Production of Industrial Sugars from Petai Belalang

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ABSTRACT

Leucaena leucocephala or locally known in Malaysia as ‘petai belalang’ was studied as an alternative material to produce sugar from cellulose. Analyses of the dried samples contain about 25% lignin, 50% cellulose and 17% hemicellulose. This dried sample was pulverised, mixed with water (10% w/v) and pre-treated by autoclaving. The resulting liquid and solid fractions were enzymatically hydrolysed and analysed for sugars, with a yield of 30% and 17%, respectively. Further analyses revealed that the sugars consisted mostly of glucose at 30% from the liquid and 40% from the solid fractions, clearly exhibiting higher content of glucose in the solid fraction. Accordingly, 1kg of fresh petai belalang biomass (84% dry matter) produces 395g of raw sugar, almost 50% of the total fresh weight. In a circular economy, this exhibits the potential of using waste biomass from petai belalang that can be utilised to produce raw sugar. Other sugars were traces of arabinose at 2% and cellulobiose at 1%. Purification of this raw sugar by filtration on PAC removed about 80% of the sugar, with a remaining amount of 87g (22%) purified glucose, at 55g and 32g from the liquid and solid portions.

Keywords: *Leucaena leucocephala*, glucose, enzymatic hydrolysis, powdered activated charcoal.

INTRODUCTION

Leucaena leucocephala is a small, fast-growing mimosoid tree native to Southern Mexico and northern Central America (Belize and Guatemala) but is now naturalised throughout the tropics. Common names include river tamarind, white lead tree, jumbay, and white popinac. (Wikipedia, 2023). The legume is promoted in several countries of Southeast Asia as a source of quality animal feed, since it provides an excellent source of high-protein cattle fodder due to the high protein content, easily digestible and fast growth.

Petai Belalang is one of those plants which contain high content of lignocellulosic materials consisting of cellulose, hemicellulose and lignin. Lignocellulosic material is a renewable and cheaper source of sugar for biofuel production. Normally, these materials need to undergo pre-treatment and enzymatic hydrolysis to open up the structure of lignocellulosic materials to ease the conversion of cellulose components into sugars, as reported elsewhere with sago fibre (Adeni *et al*., 2010). A similar approach was attempted to this plant in our effort to obtain non-table sugars.

In Malaysia *Leucaena leucocephala* has come to prominence after being identified as a major feedstock for power generation, animal feed and downstream biochemical production. A major component of the value chain involves the conversion of woody biomass from *Leucaena leucocephala* into industrial sugar, which will then be converted to various downstream biochemicals (MOSTI, 2012).

(a) **(b)**

FIGURE 1: *Leucaena leucocephala* or petai belalang (a) top part of young shrubs (b) fruiting portion.

The purpose of this study is to observe the possibility of producing sugar from petai belalang twigs and branches. This prospect will be useful, if the cellulosic contents is high, of at least 50% to be commercially viable. The cellulosic components (cellulose and hemicellulose) under proper and economical pre-treatment procedures can be enzymatically hydrolysed into sugars.

MATERIALS AND METHODS

SAMPLES

Leaves from locally obtained petai belalang samples were removed and the twigs (5-8mm diameter) were sliced into smaller pieces. This size reduction enhanced the drying process but may increase to the total volume to be dried in the oven. Oven drying was performed at 70-80^{o}C for a couple of days to assist in its pulverisation using a stainless steel grinder. Ground samples were filtered (710 µm) and stored at room temperature prior to use.

PRE-TREATMENT OF GROUND AND DRIED SAMPLES

The pre-treatment process (10% w/v) was modified from the method used by Thomsen and Schmidt (1999) and performed using an autoclave (121°C for 30 minutes). The solid and liquid fractions were separated and hydrolysed for extraction of sugars. Starch was quantified using iodine starch colorimetric method (Nakamura, 1981), respectively.

