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Physicochemical Investigation and Analysis of Nypa Sap (*Nypa fruticans* Wurmb) using a Novel Collecting Device

ANA SAKURA ZAINAL ABIDIN[1], HANIF MOHD TEDDY[2], MOHD RAZIP ASARUDDIN[2], MOHD ZULHATTA KIFLI[1], RASLI MUSLIMEN[1], MARINI SAWAWI[1], ABANG AHMAD DZULFAKHRI[1], KASYFUL AZHIM YUSSOF[1], FREDY MUHAMAD FIRDAUS[1], & KHAIRUL FIKRI TAMRIN[1*]

[1]Faculty of Engineering, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia, [2]Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia
*Corresponding author: tkfikri@unimas.my
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ABSTRACT

A sweetener from *Nypa fruticans* Wurmb offers significant health benefits. The traditional process of sap tapping requires careful attention to enhance cleanliness. In this research, a nypa sap harvesting device has been developed, and a time study for its installation has been conducted. The physicochemical properties of the sap gathered from the device were evaluated and compared with the traditional method in terms of total soluble solids(TSS) reading, pH level, total dissolved solid (TDS) and electrical conductivity (EC) value, and glucose content. Both methods showed no significant difference, with an average TSS reading of 14 to 15 °brix, pH of 3.57-3.97, glucose content of 25.9 to 32.4, TDS of 2340-2726 ppm, and EC reading of 4679-5472 µs/cm. Slight differences in physicochemical properties were observed due to the random selection of trees for the experiment. The use of the device was found to improve the physical cleanliness of the sap by 97% and chemical purity by 7% to 11%. The colour of the sap collected with the device was milky white compared to the traditional bamboo method, resulting in a yellowish-white sap. The time study showed a 40% improvement compared to the first trial, indicating the practicality of the device, which can be easily installed by the operator. The physicochemical properties of the sap collected using the device can be further improved with the addition of preservatives. The findings are expected to enhance sap harvesting hygiene and the quality of sap, directly affecting the quality and benefits of the derived products.

Keywords: Agrotechnology, collecting device, nypa sap, physicochemical

INTRODUCTION

Sugar is a primary ingredient in most food and beverages. However, diets high in sugar can contribute to chronic, long-term health conditions that can lead to diabetes and obesity. As consumer awareness about health increases, there is a growing demand for healthier and organic sweeteners as an alternative to commercial table sugar (Reed & McDaniel, 2006. In particular, the European market is willing to pay a premium price for such sweeteners. However, entry into the European market is not easy, as all products must meet the food production standards imposed by the European Commission (Somorin *et al*., 2021).

Nypa fruticans Wurmb is a type of palm tree that can be found abundantly in muddy soils along rivers. It forms extensive stands in brackish to tidal freshwater, demonstrating wide ecological tolerance. In terms of chemical composition, nypa sugar contains less reducing sugar than table sugar (Radam *et al*., 2014). The presence of sugar in nypa sap can have an adverse effect due to the hydrolysis of sucrose into glucose and alkaline invertase enzymes that consequently ferment and deteriorate the sap and its derived products (Tamunaidu & Saka, 2012; Sainz-Polo *et al*., 2013). Nypa sugar, made from nypa sap, is the main source of income for communities in Kampung Tambirat Kota Samarahan, Sarawak, Malaysia. In Sarawak, the community still produces the sweetener using a traditional method by which the sap is extracted through a tapping process by chopping off the thin palm inflorescence. A traditional bamboo container, as shown in Figure 1, is used to store the nypa sap during the overnight collection process. However, this method is deemed unclean as the collected sap is exposed to foreign particles such as insects, rodents, dust, pollen, rainwater and other debris that can contaminate the sap (Madigal, 2020).

Figure 1. Nypa sap collection vessel using a traditional bamboo container

In order to meet the premium market requirements, such as those in Europe, traditional sugar producers in Sarawak would need to improve the cleanliness standards of the nypa palm production process. The sap harvesting process is particularly critical in production. Therefore, a device has been developed to enhance the cleanliness level while maintaining the sap quality.

