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FULL PAPER

Physicochemical and microbiological assessment of *Nypa fruticans* sap collected in Sarawak, Malaysia

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Abstract

Nipa sap is a sweet and translucent beverage that originated from nipa palm (*Nypa fruticans)* tree. In Sarawak, nipa sap become raw material for nipa sugar or locally known as *gula apong*. However, nipa sap undergoes natural fermentation that alters the properties of nipa sap including taste, aroma and quality. Fermented nipa sap is whitish colour with an unpleasant aroma and taste, which makes it unacceptable for consumption. Hence, it is no longer suitable to make nipa sugar. This study aimed to determine the physicochemical and microbiological changes of nipa palm sap from fresh to fermented. The nipa sap was allowed to undergo natural fermentation at room temperature for 56 days. Samples were collected every 24 hrs for the first week and once a week in the subsequent week. The selected physiochemical qualities were analysed using high-performance liquid chromatography (HPLC) whereas the microbial content was analysed using spread plating. Fresh nipa sap showed the highest load of sugar (334.2±12 g/L) with sucrose as the main sugar found (231.5±4.3 g/L), followed by fructose (42.1±1.2 g/L), and glucose (29.7±3.2 g/L). Fresh nipa sap also possessed the lowest load of ethanol (0.08±0.03 g/L), lactic acid (1.09±0.06 g/L), and acetic acid (0.05±0.01 g/L) as well as microbial and yeast concentration. Later, ethanol started to accumulate on day 4 (9.80±0.1 g/L) and the highest peak was on day 21 (19.1±2.01 g/L). The microbial concentration changed as well, affecting the quality of nipa sap. As nipa sap plays such an important role in the lifestyle of people in Sarawak, this study provides a better understanding of the microbiology and biochemistry of its fermentation process. Hence, proper planning for handling fresh nipa sap should be considered to ensure the quality of value-added product production.

1. Introduction

Nipa sap or locally known as *air sadap* or *air nira* is a traditional beverage consumed by people in Sarawak. Nipa sap is a refreshing beverage that is rich in sugar, translucent, and fruit-like odour juice obtained from *Nypa frutican* tree (Minh *et al*., 2014). It is obtained by cutting the mature infructescence and inflorescence nipa palm. Nipa palm (*Nypa fruticans*) is a fast-growing palm that thrives in brackish water environments such as river estuaries and mangrove forests. Nipa palms have been known to yield excellent sugar-sap from their infructescence for more than 50 years (Tamunaidu and Saka, 2011). The shoots of 9-12 years old stems are reported to be the highest yielding, providing up to 1500-1900 mL of sap per stem per season. The stems of 15 years or more were reported to yield lower sap production (Farid *et al*., 2015). Nipa sap can be utilised to produce many products such as fresh beverages, syrup, alcohol, molasses, and traditional vinegar. These have become sources of income for the community. In addition, some local communities inherit traditional techniques for generations to utilise nipa palms that remain a part of the community's livelihood.

The natural fermentation of nipa sap involves the breakdown of carbohydrate materials under an anaerobic condition with the help of microbial activities. Since the microorganisms in nipa sap are alive, they metabolise away the pleasant sugary taste of the fresh nipa sap and produce ethanol and various organic acids in a short period (Radi *et al*., 2013). Hence, the process of fermentation of nipa sap will alter the properties of nipa sap including taste, aroma, contents, and quality. Fermented nipa sap is whitish colour with an unpleasant aroma and sour taste, which makes it unacceptable for

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local consumption and sale (Minh *et al*., 2014). Once it becomes unacceptable, it is no longer suitable to make nipa sugar. Hence, the local villagers will use it as a raw material to make nipa vinegar. Nipa vinegar is one of the traditional vinegar produced by fermentation. The ethanol developed as a product of natural fermentation in nipa sap and vinegar also becomes a great concern to halal requirement and quality.

In Sarawak, nipa palm related food small industries are predominantly produced by coastal Malay communities. The process and utilisation of nipa sap by local people are shown in Figure 1. Local people utilise nipa sap as a raw material to produce nipa palm sugar or locally known as *gula apong* for decades. *Gula apong* is a sugary block that can be easily distinguished by its golden caramel colour and in some cases, reddish brown colour. Nipa sugar is produced by boiling nipa sap for a few hours and is usually used as a sweetener and food seasoning. Nipa sugar has been exported to several regions such as Europe, the Middle East, South Korea and Japan (The Borneo Post, 2019; Sarawak Voice, 2020). Hence, it is important to assess the physiochemical and microbiological status of nipa sap as raw material for nipa sugar production to determine the level of biological quality and safety of nipa sugar produced. This will not only facilitate marketing abroad but also guarantee the safety of nipa sugar produced.

