Faculty of Resource Science and Technology

EFFECT OF STORAGE ON NIPA PALM SAP AS FEEDSTOCK FOR BIOETHANOL FERMENTATION

Nur Afiiqah binti Taib

(50742)

Bachelor of Science with Honours
(Resources Biotechnology Programme)
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Effect of Storage on Nipa Palm Sap as Feedstock for Bioethanol Fermentation

Nur Afiiqah Binti Taib

(50742)

This project is submitted in partial fulfilment of the requirement for degree of
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FACULTY OF RESOURCE SCIENCE AND TECHNOLOGY

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Effects of Storage on Nipa Palm Sap as feedstock for Bioethanol Fermentation

Nur Afiiqah Bint Taib (50742)

Resources Biotechnology Programme
Faculty of Sceince and Technology
Universiti Malaysia Sarawak

ABSTRACT

Nipa Palms *(Nypa fructicans)* is one of the most widely distributed and beneficial plants in the mangrove forest of Malaysia. It permits diverse uses traditionally. From the leaves of the palm to the sap it confers various function to users. In Sarawak, Nipa palm saps are mainly utilized to produce Gula Apong which are used as flavour enhancer in every cooking. However, the sap can get spoiled easily without proper method of storage. Therefore, it is discarded as it serves no more purpose in making Gula Apong. This paper attempted to review the sugar and ethanol profile in Nipa palm sap stored in different surrounding temperature and conditions. The most economical method to store Nipa sap for bio ethanol production is at ambient room temperature without any sterilization process. 30 g/L of ethanol was produced through such method. Sugar profile of glucose showed drastic reduction on the 48 hour mark post to storage. Other than glucose, xylose and sucrose was distinguished and displays similar pattern..

Key words : Nipa Palm sap, *Nypa fructicans,* Storage method, Sterilization process, Bio ethanol profile, Sugar profile

Abstrak

Pokok Nipah (Nypa fructicans) merupakan antara pokok yang mempunyai populasi yang tinggi and bermanfaat di hutan bakau Malaysia. Ia banyak digunakan dalam kehidupan masyarakat secara tradisional. Seluruh bahagian pokok Nipah ini menghasilkan pelbagai fungsi kepada pengguna. Di Sarawak, air Nipa digunakan dalam penghasilan Gula Apong yang dugunakan sebagai penambah rasa dalam masakan. Walaubagaimanapun, air nipah ini boleh rosak dengan mudah tanpa kaedah penyimpanan yang betul. Oleh itu, air Nipah akan dibuang kerana air Nipah yang basi tidak boleh digunakan dalam pembuatan Gula Apong. Kajian jini dihasilkan bertujuan untuk mengkaji semula profil gula dan bio etanol yang disimpan dalam suhu dan keadaan sekitar yang berlainan. Kaedah yang paling ekonomik untuk menyimpan Nipa sap untuk pengeluaran bio ethanol adalah pada suhu bilik ambien tanpa sebarang proses pensterilan 24 g / L etanol dihasilkan melalui kaedah sedemikian. Profil gula glukosa menunjukkan pengurangan drastik pada tanda 48 jam penyimpanan. Selain daripada glukosa, xilosa dan sukrosa juga ditemui dan kedua dua gula tersebut memaparkan corak yang sama.

Kata Kunci : Air Nipah, Nypa fructicans, Kaedah penyimpanan, Proses pensterilan, Profil bioethanol, Profil gula

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LIST OF ABBREVIATION

HPLC	High Performance Liquid Chromatography
Na	Sodium
Cl	Chloride
g	Gram
L	Litre
°C	Degree celsius
%	Percentage
h	Hectare
mmol	Milimolar (Concentration)
CO_2	Carbon dioxide
mL	mililitre
rpm	Revolution per minute