Experiments and a series of analyses have been conducted to validate the performance of the developed nypa sap collecting device. Due to the nature of nypa sap, which hydrolyses immediately after collection, on-site total sugar determination is essential. On-site sugar analysis by brix and inverted sugar measurement using a portable refractometer and glucometer was established in this study as a quick and simple analytical on-site method based on the settings by Tamunaidu & Saka (2012).

MATERIALS AND METHODS:

The development of the collecting device involved basic studies of nypa plant anatomy and physiology. The study focused on nypa trees located at Kampung Tambirat, Sarawak, Malaysia (Coordinate: 1.551554 DD, 110.509297 DD). Accuracy was crucial to ensure the device fit perfectly, preventing any foreign substances from contaminating the sap. The dimensions of the nypa stalk diameter were measured at three positions – the tip, middle and end of the stalk (within its sap-producing capacity) using a Vernier caliper, as illustrated in Figure 2.

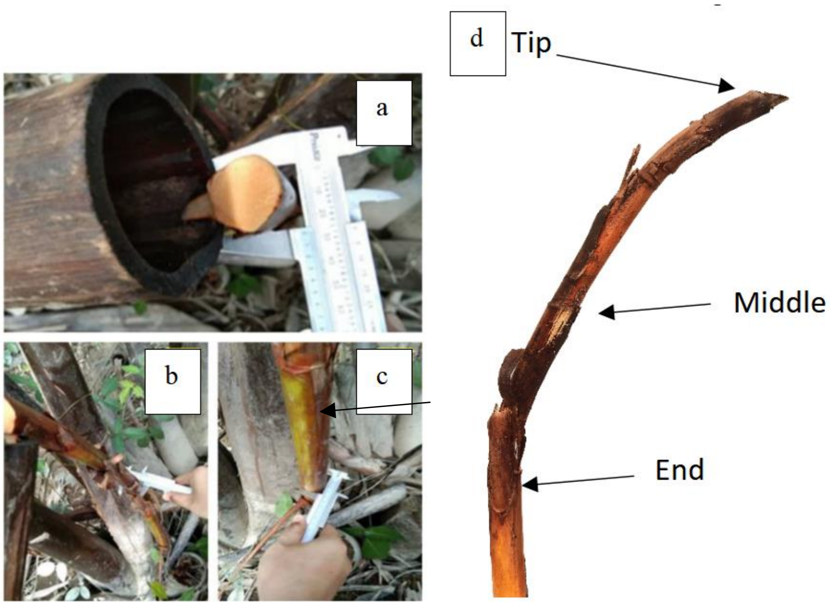

Figure 2. Diameter measurement of the tip (a), middle (b), and end (c) parts of the nypa palm stalk, along with a schematic diagram of the measurement process (d)

On-site experiments were conducted to verify the functionality of the developed nypa sap collecting device. The experiments were carried out at Kampung Tambirat in Kota Samarahan, Sarawak, Malaysia, on three different dates: 15th April 2022 (Time: 1700 hrs, Atmospheric Condition: Cloudy, Temperature: 30 ℃, Humidity: 85%, Wind Speed (mph): 5.6NW), 18th May 2022 (Time: 1700 hrs, Atmospheric Condition: Cloudy, Temperature: 30 ℃, Humidity: 77%, Wind Speed (mph): 5.8E), and 24th May 2022 (Time: 1700 hrs, Atmospheric Condition: Clear, Temperature: 32 ℃, Humidity: 81%, Wind Speed (mph): 5.8NW). Ten nypa trees were randomly chosen on each date, resulting in a total of 30 sets of data in total (3 days x 10 trees), along with three control data sets (3 trees). The assembly drawing of the nypa sap collecting device is shown in Figure 3, and details of the components, materials and functions are summarised in Table 1.

