Faculty of Resource Science and Technology

FRESH NYPA PALM SAP AS A SOURCE FOR PRODUCTION OF SELECTED VALUE ADDED PRODUCT

NORADILA BINTI OTHMAN

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FRESH NYPA PALM SAP AS A SOURCE FOR PRODUCTION OF SELECTED VALUE ADDED PRODUCT

NORADILA BINTI OTHMAN (58909)

This project is submitted in partial fulfilment of the requirement for the degree of Bachelor of Science with Honours (Resource Biotechnology)

Supervisor: Dr. Dayang Salwani binti Awang Adeni

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List of Abbreviations

N. fruticans	*Nypa fruticans*
°C	Degree Celcius
g/L	Concentration (Gram per Litre)
L	Liter
OD	Optical Density
rpm	Revolutions per minutes
HPLC	High Performance Liquid Chromatography
mL	millilitre
L/hectare/year	Litre per hectare per year
G	Gram
%	Percentages
UNBNS	Unsterilized Bottle with Nipa Sap
SBNS	Sterilized bottle with Nipa Sap

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FRESH NYPA PALM SAP AS A SOURCE FOR PRODUCTION OF SELECTED VALUE ADDED PRODUCT

Noradila Binti Othman

Programme of Resource Biotechnology
Faculty of Resource Science and Technology
University Malaysia Sarawak

ABSTRACT

Nypa fruticans are common widely distributed and useful palms in the mangrove forest of Southeast Asia. Nipa saps are source of sugars, minerals yeasts and nutrients which can be suitably used as raw for bioethanol fermentation. In addition, it is due to the really high percentage of sugars in nipa sap. Nipa sap sugar material consists of sucrose, glucose and fructose. Thus, no additional carbon sources were added in this study. The naturally existing microorganisms in nipa sap will act as the inoculum and the naturally existing sugar will act as the substrate for the production of bioethanol and others value added product such as lactic acid and acetic acid. Two different conditions were introduced to obtain the most economical way in producing value added product from nipa sap fermentation with the least amount of investment needed such as unsterilized bottle with unsterilized sap and also sterilized bottle with unsterilized sap. Although these two methods consumed almost similar level of energy for fermentation, sterilized condition was found to be more efficient. The profile of sugars and bioethanol was analysed by using High Performance Liquid Chromatography (HPLC). At the end of this experiment, the initial carbohydrate content in sterilized and unsterilized bottle were at 121.207 g/L respectively while the ethanol obtained was 13.181 g/L and lactid acid was 6.644 g/L and acetic acid was 0.855 g/L. The results showed that nipa sap actually can be used as alternative feedstock for bioethanol production and other values added product without any supplementation. Hence, those characteristics make nipa sap an interesting feedstock to be exploited for sustainable ethanol productions and other value added products.

Keywords: *Nypa fructicans*, nipa sap, bioethanol, glucose, lactic acid, sterilized unsterilized.

ABSTRAK

Nypa fructican *adalah pokok yang biasa diedarkan dan berguna di hutan bakau di Asia Tenggara. Jus nipa adalah sumber gula, yis mineral dan nutrien yang boleh digunakan sebagai bahan mentah untuk penapaian bioetanol. Gula dalam jus nipah terdiri daripada sukrosa, glukosa dan fruktosa. Oleh itu, tiada sumber karbon tambahan telah ditambah dalam kajian ini. Mikroorganisma yang wujud secara semulajadi dalam nipa sap akan bertindak sebagai inokulum dan gula secara semula jadi yang sedia ada akan bertindak sebagai substrat untuk pengeluaran bioetanol dan produk nilai ditambah produk seperti asid laktik dan asid asetik. Dua keadaan yang berbeza telah diperkenalkan dalam menghasilkan produk tambah nilai dari penapaian nipa sap dengan jumlah pelaburan yang paling sedikit yang diperlukan seperti botol tidak tersterilisasi dengan sap tidak disteril dan juga botol yang disterilkan dengan sap tidak disteril. Walaupun kedua-dua kaedah ini menggunakan tahap tenaga hampir sama untuk penapaian, keadaan yang disterilkan didapati lebih cekap. Profil gula dan bioetanol dianalisis dengan menggunakan Cecair Kromatografi Berpretasi Tinggi atau dikenali sebagai (HPLC). Pada akhir eksperimen ini, kandungan karbohidrat awal dalam botol steril adalah 121.20 g / L manakala etanol yang diperolehi ialah 13.18 g / L dan asid laktid ialah 6.64 g / L dan asid asetik adalah 0.85 g / L. Hasilnya menunjukkan bahawa jus nipah sebenarnya boleh digunakan sebagai bahan baku alternatif untuk pengeluaran bioetanol dan nilai tambah produk lain tanpa sebarang suplemen. Oleh itu, ciri-ciri tersebut menjadikan nipa sap satu bahan makanan yang menarik untuk dieksploitasi untuk pengeluaran etanol yang mampan dan produk tambah nilai yang lain.*

