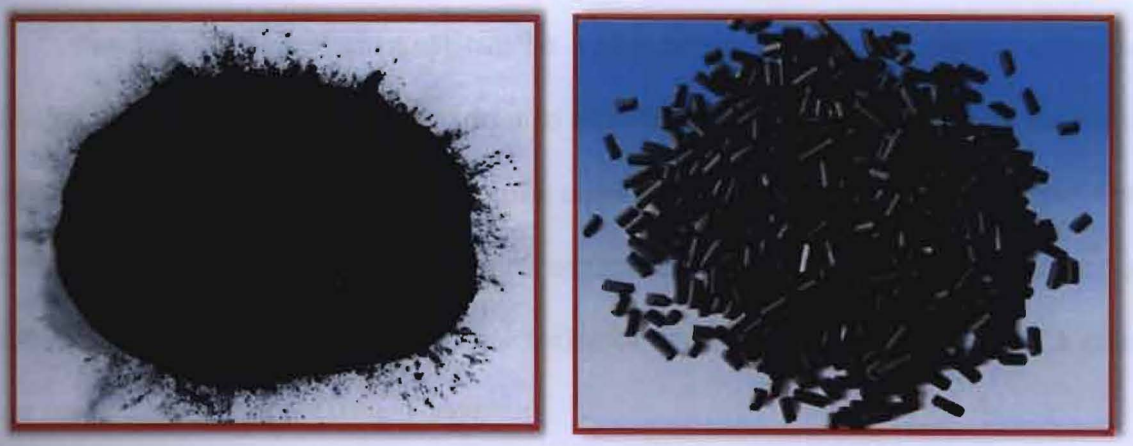
Activated charcoal has been long recognized as one of the most versatile adsorbents to be used for the effective removal of low concentrations of organic substances from solution (Robert, 2008). According to the first documented use of charcoal as written on papyrus by 1500 before century (B. C), the Egyptian's use of charcoal had progressed, using the material to cure intestinal ailments, preserve the dead and even to absorb unpleasant odours. Around 400 B. C. Christopher Columbus, the seafarers throughout history had adopted this practice and continued until the 1800s (Flexicon Corporation, 2014).

As todays, the uses of activated charcoal continue to grow in a variety of industries such as gas adsorption, corn and cane sugar refining, fat and oil removal, pharmaceuticals, dry cleaning, alcoholic beverage production and much more. The purification of municipal water supplies is the biggest market for activated charcoals.

2.6.2 Physical characteristics

Activated charcoal is a fine black powder produced by the activation (i.e., pyrolysis, oxidation, and purification) of carbon-containing materials such as bone, coal, peat, petroleum and wood. It is odourless, tasteless and insoluble in liquids. The activation process yields particles that have an extensive internal networks of minute, branching, irregular, interconnecting channels (i.e., pores) that range in size from approximately 10-100 nm in diameter and account for the extremely large surface area of activated charcoal.

Below are the examples of activated charcoal in form of powdered activated charcoal (PAC) and granular activated charcoal (GAC) as shown in **Figure 3**:



(a) (b)

Figure 3: Examples of activated charcoal in the form of (a) PAC, (b) GAC.
(Source: http://www.mohiexporttraders.com/coconut-shell-activated-carbon-powder.htm)

2.6.3 Adsorption

Adsorption is a surface phenomenon where certain molecules attach themselves to a solid surface based on intermolecular forces. The important properties of an adsorbent, which will affect the adsorption characteristics, are: the pore size distribution, surface area, surface qualities (hydrophobic/hydrophilic nature and function and physical properties such as hardness, bulk density and particle-size distribution. These properties are generally controlled by fine tuning the different parameters of manufacturing process (Mussatto *et al.*, 2006).

In sugar refining, undesired colour compounds, which are extracted from the sugarcane or sago sugar along with the sugar, are removed by passing the juice across a bed of activated charcoal. This is a microcrystalline, no graphitic form of carbon with high porosity and surface area to enhance adsorption capacity.

2.6.4 Application of PAC

2.6.4.1 Drinking water treatment

Powdered activated charcoal can be added before coagulation, during chemical addition, or during the settling stage, prior to sand filtration. It is removed from the water during the coagulation process, in the former cases and through filtration, in the latter. One of the advantages of PAC is that it can be applied for short periods. With problems that may arise only periodically such as algal toxins or tastes and odours, this can be a great cost advantages (David & Gayle, 2006).

2.6.4.2 Decolourization process in food industry

Adsorption by activated charcoal finds numerous applications in several food processing industries to remove unwanted colour and to improve the quality and consumability of the food material. The more important industries where activated charcoal have applications are the sugar industry for decolourization of sugar syrups, in the preparation of alcoholic beverages for removing unwanted compounds to improve the taste,colour, and other properties (Tascon, 2012).

Powdered activated charcoal is usually used for the decolourization of thick juice or standard liquor because these in-process products have the right dry substance content (55 to 65%) for PAC operation. In comparison with the decolourization with granular activated charcoal, the installation of PAC decolourization proess has the advantage of requiring low capital without major plant changes (Mosen, 2006).

A pH of 4.5 is optimum for decolourization. The treatment of sugar solutions with active carbons only slightly increases the purity of the solution, usually 0.1 percent at most, but gives the solution better optical appearance (Roop & Meenakshi, 2005).