Table 1. Components of nypa sap collecting device, materials and functions

No	Part	Material	Function
1	Netting-cap	PVC	• To prevent insects/rodents from entering the assembly • To equalize inside assembly temperature with ambient
2	Chamber	PET	• Main body: encloses the assembly to protect it from insects/pesticides. • Upper part; holds the net. • Hole; provides access for the gripper to the chamber. • End part; supports and secure casing position to the tapped stalk
3	Gripper	Food grade FDA Silicone	• To attach the assembly to the nypa stalk • To prevent insects/rodents from entering the assembly via the stalk • To accommodate various diameter sizes of nypa stalk • Has a remarkable degree of elasticity and stretch
4	Plastic Bag	HDPE Food grade	• To store the dropped sap
5	Clip	Stainless Steel 304	• To lock the position of the plastic bag (4) to the casing (6) • To hold the position of the casing (6) to the stalk
6	Casing	Bamboo	• To hold the plastic bag for nypa sap storage • To protect the plastic and nypa sap from pests' attack

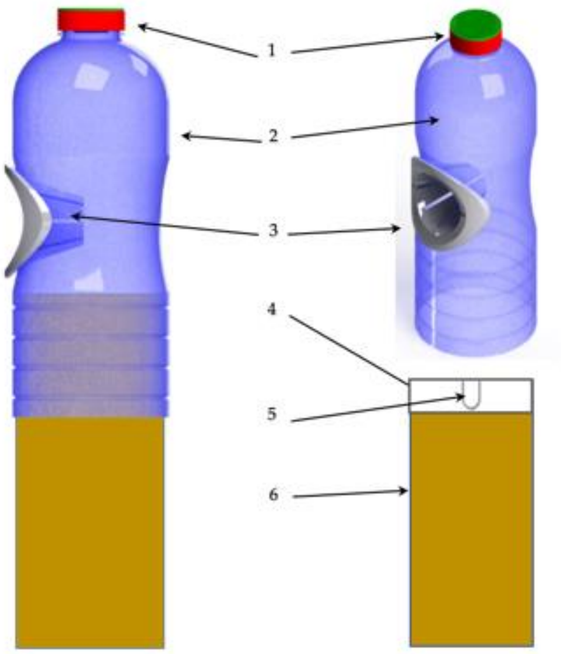

Figure 3. Assembly diagram of the nypa sap collecting device

Compounds such as polyethylene terephthalate (PET) have provided plastic bottles with several advantages such as toughness, energy savings and ease of production (Nistico, 2020). These advantages significantly contribute to the higher consumption of single-use plastic. Research by Ballerini *et al*., 2022 has found that single-use plastic bottles are the most abundant pollutant at riverbanks, posing harm to marine life, water quality, and indirectly affecting the livelihoods of fishermen (Saxena *et al*., 2022). Therefore, there is a need to educate and encourage the Apong community in Sarawak about the recycling and reuse of plastic bottles in Apong production. Furthermore, the reuse of the plastic bottle as a nypa sap collection vessel is convenient, easy to manufacture and cost-saving. Compared to glass, PET plastic bottles are lightweight (Benyathiar *et al*., 2022). Recycling plastic bottles provides significant environmental benefits, including the reduction of CO_2 emissions, mitigation of greenhouse effects, minimization of carbon footprints, and substantial savings in cost and pollution (Biron, 2017).

Time Study

A time study was conducted to determine the installation duration of the nypa sap collection device in a real-world environment. The identified traditional nypa sap tapper was provided with brief training prior to the experiment. The time study consisted of three primary processes, as summarised in Table 2, and the installation process and setup of the nypa sap collecting device are depicted in Figure 4.

Table 2. Installation process and description of the nypa sap collecting device

Process	Description
1.	Beat the inflorescence stalk.
2.	Scrape off the nypa stalk tip.
3.	Attach the gripper to the stalk (Item No. 3 in Table 1) follow by the chamber (Item No. 2 in Table 1).
4.	Place the plastic on the bamboo and secure the assembly with clips or string.
5.	Insert the bamboo from Step 4 into the chamber (Item No. 2 from Table 1).
6.	Sap collection (the following day): Remove the plastic from the bamboo (in Step 4) and tie it up.

Figure 4. Installation process and setup of nypa sap collecting device

A stopwatch was used to measure the time taken for each process, and the results were recorded. Steps 1 through 5 were executed consecutively, one after the other. Subsequently, the assembly was left overnight to allow for the accumulation of nypa sap droplets. Step 6 was performed the following morning for sap collection. The time taken to remove and tie up the plastic from the assembly was also recorded, with all plastic samples appropriately labelled. Meanwhile, the installation of the traditional process (control) involved a similar procedure for nypa sap collection. Following a 16-hour period, the sap was harvested for analysis.

