Faculty of Resource Science and Technology

Palm Sap As An Alternative Substrate For Bioethanol Production

Anis Jasmin Binti Azimin (72116)

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Anis Jasmin Binti Azimin

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SUPERVISOR: DR. DAYANG SALWANI BINTI AWANG ADENI

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Palm Sap As An Alternative Substrate For Bioethanol Production

Anis Jasmin Binti Azimin

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

ABSTRACT

Palm sap is a promising commercial fermentation substrate for bioethanol production since it contains free sugar content without requiring any pre-treatment, readily available and abundant. However, to have a place in this competitive industry, palm sap must be of high concentration of fermentable sugar to be used as a raw material in bioethanol industrial applications. In this study, nipa sap and sago fond sap are used to study the potential of palm sap as a raw material to produce bioethanol. Analysis of starch and sugar content in both saps was done to determine if they were suitable for fermentation. It was found that nipa sap and sago frond sap is high in sugar which are 66.87 g/L and 19.16 g/L respectively. On the other hand, available starch found in sago frond sap was also converted to sugar through enzymatic hydrolysis. Both saps were further used as fermentation media for bioethanol production using commercial baker's yeast and undergoes fermentation for 24 hours. The ethanol concentration produced in both saps was determined using High-Performance Liquid Chromatography (HPLC). Based on the result of this study, the ethanol produced from nipa sap and sago frond sap were 2.50 g/L and 6.64 g/L respectively. Both palm sap has high fermentable sugar which is a suitable fermentation substrate as it is renewable and can be obtained easily.

Key words: Palm sap, Bioethanol, Starch, Fermentable sugars, Fermentation.

ABSTRAK

Nira pokok palma adalah substrat fermentasi yang berpotensi untuk penghasilan bioetanol kerana ia mengandungi gula asli tanpa memerlukan sebarang pra-rawatan, mudah didapati dan dalam kuantiti yang banyak. Namun, untuk mendapat tempat dalam industri yang kompetitif ini, nira pokok palma mestilah mempunyai kepekatan gula fermentasi yang tinggi untuk digunakan sebagai bahan mentah dalam aplikasi industri bioetanol. Dalam kajian ini, nira pokok nipah dan nira pelepah sagu digunakan untuk mengkaji potensi nira pokok palma sebagai bahan mentah untuk menghasilkan bioetanol. Analisis kandungan kanji dan gula dalam kedua-dua nira dilakukan untuk menentukan sama ada ia sesuai untuk fermentasi. Kajian ini juga mendapati bahawa nira nipah mengandungi kandungan gula yang tinggi iaitu masing-masing adalah 66.87 g/L dan 19.16 g/L. Selain itu, nira pelepah sagu mengandungi kanji yang boleh dirawat melalui hidrolisis enzimatik untuk menukarkan kanji kepada gula. Kedua-dua nira tersebut seterusnya digunakan sebagai media fermentasi untuk penghasilan bioethanol menggunakan yis roti dan difermentasi selama 24 jam. Penghasilan etanol dalam kedua-dua nira pokok palma ditentukan menggunakan Cecair Kromatografi Berprestasi Tinggi atau dikenali sebagai (HPLC). Berdasarkan hasil kajian ini, etanol yang dihasilkan daripada nira nipah dan nira pelepah sagu masing-masing adalah 2.50 g/L dan 6.64 g/L. Kedua -dua nira pokok palma ini mempunyai potensi sebagai substrat fermentasi kerana ia adalah daripada sumber yang boleh diperbaharui dan dapat diperolehi dengan mudah.

***Kata kunci**: Nira pokok palma, Bioetanol, Kanji, Gula fermentasi, Fermentasi.*

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LIST OF ABBREVIATIONS

%	Percent
ha	Hectares
m	Metre
cm	Centimetre
ton	Tonne
kg	Kilograms
°C	Degree Celsius
wt%	Weight Percent
L	Liter
w/v%	Percent Weight per Volume
g/L	Gram per liter
UV.	Ultraviolet
HPLC	High Performance Liquid Chromatography
mL	Mililiter
µl	Microliter
tons/ha	Tonnnes per Hectares
nm	Nanometer
sp.	Species
mmol	Milimole
rpm	Revolution per Minute
M	Mole
min	Minutes

mL/min	Milliliter per minute
g/kg	Gram per Kilograms
mg/g	Milligram per Grams
g	Grams
mg/mL	Milligrams per Milliliter