Apart from that, nipa sap can be used to produce nipa vinegar by using fermented nipa sap. Nipa sap is allowed to undergo natural fermentation in a glass jar or bottle at room temperature to produce nipa vinegar. The period of fermentation to produce traditional nipa vinegar varies from 3 to 44 days. Nipa vinegar is commonly used as a food condiment and even as traditional medicine (Yusoff *et al.,* 2015). Unlike apple vinegar, which had been studied extensively, there is not much information on the physiochemical and nutritional contents of nipa vinegar. Thus, the research aimed to evaluate the physicochemical and microbiological changes of fresh to fermented nipa palm sap during natural fermentation.

2. Materials and methods

2.1 Collection of nipa palm sap

Freshly tapped sap of nipa palm was collected from local collectors in Sarawak, Malaysia. Nipa palm sap was tapped in the evening and harvested 12 hrs later. Nipa sap was collected in bamboo vessels according to traditional methods. Then, the collected sap was poured into a sterilised bottle and stored at 4°C. The samples were then brought to Biochemistry Laboratory UNIMAS for further analysis.

2.2 Fermentation of nipa sap

The fermentation process was adapted from Radi *et al.* (2013) with modifications. A total of thirty bottles consisting of 250 mL of nipa sap were stored at ambient temperature and allowed to undergo natural fermentation for 56 days (8 weeks). For the first week, the samples were collected at an interval of 24 hrs while for the rest of the weeks, the samples were collected once every 7 days. Each sample collection was done in duplicate. The samples were kept at -20°C before further analysis.

2.3 Determination of pH

The pH of nipa sap was determined using a pH meter (Biobase, China) after calibration with a standard buffer.

2.4 Determination of total sugars and reducing sugars

Total sugars in nipa sap samples were determined using the phenol sulphuric acid method as previously described by Dubois *et al.* (1956) while reducing sugars

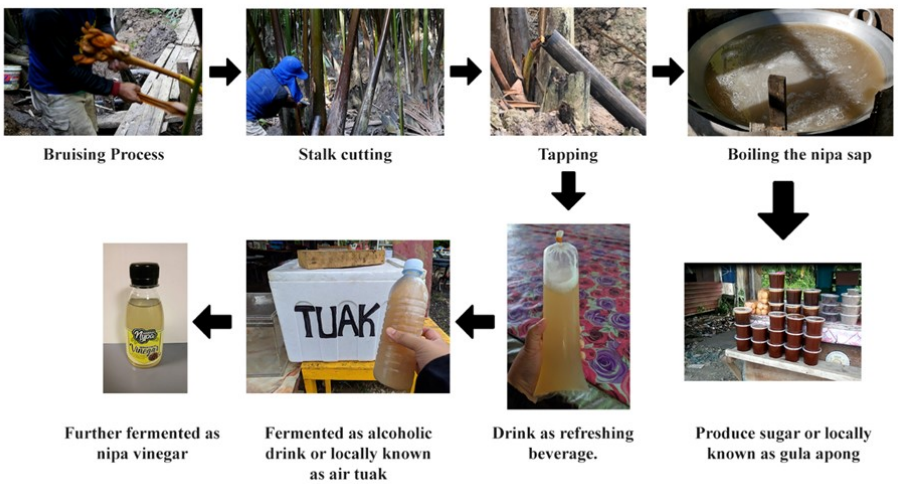

Figure 1. Flowchart indicates the process of nipa sap collection and the production of nipa sugar and vinegar by the local villagers.

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were analysed using dinitrosalicylic acid (DNS) or termed the DNS method as previously described by Karamoko *et al*. (2012).

2.5 Determination of sugar profiles, ethanol, and organic acid using high-performance liquid chromatography (HPLC)

Samples were centrifuged at 10,000 rpm for 10 mins and supernatants were filtered through a 0.45 µm millipore membrane filter (EMD Millipore, Billerica, MA). Sugar (sucrose, glucose, and fructose), organic acids (lactic and acetic acid), and ethanol analyses were performed using high-performance liquid chromatography (Shimadzu, Japan) as described by Phetrit *et al*. (2020). Index (RID-10A) detector with a column of prominence CTO-20A (Shimadzu, Japan) and 0.005 M H_2SO_4 as mobile phase at 0.8 mL/min flow rate for glucose, ethanol and lactic acid determination at 60° C column temperature.