ENZYMATIC HYDROLYSIS OF THE LIQUID PORTION TO SUGARS

About 500ml separated syrup (supernatant) and 500g hydrolysed solids were obtained, to undergo cellulosic enzymatic hydrolysis for 16 hrs, using Thermamyl 120-L (pH6.5, 90-95^{o}C) and Dextrozyme (pH4.5, 60-65^{o}C) for liquefaction and saccharification, respectively (Ugam and Bujang, 2009; Bujang, 2011; Adeni et al 2013).

ENZYMATIC HYDROLYSIS OF THE SOLID PORTION TO SUGARS

The solid residual was treated with a combination of 20% cellulase and 0.5% β-glucosidase, followed by 0.1% enzyme complex to release cellulose from the fibres and subsequently hydrolysing this into sugars. These were done at the pH5.0 and 37°C in distilled water, at 100g sample/L of water (Ugam and Bujang, 2009; Bujang, 2011; Adeni et al 2013).

ANALYSIS OF SUGARS AND ITS BY-PRODUCTS

In order to observe different sugar components in petai belalang, sugar samples obtained from both liquid and solid portions were analysed using an HPLC (Shimadzu), at the standard parameters and compared with standard sugar solutions (Bujang *et al*., 2012).

PURIFICATION OF SUGARS

The sugar syrup was centrifuged, sterilised and course-filtered to remove residual micro-solids, and then purified by filtration on powdered activated charcoal (PAC) for colour removal. The sugar concentration was analysed before and after this filtration (Bujang *et al*., 2012).

RESULTS AND DISCUSSION

GRINDING AND DRYING OF SAMPLES

It was observed that it was almost impossible to grind fresh samples due to the pliable nature of the stems and twigs of this woody material. Fresh stems and twigs simply fused and swivelled together with the blade of the grinder and hence unable to be pulverised. Sliced and dried and samples were pulverised using a steel grinder (Figures 2a & 2b) and filtered (710 µm) prior to use.

Figure 2a: Dried stems and twigs of Petai Belalang

Figure 2b: Grinding of dried sample

ANALYSES OF LIGNOCELLULOSIC COMPONENTS

Upon analyses on HPLC, the lignocellulosic component of petai belalang used in this study was 51.7% cellulose, 16.8% hemicellulose and 24.8% lignin, as shown in Table 1 below.

TABLE 1: Characterisation of lignocellulosic components of Petai Belalang samples

Component	Percentage (%)
Lignin	24.8
Cellulose	51.7
Hemicellulose	16.8
Others (ash, starch, free sugars, etc.)	6.7

From this table, the potential use of petai belalang for the production of sugar will be limited by the amount of cellulose and hemicellulose (a total of about 69-70%). However, it is well known that the process is also highly dependent on the types of pre-treatment processes, the ability of the enzymes to degrade the cellulosic components into digestible starch and subsequently to fermentable sugars.

PRE-TREATMENT OF GROUND (DRIED) SAMPLES

A total of 100g ground samples (from Figure 2b) was autoclaved in 1L of water (10% w/v). The resulting products are a mixture of liquid and solid portions, and sugars were anaylsed in each portion.

ENZYMATIC HYDROLYSIS TO SUGARS

The liquid portion (approximately 1L) was analysed for starch, and followed by enzymatic hydrolysis to obtain the reducing sugar. The results were very small amount of starch (0.89%) and the sugar content was at 30.46% as shown in Table 2 below.

TABLE 2: Starch and reducing sugar content of the liquid portion upon pre-treatment by autoclaving 100g of sample (approx. 84% dry matter) in 1L distilled water.

Contents	Recovery (%)
Starch	0.89 ± 0.09
Reducing sugars	30.46 ± 1.2

The solid portion was treated with 20% cellulase, 0.5% β-glucosidase and 0.1% enzyme complex. Different sequence of treatments was performed such as addition of cellulase followed by β-glucosidase, or addition of cellulase followed by β-glucosidase together with enzyme complex. The resulting sugar yield was similar for both sequences, at about 17g/100g sample, or approximately 17% recovery.