CHAPTER 1

INTRODUCTION

1.1 Study Background

Malaysia boasts a diverse range of palm tree species as a tropical country, including the coconut and sugar palm, the sago palm, the nipa palm, and palm oil. Palms are monocotyledonous angiosperms that belong to the *Arecaceae* family (also known as *Palmae*). Palms are beneficial to the environment in terms of ecology because they restore damaged soil and require little water. Palm trees are also economically vital since they have a high commercial value (Srikaeo et al., 2019).

An agro-industrial crop like palm trees has multipurpose uses such as oil extraction from its seeds, thatched roofs, and building materials made from mature leaves and cigarette wrappers from the young leaves. In addition, young palm fruits are collected for food supply, aromatic tea is made from the buds, leaflet midribs are used as broom material, stems are used for firewood, and roots are used to treat various ailments (Nguyen et al., 2016).

Palm sap is a liquid fluid that carries photosynthetic products from plants to different tissues to sustain growth. Palm sap is also in high demand because they have multipurpose uses to make a variety of products, such as fresh juice (sweet toddy), fermented beverages such as “tuak”, syrup, brown sugar, and refined sugar and bioethanol (Dalibard, 2007).

Depending on the species and individual variation, palm trees usually provide sap high in sugar (10-20%). The yields vary greatly depending on the species and how they are managed. When compared to the production of sugarcane, which ranges from 5 to 15 tonnes of sugar per hectare per year, the major tapped palm species (*Borassus flabellifer, Arenga pinnata, Nypa fruticans and Cocos nucifera*) have the potential to yield around 20 tonnes of sugar per hectare when managed in the most efficient manner (Dalibard, 2007).

After being exploited, these palm trees will leave an agricultural waste such as old tree trunks and palm fronds. Zahari et al. (2012) found that agricultural and agro-based-industry waste are the most commercially renewable resources used as raw material for fermentable sugar production. These wastes are plentiful, non-edible, and involve a cost-reducing process. Furthermore, the carbon sources found in agricultural leftovers may reduce the cost of the fermentation substrate, making it perfect as a fermentable substrate and a chemical option for bioethanol production.

Recent years have seen a surge in interest worldwide in developing bioethanol as a sustainable energy source. It has the potential to be employed in the fuel industry as an alternative source of energy and to help reduce emissions of greenhouse gases. Bioethanol is the most widely utilised biofuel since it is environmentally friendly and does not affect the environment (Sakamoto et al., 2012). In addition, Malaysia is a country that is well-known for its agricultural operations, and it is indisputable that the agriculture industry produces a substantial quantity of waste. Thus, due to its abundance, agricultural waste has the potential as a suitable feedstock for bioethanol production.

In this study, the starch and sugar content of nipa palm sap and sago frond sap are analysed to evaluate if they are suitable for bioethanol production using *Saccharomyces cerevisiae* as an inoculum. The sap of palm trees has the greatest potential as a raw material for the production of bioethanol due to its ready availability throughout the year, and it was also abundance (Ilyas et al., 2019; Izwan et al., 2020).

1.2 Objectives

The aim of this study includes:

1. To determine starch and free sugar content in nipa sap and sago frond sap.
2. To increase sugar production from residual starch in palm sap by conducting enzymatic hydrolysis.
3. To study bioethanol production by utilising nipa sap and sago frond sap as sole fermentation media using Baker's Yeast (*Saccharomyces cerevisiae)*.

CHAPTER 2

LITERATURE REVIEW

2.1 Nipa Palm (*Nypa frutican*)

Nypa fruticans is a monoecious palm belonging to the Arecaceae family and a species under the *Nypa* genus that grows well in riverine and marine intertidal. However, it is the only palm adapted to the typical mangrove biome that extends in brackish water and muddy estuaries (Chin, 2018).

Nipa palm can be found in swampy areas across Southeast Asia, including the Philippines, Myanmar and Thailand, Sri Lanka, and Malaysia, especially in Kedah, Perlis, Terengganu, and Sarawak (Chin, 2018; Othman, 2019). For example, in Sarawak, nipa palm is growing naturally and abundantly in the mangrove and river areas of Kota Samarahan, Pusa, Asajaya and Betong Sebuyau. As a result, Sarawak had a lot of nipa palm, covering about 47 000 ha with concentrated wetland, mostly in Rajang Delta (Othman, 2019).