2.6 Total bacterial count and total yeast and mould count

The total bacterial count (TBC) and total yeast and mould count (TYMC) were determined by surface plating using the standard spread plate method. Nipa sap was serially diluted using sterile saline (0.85% w/v) and appropriate dilutions were spread plated on respective agar plates. TBC was determined using plate count agar (Sigma-Aldrich, USA) with incubation at 37°C for 24 hrs. TYMC was determined using potato dextrose agar (Sigma-Aldrich, USA) with tartaric acid which was incubated at 28°C for 36 hrs.

3. Results and discussion

3.1 Physical characteristics of nipa sap during the fermentation process

Figure 2 shows the physical appearance of fresh and fermented nipa sap. Upon collection, fresh nipa sap was observed to be yellowish transparent in colour. The aroma was nice, and it has a sweet taste. The yellowish transparent colour of nipa sap was characterized as fresh and good-quality of sap (Radi *et al.*, 2013). It is acceptable for consumption by the local people and can be used as raw materials to produce nipa sugar or locally known as *gula apong*. In contrast, the colour of fermented nipa sap turned whitish with vigorous effervescence while the aroma became sour and unpleasant. Vigorous effervescence production is due to the production of carbon dioxide as a product of natural fermentation by yeast (Madigal *et al.,* 2019). Yeast metabolised glucose and converted it into ethanol and carbon dioxide (Madigal *et al.,* 2020). This is the consequence of endogenous sugar fermentation by the natural flora of the fermenting sap, which appears whitish and has a robust effervescence. Differences in the aroma of fresh and fermented nipa sap were due to differences in volatile organic compounds (VOC) in nipa sap. Fermented nipa sap contains several VOCs such as acetoin, ester, diacetyl and alcohols which are responsible for giving a crude and malty aroma (Radi *et al.,* 2013).

Figure 2. Physical appearance of fresh (A) and fermented nipa sap (B). The image of fresh nipa sap was taken on Day 1 (collection day) whereas fermented nipa sap was taken on Day 56.

3.2 Chemical characteristics of nipa sap during the fermentation process

The chemical composition of fresh nipa sap (day 1) is shown in Table 1. It was clearly shown that fresh nipa sap was rich in sugar and low in ethanol, lactic acid, and acetic acid content. The pH was also slightly acidic (pH 5.21±0.3). In this study, the initial concentration of total sugar of the nipa sap was 334.2±12.0 g/L with sucrose as the main sugar presented (231.5±4.3 g/L), followed by fructose (42.1±1.2 g/L), and glucose (29.7±3.2 g/L). According to Granot and Stein (2019), most plants produce mainly sucrose as the end product of photosynthesis and the primary sugar transported in the phloem of most plants. Then, the sucrose is hydrolysed into glucose and fructose before it is converted to ethanol (Tamunaidu and Saka, 2011). The sugar composition of nipa palm sap correlated with the studies in Thailand (Phetrit *et al.,* 2020), Indonesia (Tomomatsu *et al*., 1996; Hidayat, 2018), and Peninsular Malaysia (Tamunaidu *et al*., 2013). However, the total sugar composition of nipa sap in this study was higher than that in those studies (15 - 23% w/w). The sugar composition could differ from

Table 1. The chemical composition of fresh nipa palm sap (day 1)

Parameters	
pH	5.21±0.3
Total sugar (g/L)	334.2±12.0
Sucrose (g/L)	231.5±4.3
Glucose (g/L)	29.7±3.2
Fructose (g/L)	42.1±1.2
Ethanol (g/L	0.09±0.03
Lactic Acid (g/L)	1.09±0.06
Acetic Acid (g/L)	0.05±0.01

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place to place depending on the tapping practices, climates, and environment (Tamunaidu *et al*., 2013). It was also suggested that dry places may contribute to the higher content of sucrose (Radam *et al*., 2014).

Throughout the storage, the total sugar content decreased significantly (p<0.05) and produced a different composition of sugar each day as shown in Figure 3. Hence, at the end of storage, the total sugar decreased to 54.1 g/L with fructose as the main composition (15.1 g/L), followed by sucrose (12.4 g/L), and glucose (4.1 g/L). The reduced sugar content was a clear indication of the fermentation of a large proportion of sugars, especially during the early stages of storage. The sugar content was metabolised by natural flora from the environment (Atputharajah *et al*., 1986; Chanthachum and Beuchat, 1997; Amoa-Awua *et al*., 2007). It was also suggested that microbes could be introduced into the sap by unhygienic tapping utensils, procedures, and collection (Naknean *et al*., 2010). Tapping utensils, particularly bamboo vessels are used repeatedly in the traditional context without cleaning the inner surfaces to remove microbiological deposits. The sucrose content in the nipa sap dropped, which might be attributed to an inversion reaction produced by invertase activity and an acid state in the medium caused by microbial metabolic activity (Naknean *et al*., 2010; Santiago-Urbina *et al*., 2013; Flores-Gallegos *et al.,* 2019).