The quantity of sugars in petai belalang was approximately 30g and 17g, extracted from 100g of pre-treated ground samples, in the liquid and solid portions, respectively (Table 3). This gave a final yield of 30% from the liquid, while the residual sugars can be extracted at about 17% from the solid portion.

TABLE 3: Yield of sugars from the liquid and solid portions of petai belalang.

Portions	Recovery (%)
Liquid	30.46 ± 1.20
Solid	17.24 ± 0.66

It is exhibited here that, from 100g of treated ground samples which contain 70% cellulosic components (Table 1, before), the total yield of sugars from petai belalang can be expected to be approximately 50% by weight, as a cumulative amount from the liquid (31%) and solid (17%) portions. This is a considerable amount that can be obtained from a biomass residue from animal feed.

ANALYSES OF SUGARS AND ITS BY-PRODUCTS

Sugar samples obtained from both liquid and solid portions were analysed on an HPLC (Shimadzu). Sugar samples from the liquid and solid portions were analysed separately on the HPLC, but using the same sugar standards, and the results is shown in Table 4 below.

TABLE 4: Types and yield of sugars from the liquid portions.

Parameters	Liquid portion (%)	Solid portion (%)
Glucose	**30.20 ± 0.16**	**38.66 ± 1.96**
Arabinose	2.29 ± 1.79	0.08 ± 0.11
Cellulobiose	1.01 ± 0.02	1.23 ± 0.17
Acetic acid	0.13 ± 0.002	0.10 ± 0.03
Lactic acid	0.59 ± 0.11	0.17 ± 0.08
Ethanol	0.31 ± 0.01	0.23 ± 0.05

The major sugars which made up the bulk of the sugar content in petai belalang (about 65-70%) have been reported to be galactose, mannose, rhamnose and arabinose (Rincon *et al*., 2007; Gamal-Eldeen *et al*., 2007; Singh and Bahadur, 2010). Maltose was analysed but not detected in our samples of petai belalang, while galactose, mannose and rhamnose were not detectable due to the absence of specific columns and standards for our HPLC system.

Evidently, glucose is one of the main components of all the reducing sugars extracted from both the liquid and solid portions, when hydrolysed using the standard enzymatic method, at 30% and 39%, respectively. Apart from these, there are residual amount of arabinose, cellobiose, lactic acid, acetic acid and traces of ethanol, as shown in Figure 3 below.

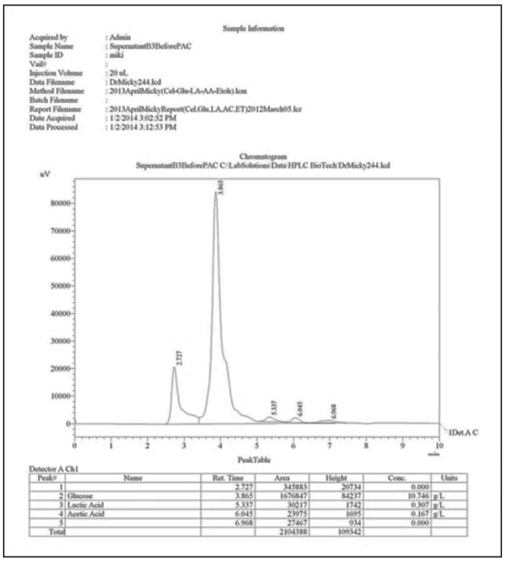

Fig. 3: Analyses of sugars from enzymatic hydrolysis of petai belalang.

One of the oldest sweetness ratings is the comparison of the unknown sample in relative sweetness to that of sucrose (common table sugar). Sucrose is rated at a 1.0 rating and all other sweeteners are rated either more (fructose 1.1-1.8, Aspartame 180) or less (maltose 0.4, lactose

0.4). Even then, various reports still have various results when compared to other researchers (Ramsey, 2000).