Collected Nypa Sap Profile and Physicochemical Properties

The collected sap from the time study was transported to the laboratory at Universiti Malaysia Sarawak, which is located approximately 30 minutes from Kampung Tambirat. The samples were stored in a cooler box filled with ice cubes to maintain a temperature of 20 °C and to slow down the fermentation process. Subsequently, all samples were subjected to a series of analyses to check for impurities, and to determine total soluble solids (TSS), pH, total dissolved solids (TDS), electrical conductivity (EC) and glucose content. The following procedures were followed for the analyses:

a) Impurities and colour observation

The sap samples were placed in glass bottles and labelled accordingly. The colour of the sap was observed and compared with each other using the naked eye. Drops of sap from each sample were examined under the LED Lab Trinocular Compound Microscope Model T120C to identify impurities. The microscope's magnification was set from 4X to 10X and 40X to obtain clear images. The images were saved and labelled according to the corresponding sap samples harvested from chosen nypa trees. Furthermore, the images were compared with existing literature to identify any foreign entities present in the samples.

b) Determination of TSS

The total soluble solids content of the sap was determined as °brix using a handheld refractometer. The refractometer measured the refractive index of the liquid (Sulaiman *et al*., 2020).A drop of the collected nypa sap was placed on the refractometer's sample zone, and the TSS reading in °brix was recorded. The brix scale, widely utilised in the food and beverage industries, represents the percentage of sucrose content in a solution (Toledo, 2014).The correlation between °brix and sucrose content is given as follows (Elewa *et al*., 2020):

1 °brix = 1 g of sucrose/ 100 g of solution (Eq. 1)

c) Determination of pH level, TDS, and EC

In this experiment, a Hanna HI98130 Combo pH, TDS and EC meter were utilised to determine the pH, TDS and EC value, respectively, of the nypa sap. EC and TDS are distinct parameters. Typically, TDS concentration is measured in ppm, while μs represents the EC (Rusydi, 2018). The correlation between TDS and EC is determined by the characteristics and nature of the dissolved cations and anions in the water. The relationship between TDS and EC of a solution can be described as follows (Thirumalini & Joseph, 2009):

$$TDS = A \times EC \qquad \text{(Eq. 2)}$$

where A is a constant that typically ranges between 0.55 and 0.9 in conductivity measurements.

The meter was calibrated before use by dipping the probe into buffer solutions of pH 7.01 and 4.01 for pH calibration. For TDS/EC calibration, the probe was immersed in a calibration solution of 12.88 ms/cm. Next, a portion of the collected nypa sap was placed in a beaker, and the probe was immersed into the nypa sap sample. The pH, TDS and EC readings were recorded. After each measurement, the probe was removed and thoroughly rinsed with distilled water to prevent cross-contamination before the next use.

d) Glucose content

Traditional laboratory methods typically require specialised equipment and trained personnel. However, the use of a glucometer is relatively inexpensive, compact and easy to use in the field. According to a study by Fundador and Calumba (2020), the glucometer was able to accurately measure the sugar content in tested fruits, with an average error of only 3.8%. The ACCU-CHEK® Instant S blood glucometer was employed to determine the glucose content of the nypa sap. The two essential components of the glucometer are the sensor (test strips) and the detector (Tonyushkina & Nichols, 2009). A drop of sap was placed on the strip dosing area inserted in the glucometer. A chemical reaction occurred when glucose was detected, producing a small electrical current proportionate to the amount of glucose content (Yanez, 2013). The value of the glucose content of the sample appeared on the LCD screen. The glucose concentration reading in mmol/L was recorded.

RESULTS AND DISCUSSION

The results of the stalk dimensions are summarised in Table 3. The results provide important input for defining the gripper size. The data were gathered from 33 trees representing a variety of stalk conditions from different locations. The minimum stalk diameter recorded was 21.38 mm, while the maximum stalk diameter was 55 mm. The variety of stalk sizes, as well as sap production, as also influenced by the age of the nypa tree, in line with Matsui *et al*. (2014).