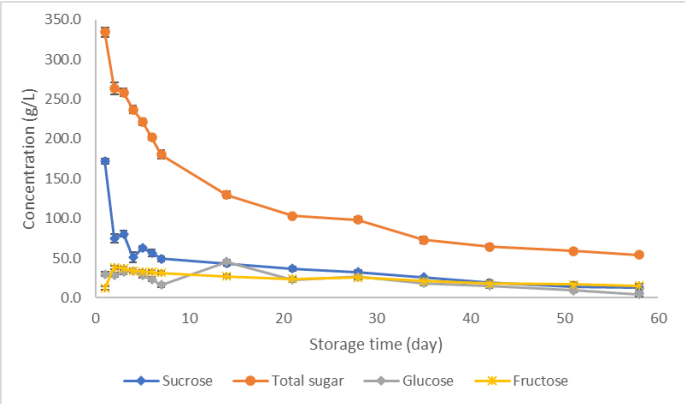

Figure 3. Changes in the sugar composition during storage of nipa sap.

Besides, the pH value of nipa sap decreased from the initial pH of 5.14 (Day 1) to 3.14 (Day 58) as shown in Figure 4. The decrease in pH value was correlated with the increase of acetic acid and lactic acid in the samples from 0.05-9.10 g/L and 1.09-12.10 g/L, respectively. The rise in acidity observed is explained by the concurrent production of lactic acid and acetic acid in the fermentation sap with the production of ethanol, which proves the occurrence of ethanol biochemical reaction. Nipa sap fermentation has been reported to be an alcoholic, lactic and acetic fermentation (Amoa-Awua *et al*., 2007; Ouoba *et al*., 2012; Santiago-Urbina *et al*., 2013). Therefore, yeasts, lactic acid bacteria, and acetic acid bacteria are considered to play the most important roles in the natural fermentation of nipa sap.

On the other side, the concentration of ethanol increased rapidly from 0.08 g/L on Day 1 to 15.18 g/L on Day 5, then slowly increased from Day 7 (15.65 g/L) to Day 21 (19.01 g/L) before it dropped slightly on Day 22 (19.01 g/L) to Day 58 (12.85 g/L). The concentration of ethanol in nipa sap changed across samples, most likely due to a rise in the yeast population responsible for ethanol fermentation. It was supported by the increase in the concentration of yeast starting from week 1 (Figure 5).

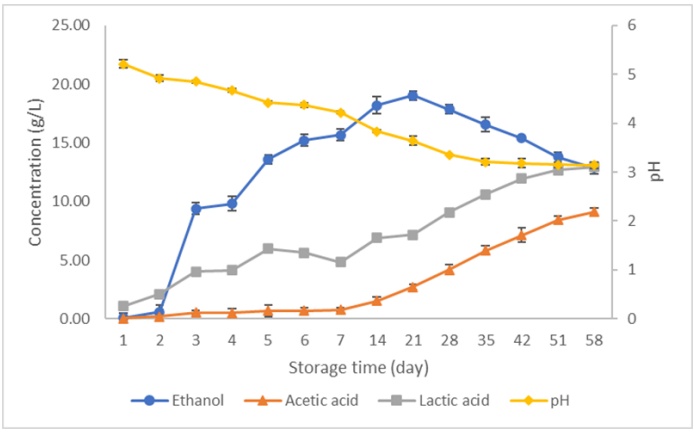

Figure 4. Changes of the pH, ethanol, acetic acid and lactic acid during storage of nipa sap.

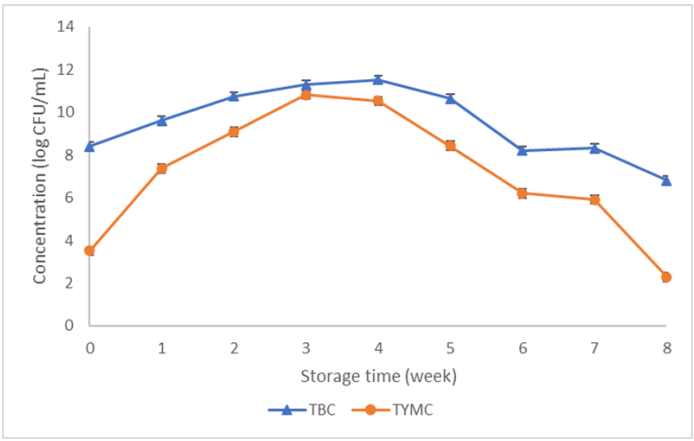

Figure 5. Concentration changes of microbial communities in nipa sap throughout the storage