Nipa palm grew in clusters as they formed large colonies with no visible stems above the ground. The nipa frond is large and has a feather-like appearance. A mature nipa frond can reach more than 7 meters long, and the rhizome, or subterranean stem, sits horizontally beneath the ground and grows to half a meter long (Tsuji et al., 2011). Nipa differs from most palms because it lacks an upright stem, has no trunk, and displays its fluorescence at around 1 meter (Uhl, 1972).

Nipa is usually exploited for their sweet sap from the cut stems of mature or immature inflorescences after the flower or heads of fruit have been removed to produce a fermented beverage called "tuak" and palm sugar, commonly known as "gula apong" by Sarawak's local (BorneoTalk, 2021). In addition, the high sugar content of nipa, which has up 14 to 17% of sucrose, is helpful as a biofuel source and can be made into a suitable bioethanol product (Othman, 2019).

2.1.1 Nipa Palm Sap

Nipa palm sap is a watery fluid, a phloem sap that functions as a medium for transporting nutrients throughout the entire plant (Nguyen et al., 2016). These saps are rich in free sugars, including sucrose, glucose, and fructose, which may be fermented to make bioethanol (Tamunaidu & Saka, 2012). Furthermore, the nipa palm is considered a sustainable resource for producing biofuels and other value-added products since this species can be harvested anytime throughout the years. As a result, the nipa palm is an excellent medium for fermentation substrate and a suitable feedstock for bioethanol production (Okugbo et al., 2012).

In the nipa palm, the sap is produced without starch build-up. Instead, it comes straight from the leaves, where photosynthesis turns water-soluble sugars into sugars. (Die & Tammes, 1975). Nipa sap may be acquired in a non-destructive manner by tapping the palm once it has reached the age of five years, and the palm will continue to generate sap until it is 50 years old (Nguyen et al., 2016). The sap is harvested by removing the palm's inflorescence and tapping it on the stalk. Sap production per palm might reach 1.3 liters per day after 60 to 340 days after tapping. Additionally, tapping results in a significant waste reduction, and the sap could be drained daily without needing to harvest the plant (Nguyen et al., 2016).

Figure 1: Nipa palm sap is rich in sugars that were extracted using the tapping technique.

In addition, the sugar content of nipa palm sap is greater than that of other crops, making it the most economical source of sugar available. Hamilton and Murphy (1988) discovered that nipa palm saps have higher sugar content generated during hot days since water inundation might lower sap output and sugar content. Because sugar yield and bioethanol production are directly related, an increase in sugar yield will increase bioethanol production.

Although sugarcane has a higher sugar content, Hamilton and Murphy (1988) stated that traditional handling of nipa palm sap could provide a higher ethanol production than sugarcane. According to Halos (1981), the present technology will allow a 40% improvement in bioethanol production because nipa sap's three bioconversions are more efficient than sugarcane, even though sugarcane has 150.3 g/L sugar compared to nipa sap, which contains only 144 g/L sugar. The yield of ethanol in various crops is shown in **Table 1**.

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

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

Additionally, fresh nipa palm sap contained ethanol, diacetyl, and esters as volatile chemicals, according to Nur Aimi et al. (2013), which were identified using gas chromatography-mass spectrophotometry. The sap of the nipa palm also contains significant concentrations of inorganic chemicals, such as sodium (Na) and chloride (Cl) (Tamunaidu et al., 2013).

2.2 Sago Palm (*Metroxylon sagu*)

Metroxylon sagu, locally known as sago palm, belongs to the family Arecaceae and is a species under the *Metroxylon* genus primarily found in hot and humid tropics like Southeast Asia (Ahmad, 2017) including Papua New Guinea, Malaysia, and Indonesia (Lim et al., 2019). Even in swampy or peaty soil conditions, the palm can survive and develop naturally without pesticides or herbicides (Pei-Lang et al., 2006). The palm can thrive in tropical environments with temperatures of 25 ˚C and humidity levels average around 70%. For optimal growth and development, a low salt level in the soil and light exposure of over 800 k/cm^2 every day are desired (Singhal et al., 2008). Apart from that, the sago palm is not susceptible to severe disease, fire, flood, drought, or water environment salinity (Bujang, 2015).