CHAPTER 1 : INTRODUCTION

Nipa palm *(Nypa Fructicans)* saps are exclusively use by the local citizens to produce Gula Apong which are used as flavour enhancer in various cooking. In the process of making good quality gula apong, fresh Nipa sap is required. Nipa sap collected is cooked at high temperature at least 5 hours post to collection observed through gula apong processing in Kampung Pinggan Jaya, Kota Samarahan, Sarawak. It is recorded that 0.5 to 2.5 L/palm/day of Nipa sap is collected and processed immediately as the sap can get spoiled easily (Tamunaidu & Saka, 2012). Spoiled sap will then discard as the sap no longer serve any purpose in gula apong production. According to Tamunaidu and Saka (2012), Nipa sap is able to self-ferment without nutrient supplements resulting production of bio-ethanol. Therefore, unused sap or spoiled sap can be further ferment to produce bio-ethanol.

Global utilization of biofuel has come to its peak as it is an excellent substitute for fossil fuel. Rapid depletion of fossil fuels due to exploitation in meeting world's demand has induced production of biofuel and bioethanol (Deenanath *et al.*, 2000). Petroleum-based fuels have generated massive environmental concerns such as global warming, health hazards and ecological imbalance (Nguyen *et al.*, 2016). Therefore, sustainable and environmentally-friendly energy sources have generated significant interest in developing biofuel production from plant biomass (Kim & Dale, 2003).

Generally, bioethanol is used in the production of biofuel that is widely used as biofuel additives in gasoline production. Hence, production of bioethanol is much needed in order to produce biofuel. According to Walker (2010), bioethanol is produced via fermentation process where microorganism that is capable of converting simple sugars to alcohol and carbon dioxide is used. Nipa Palms are common for its diverse commercials and services benefits. It is well preserve as it brings about food and sources of materials for the local people to utilize (Tsuji *et al.*,2011). From the inflorescences to the sap of the palm and the seeds are collected as it is edible and have become staple food sources for the local people. However, few attentions have been given to its sap as particularly as a potential feedstock for bioethanol production.

As stated by Hamilton & Murphy (1988), Nipa could produce a higher amount of bio-ethanol than sugarcane and cassava at lower cost. This research advocates that Nipa sap, which is readily simple sugars would produce higher yield of bioethanol in contrast to other crops. However, restrictions on immediate fermentation on sap can also indirectly affect production on bioethanol from Nipa sap. Therefore this research is conducted to further study the effect on its sugar profile particularly if it has been stored for certain

period of hours.

1.2. Objective of this study:

I. To examine the sugar profiles of Nipa Palm Sap which varies in period and method of storage.

II. To study bioethanol production by utilizing Nipa palm sap as sole fermentation media via existing microorganisms present in the sap.

CHAPTER 2 : LITERATURE REVIEW

2.1. Nipa Palm (*Nypa Fructicans)*

Nipa (*Nypa fructicans* Wurmb) is a part of the Palmae or Arecaceae family. The genus *Nypa* is placed in the subfamily of Nypoideae (Moore 1973; Uhl & Dransfeld 1987; Whitmore 1973). Nipa is the only palm considered to be mangrove because it prospered well in the mangrove environment specifically in upper tidal areas with high water salinities. Nipa palms are widely distributed across South East Asia; Malaysia, Singapore, Indonesia and Brunei. Okugbo *et al.* (2014) reported that the trunk of Nipa Palm cultivate horizontally beneath the ground permitting only the leaves and flower stalk to grow upwards above the surface.

Nipa palms confer several qualities in terms of sustainability for bio-fuel production such as it is easily available and has a year round supply. Nipa palms are perennials which indicate that it can be tapped all year round (Okugbu *et al.*, 2014). Furthermore, tapping produce less waste production and the sap can be drained every day without the need to harvest the plant (Nguyen *et al.*, 2016). Other than that, Nipa Palm sap have the highest sugar yield as the sap sugar content is higher compared to other crops. High sugar yield will result high yield of bioethanol production as it is directly proportional to one another. According to Hamilton and Murphy (1998), Nipa Palm sap can produce higher ethanol yield by traditional management than sugarcane although sugar cane have higher sugar content. Total sugar content of sugarcane sap is recorded as 150.3 g/L whereas Nipa Palm sap contains approximately 144 g/L. According to Halos (1981), the amount of bioethanol production is expected to increase by 40% by current technology. This proves that bioconversion performed by Nipa Sap is more efficient than sugarcane. Table 1 shows production of ethanol of different crops.