However, towards the end of the storage, there was a large amount of ethanol residual left and low acetic acid produced. This finding illustrated the real picture of the traditional nipa vinegar's composition produced by local villagers. According to the local villagers, the nipa vinegar was produced by allowing nipa sap to ferment spontaneously at room temperature for 3 to 44 days, whereby the number of days varies between places. Traditional methods for making vinegar from nipa sap use a two-stage fermentation process in which yeast produces alcohol, which is then transformed into acetic acid by aerobic bacteria (Van *et al.,* 2019). The inefficient production of acetic acid in traditional nipa vinegar production was due to low carbon conversion

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efficiency. Two moles of carbon are lost during this process in the form of CO_2 (Kondo and Kondo, 1996). Nipa vinegar produced by traditional methods is not suitable for industrial and commercial use due to low acetic acid content and high ethanol residual concentration. This high ethanol residual concentration in traditional nipa vinegar also raised halal concerns among Muslim consumers. According to JAKIM the maximum amount of permissible amount in food and beverages is 1% v/v (Ghani and Ismail, 2010).

3.3 Microbiological changes of nipa sap during storage

The microbiological quality of nipa sap was analysed by total bacterial count (TBC) and total yeast and mould count (TYMC). Nipa saps were analysed every 1 week for 8 weeks. The results are shown in Figure 5.

The TBC on day 1 was 8.41 log CFU/mL and increased gradually until week 4 (11.52 log CFU/mL) before it gradually decreased from week 5 (10.63 log CFU/mL) to week 8 (6.81 log CFU/mL). On the other hand, the TYMC also showed the same trend but with a lower concentration of yeast and mould. On day 1, TYMC of nipa sap was 3.51 log CFU/mL. The concentration of yeast and mould increased rapidly until week 3 (10.82 log CFU/mL) before slightly reducing in week 4 (10.53 log CFU/mL). The TYMC of nipa started to reduce rapidly from week 5 (8.43 CFU/mL) to week 8 (2.28 log CFU/mL).

The results here were concurrent with those reported by Santiago-Urbina *et al*. (2013). The reduction in the quantity of the microbial population in the study was probably caused by the reduction of the amount of sugar present in the sap. In addition to the selective conditions regarding pH and ethanol concentration in the fermented sap only favour the growth of the fermenting microorganisms (Stringini *et al*., 2009). The acidic environment had inhibitory effects on total coliform and faecal coliform loads. Total coliform loads showed a significant reduction over time, whereas total faecal loads disappeared. Nevertheless, lactic acid and acetic acid bacteria only exhibited a slight reduction through time which explained why lactic acid and acidic concentrations kept increasing throughout the storage (Karamoko *et al.,* 2012).

According to Odunfa and Oyewole (1998), *Saccharomyces cerevisiae* was the dominant yeast species in palm sap fermentation whereas high concentrations of lactic acid bacteria (LAB) and acetic acid bacteria (AAB) were found. Both yeast and LAB metabolise sugar from palm sap to produce ethanol and lactic acid. On the other hand, AAB consume ethanol to produce acetaldehyde and acetic acid, which is found as major volatile acid in palm wine (Kadere *et al*., 2008). The productions of acetic acid and lactic acid are responsible for the rapid product acidification as well as the decrease in pH of nipa sap (Karamoko *et al*., 2012). From the results, it was clearly shown that the fermentation of nipa sap involved three processes, which were lactic acid fermentation, alcoholic fermentation, and acetic acid fermentation.

4. Conclusion

Natural fermentation of fresh nipa sap will change the physical appearance, aroma, and chemical properties. It is due to the presence of high sugar content in the nipa sap, which makes it favourable for the microbes to metabolise it. Generally, in fresh nipa sap, the sugar content is relatively high, whereas ethanol, lactic acid, and acetic acid are low. Through time, these sugars are metabolised by natural flora and produced an unfavourable aroma, which is caused by the presence of high ethanol, lactic acid, and acetic acid in the fermented nipa sap. This study provided a better understanding of the fermentation process of nipa sap which is important in order to improve the nipa sugar industry in Sarawak. In the future study, the identification of microbial communities that exist in nipa sap should be carried out and the introduction of a hygienic collection method to improve food quality and safety.

Conflict of interest

The authors declare no conflict of interest.

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