Table 3. Maximum, minimum, average and range of nypa palm stalk diameter

Parameter	Measurement (mm)
Maximum diameter overall	55.00
Minimum diameter overall	21.38
Range (Maximum – minimum)	33.62
Average diameter overall	32.49

As depicted in Figure 5, the nypa stalk exhibits a smaller tip diameter that gradually increases towards the bottom. This information was taken into account during the gripper design process. Figure 6 illustrates the gripper design, featuring a narrow top radius tailored to fit the minimum stalk diameter and a wider bottom radius to accommodate the maximum stalk size. The gripper incorporates several slots connected by a thin layer of material, allowing for easy tearing to adapt to a wide range of stalk diameter differences, from the smallest to the largest ones.

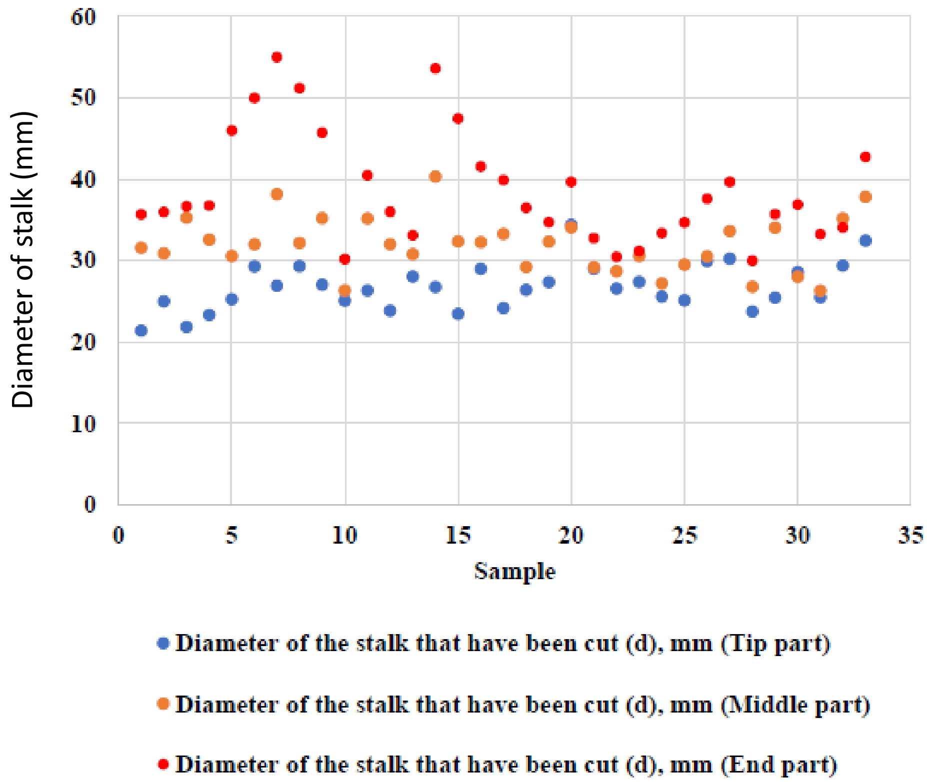

Figure 5. Tabulation of nypa palm stalk diameter at tip, middle, and end parts

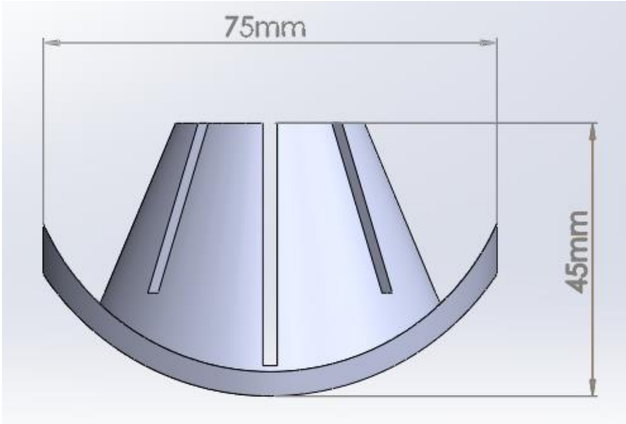

Figure 6. Gripper design of the nypa sap collecting device

As shown in Figure 5 and Table 3, the samples were randomly chosen to validate the versatility of the device, which can be adjusted to fit different stalk sizes and orientations.