3.0 MATERIALS AND METHODS

3.1 Materials and Chemicals

3.1.1 Sago starch

Sago starch was collected locally in Sarawak from Herdson Sago Mill at Pusa and 200 g of sago starch was used to make hydrolysed sago sugar (HSS) as shown in **Figure 4**.

Figure 4: Food grade sago starch (from Hedson Sago Mill, Pusa, Sarawak) used in this study.

3.1.2 Enzymes

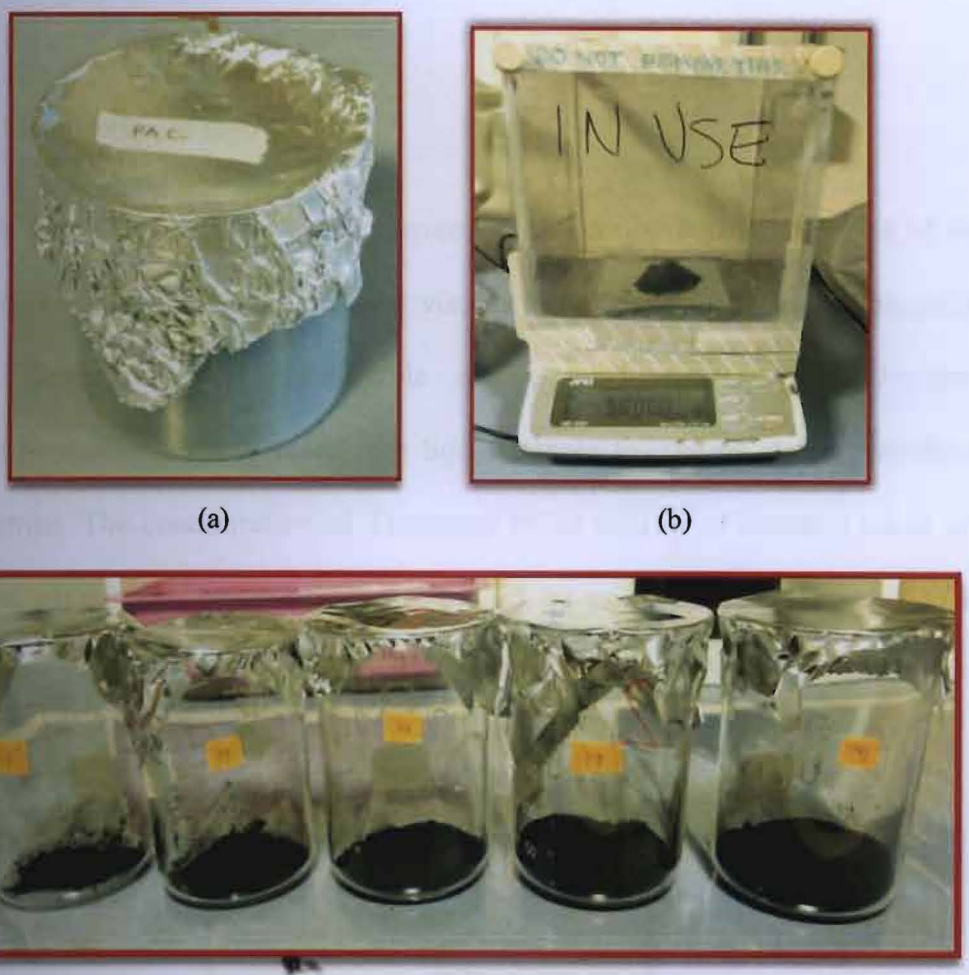
The enzymes used for enzymatic hydrolysis (Novozyme) were Termamyl SC and Amyloglucosidase (AMG) as reported by Bujang and Janggu (2009) in **Figure 5.**

(a) (b)

Figure 5: Enzymes used for enzymatic hydrolysis in HSS, (a) Termamyl SC, (b) Amyloglucosidase (AMG)

3.1.3 Powdered activated charcoal (PAC)

PAC (HmbG Chemicals) was used in this project because of its larger surface area. The PAC were weighed by using electrical balance in different amount in gram as it will investigated in this project as in **Figure 6.**



(a) (b)

(c)

Figure 6: (a) PAC collected from HmbG Chemicals , (b) Electrical balance was used to weight PAC, (c) The different amount of PAC

3.2 Experimental Methods

3.2.1Enzymatic hydrolysis of sago starch

Enzymatic hydrolysis was performed at volumes of 1 L in the 20% mixing ratio. The liquid samples were undergoing two stages of enzymatic treatment with two different enzymes.

3.2.1.1 Liquefaction of sago starch

The first step is liquefaction, which conversion of a concentrated suspension of starch granules into a solution of soluble of low viscosity for convenient handling in ordinary equipment. Termamyl SC (thermostable α-amylase from *Bacillus licheniformis*, 120KNU/g, Novoenzyme) was added into liquid sample to convert starch granules into soluble dextrins. The concentration of Termamyl SC is 0.5µl/g of sample (Adeni *et al.*, 2000). Then, pH of sample was adjusted to pH 6.5 by using 1 M NaOH and 1 M HCl. Sample was gelatinized at 90 – 95 °C for 5 – 10 minutes. Sample was liquefied at 80 – 90 °C for 2 hours as in **Figure 7.**

Figure 7: Liquefaction step on stirrer hot plate for 2 hours at 90°C.