Table 1. Bioethanol production of different crop (Nguyen *et al.*, 2016).

Crop	Bioethanol (L/hectare/year)
Nipa Palm sap	6480-10224
Sugarcane	3350-6700
Cassava	3240-8640
Sweet Potato	6750-18000
Coconut sap	5000

2.2. Nipa Palm Sap

Sap is a watery fluid that transports plant photosynthetic products throughout the plant (Harifara & Saka, 2016). These saps contain high amount of free sugar such as sucrose, glucose and fructose that can be fermented to bioethanol (Tamunaidu & Saka, 2012). Nipa sap can be obtained by non-destructive tapping after the palm have reached 5 years of age and it will continue to produce sap until 50 years old (Nguyen *et al.*, 2016). Nipa palm is considered as sustainable as this species it perennials which can be tapped all year round. Sap production in *Nypa fructicans* is formed without accumulation of starch, it is form directly from soluble sugars from photosynthesis in the leaves (Die & Tammes, 1975). It is collected removal of its inflorescence and through tapping method applied to the stalk of the palm. Tapping periods take 60 to 340 days and sap yield per palm could reach 1.3 L per day.

Factors that affect sap yields include tapping time, taping method sex and age of the palm, weather and environment (Nguyen *et al.*, 2016). Sap yield can reach a maximum just before or during flowering and fruiting. The pre-treatment of inflorescence or stalks is essential to achieve high sap yield by removing slime and P-protein from the transport system. Nguyen *et al.*, (2016) reported that these two components will disrupt the flow of sap in the phloem channel. Palm age is also able to affect sap yield where middle-aged palm have been reported to give sap yield. In addition, Hamilton and Murphy (1998) reported that Nipa palm saps with higher sugar content are produced during hot day. Water inundation can reduce sap production as well as sugar content of sap.

Sucrose, glucose and fructose are the main components of Nipa palm sap. Ethanol, organic and inorganic acids constitute 10% of the sap component. According to Nur Aimi *et al.* (2013), fresh Nipa palm sap contained ethanol, dieacetyl and esters as volatile compounds which were detected via gas chromatography-mass spectrophotometry. Inorganic compounds, particularly sodium (Na) and chloride (Cl) exist in high quantity in Nipa palm sap (Tamunaidu *et al.*, 2012).

2.3. Bioethanol Production

Bioethanol is derived from fermentation of sugar from biomass that will not produce immediate harm to the environment (Gable, 2017). Bioethanol acts as an alternative to petroleum- based fuel production to minimize demand towards non-renewable resources (Gray *et al.*, 2006). Bioethanol production confers sustainability in biofuel production which results in more bioconversion via fermentation to be pursued by developed and developing countries.

Fermentation of sugarcane molasses in Brazil have been the global model for bioethanol production and use (Okugbu *et al., 2012).* Sugarcane molasses which contain high levels of simple sugars which permits the easiest way to be converted into ethanol as saccharification is no longer needed. As explained by Dung *et al.* (2016), total sugars in gram per litre of sugarcane juice is 150.3 whereas Nipa Palm sap contains 5.8 g/L lesser. This indicates that sugarcane juice act as better feedstock for bioethanol production. However, sugarcane juices are widely utilized in Malaysia's sugar industry. Therefore, sugarcane juice is not compatible to be used as feedstock for bio ethanol production in Malaysia as it can initiate food security issues. Nipa Palm sap is a good alternative to be used as feedstock in bio ethanol production.