Kata kunci: Nypa fructicans*, jus nipah, bioetanol, glukosa, asid laktik, asid asetik , steril, tidak tersterilsasi*

CHAPTER 1: INTRODUCTION

In current state of global environment, there has been major increases of human population, thus the demands for fossils fuels are also drastically increasing year by year. One of the common widely distributed and useful palms in the mangrove forest of Southeast Asia is nipa (*Nypa fruticans*). Nipa is very useful for source of biofuel because it produces high amount of sap that can be converted to alcohol. It can produce more ethanol than sugar cane (Hamilton and Murphy, 2018). Recently, Malaysian scientists realized the usefulness of nipa and strategies for effective management of nipa were proposed (Latif, 2008). This uses continues and some have become the bases for cottage industries and commercial operations. More recently, some large-scale commercial interest has developed.

Currently fossil fuels have been major sources for certain sectors such as industrialization and also the most crucial utilization is for transportation and it has caused a very huge impact and worst effect toward the environment and human in whole country. There are many uses of bioethanol in pharmaceutical as well as medicinal purpose such as production of hand sanitizers and biofuels. This bioethanol can produce by industrial sectors within a short time compared to fossil fuels.

In other hand, the global ethanol supply is produced from main sugar and starch feedstock. Bioethanol can obtain from many type of palm. However the main highlights for this study is Nypa palm (*Nypa fructican*) type. Tamunaidu et al. (2011) has stated that besides common sugar and starch feedstock, palms have been a sugar-prospective from the ancient days. According to Dalibard (1999), main sugar yielding palms like palmyra palm (*Borassus* *flabellifer*), sugar palm (*Arenga pinnata*) and nipa palm (*Nypa fructicans*) are believed to produce more nutritious sugars than cane sugars. As well as being used as edible sugars, interests on the production of alcohol fuel also emerged from these resources. (Dalibard, 1999).

Nipa palms are selected in this experiment because not only it has a really high commercial values, according to Tamunaidu *et al*., (2013) nipa palm also produce nipa juice also known as nipa sap which contain high sugar content that consists of glucose, sucrose and fructose that can be undergo fermentation which can be used for different purpose to produce bioethanol and other organic acid such as lactic acid and acetic acid. However most of the people did not realized the potential of nipa sap itself because the local citizens have been using this nipa sap only to produce 'Gula Apong' and vinegar. Thus, in this study is to improve and increases the utilization of nipa sap of value added product other than producing sugar only. Moreover the distribution of nipa palm in Sarawak is quite high that make the accessibility easier to make this study within Sarawak.

This feedstock will be collect and will undergo fermentation in order to produce another type of useful feedstock rather than just producing sugar for bioethanol. Therefore this research is conducted to further study to production of ethanol and other value added products that can commercialize. However, some factors need to be taken into consideration including pH, unsterilized and sterilized condition and also the method of fermenting nipa sap.

Thus, the objectives of this study are:

I. To analyse the profile of selected value added products exist in fresh nipa sap due to natural fermentation process.

II. To study the effect of selected value added product in fresh nipa sap that stored in different condition, sterilized and unsterilized bottle.