The sago palm dominates lowland freshwater swamps with a pH of 4.5 and grows taller on dry soils. For example, in Sarawak, sago palm can survive on turf soil, which covers 75% of Sarawak's coastal and lowland river basins, minimizing competition with other farming crops and making sago palm superior to other commercial agriculture (Matnin et al., 2021).

Sago palm can reach 10 to 15 meters in height, with a diameter of 35 to 75 centimetres. It can store starch in its trunk and yield more than corn, rice, and wheat by three- to four-fold and 17-fold that of cassava (Lim et al., 2019). Sago palms typically have 24 fronds; each month, a new frond emerges from the growth point while the oldest dies (Ahmad, 2017). Sarawak's most extensive plantation areas are in Dalat and Mukah, which have 4986 ha and 7928 ha lands, respectively, involving 15-25 tons/ha of sago starch production (Ahmad, 2017).

It is possible to make adhesives out of sago starch, which may then be utilised in the production of textiles, plywood and paper, and or converted to other foods. For example, pearl sago, famous native food and "linut" or "ambuyat", popular among Borneo locals, were made using moist sago starch. It is also a stabiliser, thickener, and substitute for modified corn starch. In sago industries, sago starch is one of the primary raw materials for biofuel production by converting sago starch into sago sugar (Bujang, 2011).

As a result, sago starch has the potential to be a substitute product to compensate for the loss in sugar supply from the processing of imported sugar cane (Bujang, 2011). In addition, the high starch content in sago palm makes it an ideal resource for starch production to produce bioethanol, lactic acid and sugar (Singhal, 2008).

Currently, Malaysia ranks as the most prominent world exporter of starch from sago palm, where 47,000 metric tons per year are exported mainly to Peninsular Malaysia, Singapore, Taiwan, and Japan (Abd-Aziz, 2002); all the sago starch is produced in Sarawak. However, as the need for sago production rises, so does the amount of waste generated by the industry, posing a waste management difficulty, and polluting the environment. In addition, Sago frond, sago trunk, and sago wastewater are generated by-products (Jenol et al., 2014).

2.2.1 Utilisation of Sago Frond

Development of the sago palm industry in Sarawak will eventually lead to environmental problems due to extremely high production of sago effluent and biomass such as sago hampas and sago frond if the sago is disposed of by-products is not conducted correctly. According to Ahmad and Bujang (2014), sago frond can be used as an alternative raw material for sugar production.

The statistic shows that sago starch production (ton) in Sarawak from 2005 to 2010 and frond production in 2005 and 2010 were 740 and 850 tons, respectively (Lai et al., 2013).

The whole branch of the sago frond consists of petiole and leaflets. Therefore, the left-over sago frond at the sago plantation will be the waste product that might cause environmental pollution. This agricultural waste needs to be utilised well because of the high potential raw material in sugar production.

In this study, sago frond (SF) was used as a source for sugar recovery as an alternative to producing glucose rather than sago starch. Sago frond, obtained from sago palm, is one agricultural waste that was potentially utilised in manufacturing industrial products, organic fertilisers, and animal feeds other than oil palm frond (OPF). Recovery of sugars from agricultural waste is produced commercially at the international level because of the high demand for biofuels, especially bioethanol production. However, to acquire the highest possible yield of monomeric sugar, it is necessary to conduct a reliable pretreatment and determine the part of the feedstock in the best possible condition (Ethaib et al., 2016).

2.3 Sugar

The substance known as sugar, sucrose, is most generally referred to as table sugar. It is an edible product usually utilised as a solid since its crystalline shape is highly stable (Knecht, 1990). Pure sucrose is colourless, has no smell, and tastes very sweet. The crystalline form is termed monoclinic in the system for classifying crystals (Mullin, 2001). Crystalline sucrose melts or decomposes between 160 °C and 186 °C, the exact temperature depending on the crystallisation solvent and purity. Sucrose is highly soluble in water and other polar solvents and insoluble in ether, benzene, and non-polar organic solvents. Since sucrose has been such an essential element in manufacture and usage from the 18th century