Fermentation process in bioethanol production involves conversion of simple sugars such as glucose, sucrose and fructose into cellular energy, producing bioethanol and carbon dioxide (CO_2) as a by-products. Such bioconversion process requires a microbe which contains specific enzymes and substrate. In this case, substrate will be simple sugars of the

Nipa Palm sap. Optimal condition of fermentation process includes constant temperature; 25 to 30 °C and water which is present in the sap. The presence of oxygen will allow the host such as yeast to oxidize sugar completely to produce CO_2 and water in a process called cellular respiration. Conversion requires enzymes needed to catalyse reaction such as invertase and zymase which are provided by microbes.

2.4. Brix Test

Brix refractometer enables identification of sugar contents of an aqueous solution (Tamunaidu & Saka, 2012). Sugar analysis via Brix test determines sucrose concentration in percent or degree Brix which is demonstrated in Quality Evaluation of Sugar Beet by Roggo *et al.*, 2004. For sugars other than sucrose, it is known as the apparent Brix which is a relative value (Toledo, 2014). In this particular research, Brix test is conducted primarily to distinguish sucrose concentration in Nipa sap throughout period of storage.

2.5. High Performance Liquid Chromatography (HPLC)

HPLC incorporates column chromatography that separates, identify and quantify components such as alcohols, sugars and major acids in a liquid solvent (López & Gómez,1996). It consists of two phase, similar with simple column chromatography that is the mobile phase and stationary phase. At high pressure, HPLC pumps sample mixture in a solvent which acts as the mobile phase through a column with chromatographic packing material as the stationary phase (López & Gómez,1996). The sample is carried by moving gas carrier which can be either helium or nitrogen. Generally, main components of HPLC system include the solvent reservoir, a high-pressure pump, a column, injector system and detector.

CHAPTER 3 : MATERIALS, APPARATUS AND METHODS

3.1. Materials & Apparatus

3.1.1. Nipa Palm Sap

Nipa palm sap was collected at Kampung Pinggan Jaya, Kota Samarahan that are freshly tapped and quickly transfer into 3 bottles; 2 are sterilized prior to sampling and 1 of it will not be sterilized.

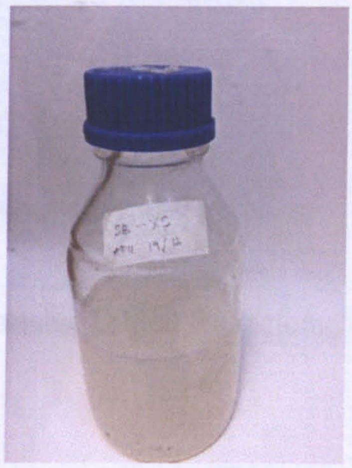

Figure 1. Unsterilized Nipa Sap

Figure 2. Sterilized Nipa Sap.

3.1.2. Brix Refractometer (% Brix)

Portable Digital Refractometer H196801 (Hanna Institution, USA) was used to measure the % Brix of Nipa sap.

Figure 3. Portable Digital Refractometer H196801.

3.1.3. High Performance Liquid chromatography (HPLC)

HPLC (Shimadzu corporation, Japan) was used in analytical procedures.

3.2. Methods

3.2.1. Site visit

On the 5th of November 2017, we have visited Kampung Pinggan Jaya, Kota Samarahan and get to know the owner of Gula Apong processing company of that area. Sensory test was conducted to differentiate grades of gula apong which include grade A being the hardest, grade B and grade C being the most liquid. Grading is done according to the texture of gula apong, emphasizing on the state of the gula apong whether it is in aqueous state or solid state.

Information on Nipah sap collection was obtained where the collection is done by using bamboo instead of plastic bottles. It is said that bamboo will produce Nipah sap with longer longevity compared to plastic bottle. Bamboo can be used up to 4 to 5 years of usage which is more economical compared to other materials. The collection process is executed on daily basis. Currently, expired Nipah sap is reuse to make gula apong with 1 to 9 ratio of expired sap to fresh sap respectively. The remaining of expired sap is discarded as the community have not found any use for expired sap.