CHAPTER 2: LITERATURE REVIEW

2.1. Nipa Palm (*Nypa fruticans)*

Nipa (*Nypa fruticans* Wrumb) 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 a monoecious palm with special characteristic it thrives in river estuaries and brackish water environment in which salt and fresh water mingle. The distributions of nipa palm can be found in the Phillipines, Myanmar, Thailand, Malaysia and also Sri Lanka. In addition, nipa palm often form large colonies as they grew in clusters and without visible stems above the ground. Tsuji *et al*. (2011) has stated that rhizome the underground stem lies horizontally underneath ground and able to reach to half meter long. Nipa is lack of an upright stem, trunkless and develops its florescence at a high about 1 meter that make nipa differs from most palms (Uhl, 1972).

Figure 1: Nipa Palm in Kampung Pinggan Jaya, Kuching, Sarawak.

Nipa are able to produce higher yield alcohol compared with other crops, (Hamilton & Murphy, 1988) also stated that nipa sap that produce by traditional methods 6,480 - 10,224 L/hectare/year, sugarcane 3,350 - 6,700 L/hectare/year, 3,240 – 8,640 L/hectare/year, sweet potato 6,750 – 18,000 L/hectare/year, coconut sap 5,000 L/hectare/year . As reported by Halos (1981), this record make nipa an attractive potential sources of biofuel is because the amount of alcohol is expected to increase up to 18,165 L through modern management. Due to high amount of sap production that can be converted to alcohol, nipa is also said to be useful for sources of biofuel because sugar that has up 14 to 17% of sucrose (Bamroongrugsa *et al.,* 2004). In addition, nipa palm has a huge contribution in food production. Furthermore, nipa palm grows in all area naturally without the requirement for fertilizers, insecticides or electricity (Tumanaidu *et al*., 2013). Hence, make it suitable for production of bioethanol and other selected value added product such as lactic acid and acetic.

2.2. Nipa Palm Sap

Nipa palm sap or also known as nipa sap is the watery fluid which is phloem sap produced by the nipa plant and the uses is to act as medium to transport the nutrients to the whole plant. Furthermore, according to Fong (1992), and Hamilton & Murphy (1988), nipa yields abundant sap from the cut stalks of fully develops young inflorescences after the floral or fruits heads have been removed. In additions, nipa saps can be yielded continuously throughout the year making it suitable to be used as medium for bioethanol fermentation and also as a sustainable feedstock for the production of bioethanol and other value added product such as acetic acid and lactic acids. Apart from that, nipa sap is an attractive feedstock due that it has high content of sucrose, glucose and fructose. It also can easily being collected causing no waste and no effect on the palm growth.

Palm sap is tapped from the matured unopened inflorescence of the palm by cutting the head of inflorescence. According to Whitmore (1973) and Hamilton & Murphy (1988), has stated that by beating the young inflorescence and kicking the base of stalk induces higher sap yield. From the study by Gupta *et* al., (1996), reported that freshly collected sap is oyster in white colour, sweet in taste and has opaque appearance with acidic pH. The sap thus collected can be stored range 1-2 hour under ambient condition and 1-2 days under refrigerated condition range between 5-8 °C. Apart from that, Nipa sap also can be marketed ready to as a fresh, ready to serve, and nutritious cold beverages. It also can be converted into different value-added product such as syrup, squash, concentrate, jaggery and sugar.

2.3. Value Added Product from Nipa Sap

2.3.1. Bioethanol Production

Bioethanol is derived from fermentation of sugar from biomass that will not produce immediate harm to the environment if put into use. Balat (2007), has stated that bioethanol, liquid biofuel, having some advantageous properties over gasoline. It can be used directly or blended with gasoline and with diesel as transportation fuel. Vincent *et* al. (2011), has stated that bioethanol have been classified into two different categories first generation and second generation. Bioethanol can be produced from three major types of feedstock, juices or molasses from sugar crops, starches from cereal crops, and lignocellulosic biomass from agricultural residues. However, according to Zabed *et al*. (2014), both sustainability and economic viability are important for the bioethanol industries, and currently the major type of feedstock is sugar crops.

The main feedstock for bioethanol are sugarcane juice in Brazil have been global model for bioethanol production and corn starch in the United State. However, growing the feedstock is relying heavily on non-renewable fossil fuels and exploitation of forest lands which has negative social and environmental impacts. In addition, the saccharification is no longer needed as sugarcane molasses that contain high levels of simple sugars which permits easiest way to be converted into ethanol.