The sweetness of galactose is about 0.32, much lesser than glucose at 0.50 when compared to sucrose (subjectively assumed as 1.0), as shown in Table 5 below. Mannose sugar is not sweet and never consumed as sweeteners but more as antibiotics for urinary tract infections (Wiessner, 2004). Rhamnose is a natural inert sugar, but as in mannose, this is not metabolised by the human body and as such, never commercialised as table sugar (Sahelian, 2014).

TABLE 5: Relative sweetness of sugars compared to sucrose (Ramsey, 2000)

Types of Sugar	**Sweetness**
Lactose	0.15
Maltose	0.30
Galactose	0.32
Maltose & Lactose	0.40
Glucose	0.50
Sucrose	1.00
Fructose	1.10 – 1.80
Sodium cyclamate	30
Aspartame	180
Saccharin	350
Neohesperidin dihydrochalcone	1000
Perillaldehyde antioxime	2000
1-n-Propoxy-2-amino-4-nitrobenzene	4000

However, in the same paper, Gamal-Eldeen *et al*. (2007) reported that starch (polysaccharide) extracted from the seeds of petai belalang has anticancer properties, produced by simple modification of the inactive starch – which should be a bonus in mass cultivation of this shrub.

PURIFICATION OF SUGARS

Centrifuged, sterilised and course-filtered sugar solution was purified by filtration on powdered activated charcoal (PAC). The sugar concentrations analysed after each filtration is shown in Table 6 below. Evidently, upon purification by filtration on PAC, the remaining glucose was about 22% (w/w) from both the liquid and the solid portions.

TABLE 6: Types and yield of sugars from the liquid and solid portions after purification on PAC

Sugars after purification	**Liquid portion (%)**	**Solid portion (%)**
Glucose	**22.04 ± 0.67**	**21.63 ± 1.36**
Arabinose	Not available	Not available
Cellulobiose	0.59 ± 0.09	0.69 ± 0.01
Acetic Acid	0.075 ± 0.01	0.09 ± 0.02
Lactic Acid	0.14 ± 0.03	0.13 ± 0.02
Ethanol	Not available	Not available

Apparently, the small concentrations of arabinose become negligible upon filtration, presumably to be fully absorbed by the PAC. Adsorption occurs between of PAC towards glucose and lactic acid was reported by Ang *et al.* (2006). Using PAC filtration to eliminate colours and impurities inadvertently also absorbs some of the glucose.

CONCLUSION

Petai belalang (stems and branches) must be dried prior to pulverisation into powder. Drying can be done under the sun, but an oven (70-80ºC for a couple of days) would provide a more uniform distribution of dried biomass. The total amount of reducing sugars is around 47% (w/w), as a cumulative amount from the liquid (30%) and solid (17%) portions.

Hence, 1kg of fresh petai belalang (84% dry matter, or 840g), dried, autoclaved and soaked in 10L of water, will generate 252g (30%) and 143g (17%) glucose from the liquid and solid portions, respectively. As such, the total amount of raw sugar (from both solid and liquid portion) from every 1kg of petai belalang is 395g, or almost 50% of the fresh weight. Purification of this raw sugar by filtration on PAC removed about 80% of the sugar, with a remaining amount of 87g (22%) purified glucose, at 55g and 32g from the liquid and solid portions. The main component of the raw sugars in petai belalang are galactose, mannose, rhamnose and arabinose (Rincon *et al*., 2007; Gamal-Eldeen *et al*., 2007; Singh and Bahadur, 2010).

Conclusively, it is possible to utilise the branches and stems of petai belalang to produce industrial grade or raw sugars at about 50% yield. This comes as an advantage in utilising agro-wastes following the harvest of leaves and twigs for animal fodder towards the concept of zero-wastes and circular economy in farms.

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