Cellular respiration is a process when the presence of oxygen will allow the host such as microbes that already contain in nipa sap to oxidize sugar completely to produce carbon dioxide (CO_2) and water. Fermentation process in bioethanol production involves conversion of simple sugars such as glucose, sucrose and fructose into cellular energy, producing bioethanol and CO_2 as by-products. Such bioconversion process requires a microbe which contains specific enzymes and substrate. The optimal condition of fermentation process includes constant temperature which is 25 to 30 °C and water is present in the nipa sap.

2.3.2. Production of Lactic acid

According to research by Aimi *et al*. (2013), the sugars found in nipa sap can be gradually fermented to various products, such as ethanol, lactic acid and acetic acid by several naturally occurring yeasts and bacteria. As reported by Nguyen *et al.* (2016). The lactic acid found in nipa sap accounts for 2.1 g/L of its composition and undoubtedly makes a significant contribution to its acidic pH (4.37) and could also act as an acid catalyst for the hydrolysis of sucrose. Although the different components of nipa sap could exhibit catalytic activity towards the hydrolysis of sucrose, however it was not possible to achieve the complete hydrolysis of sucrose (Nguyen *et al.*, 2016).

2.4. High- Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) is powerful tools in analysis of a wide range of compounds, including particular compounds that are polar and unstable (Nambara & Goto, 1988). HPLC is used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. It is a form of column chromatography that pumps a mobile phase which is sample mixture or analytes in a solvent at high pressure through a column with chromatographic packing material also known as stationary phase. The sample is carried by moving gas carrier which can be either helium or nitrogen. Basically, HPLC main component consists of solvent reservoir, high pressure pump, a column, injector system and detector. One of the advantages of using HPLC is it can give excellent resolution of the compound that can be achieved even for closely related as well as structurally disparate substance (Aguilar & Hearn, 1996),

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Nipa Palm Sap

Figure 2 shows that Nipa palm sap was collected at Kampung Pinggan Jaya, Kuching that are freshly tapped and quickly transferred into a bottle.

Figure 2: Fresh Nipa Palm Sap

3.1.2 Reagent and Chemicals

Concentrated Sulphuric acid (H_2SO_4), 5% Phenol, Glucose anhydrous reagent, ethanol and distilled water were used in this study to carry out the experiment.

3.1.3 pH meter

pH meter (Bante Instruments, China) was used in this study determine the pH of the sample).

3.1.4 Spectrophotometer

UV Mini 1240 UV-Vis Spectrophotometer (Shimadzu Corporation, Japan) was utilized to determine the concentrations of glucose by measuring the intensity of light detected.

3.1.5. High Performance Liquid chromatography (HPLC)

HPLC (Shimadzu Corporation, Japan) was used in analytical procedures to determine the total glucose amount in nipa palm sap.

3.1 Method

3.1.1 Site Visit

For sampling fresh nipa sap we have visited Kampung Pinggan Jaya, Kuching on 18th June 2019. Mahli bin Ramli is one of the owners of the gula apong processing company in that village.

Basically the saps are collected daily by the owner himself. According the owner, the collection of nipa palm sap was done by traditional way which is using bamboo. Those bamboo can be used up to 5 years. This is because sap that was collected by using bamboo will last longer than those which collected by using plastic bottles. Thus, it is more economical than other materials.

(a) **(b)**

Figure 3 (a) and (b): Location of site visit which is own Mahli bin Ramli @ Pak Mahli, Lot 224, Kampung Pinggan Jaya, Kuching.

3.1.2. Sample Collection and Preparation

Freshly tapped sap that was collected by traditional tapping method was stored into two different sampling bottles with different types of condition. The first condition is the sample was stored into unsterilized bottle and was labeled as A while, the other one was stored in sterilized bottle and was labeled as B. Sterilized here means the bottle have been autoclave. Table 1 shows the different treatments that were applied to the samples. Each condition was performed in duplicates.

Table 1: Different Type of Condition of Fermentation

	Condition	Method of fermentation
Condition I	Unsterilized sampling bottle + Fresh nipa sap (USBNS)	Natural fermentation (Static, Ambient temperature)
Condition II	Sterilized sampling bottle + Fresh nipa sap (SSBNS)	Natural fermentation (Static, Ambient temperature)