Table 4 presents the volume of nypa sap collected for experiments conducted on 15th April, 18th May and 24th May 2022. Overall, the results indicate no significant difference in the techniques used to collect nypa sap (see Figure 7), except for Sample 10 from the experiment conducted on 15th April, which recorded no volume. This was due to an improperly installed collecting device that did not fit the stalk during assembly, resulting in sap dripping away from the designated storage area. Other factors that can affect nypa sap production include weather conditions, nypa tree age, stalk thickness and length, and frond diameter (Tonyushkina & Nichols, 2009; Madigal, 2020). Larger frond diameters are often associated with higher sap yields, and the best sap production is typically observed in middle-aged palms (Matsui *et al*., 2014; Nguyen *et al*., 2016).

Table 4. Volumes of collected nypa sap.

	15th April 2022	18th May 2022	24th May 2022
Sample	Volume (mL)	Volume (mL)	Volume (mL)
1	745	420	270
2	380	300	400
3	690	430	390
4	250	280	700
5	50	100	600
6	135	690	100
7	54	40	320
8	150	470	30
9	16	650	410
10	0	790	150
11	700	550	450

Time Study Results

The experiment aimed to assess the practicality of the developed device, which was measured by the time taken by the harvester to install the collecting device. Table 5 presents the average time taken to install the device by date. The total time represents the cumulative time taken for the entire process from Process 1 to Process 3. The results of the time study demonstrated a significant improvement after several repetitions. The total time taken on the third day was reduced by approximately 40% compared to the first day, resulting in a time of 49.66 seconds. This indicates that the developed device is user-friendly and practical, as the harvester becomes more accustomed to it over time.

Table 5. Time study for installation of nypa sap collecting device

Date	Process 1 (s)	Process 2 (s)	Process 3 (s)	Total time (s)
15/4/2022	23.78	36.64	21.02	80.44
18/5/2022	18.83	31.78	20.92	71.53
24/5/2022	10.74	22.12	16.8	49.66

Results of Collected Nypa Sap Profile and Physicochemical Properties

The condition of sap samples collected using the developed device (Samples 1 to 10) was compared and discussed with those collected using the traditional method using bamboo (Sample 11) to verify the performance of the developed device in comparison to the traditional method.

Impurities and Color Observation

The freshness of nypa sap is indicated by its colour, with milky-white denoting fermented sap and pale yellow indicating fresh sap (Madigal *et al*., 2020). All samples (1-11) underwent the same tapping process, lasting over 14 hours. Based on Figure 7, all samples were fermented. Upon observation, nypa sap from Samples 1-10, collected using the developed device, appeared milky-white and clean. In contrast, the sap from Sample 11, collected using bamboo, appeared yellowish-white. This is due to the bamboo used to store the sap, which has been repeatedly used, cleaned, and dried using smoke from firewood (Gunawan *et al*., 2020). Bamboo is a natural fibre material that reacts with the acidic properties of the sap, causing a yellowish colour. Moisture absorption is a natural phenomenon that occurs when using natural fibres due to their hydrophilic properties (Madigal *et al*., 2020). High-density polyethylene (HDPE) plastic, an organic material, is well-suited for nypa sap collection due to its high chemical resistance, including resistance to the acidic properties of the sap (Liu *et al*., 2018). Hence, HDPE is recommended for use in nypa sap collection.

Figure 7. Colour of nypa sap 14 hours after tapping process

Figure 8 shows images of impurities that exist in the collected sap. Samples 1 to 10 were collected using the developed device, while Sample 11 was collected using traditional bamboo (as a control). Only microbes were detected in Samples 1 to 10, with no other impurities found. However, debris or solid particles were found in Sample 11, likely due to the open-top bamboo being exposed to the environment. The sweet smell of the fresh sap attracts pests and rats, and without a cover, insects, rainwater and other solid particles (debris) can easily enter the bamboo.

The microbes observed under the microscope resemble yeast, which is consistent with previous studies. Yeast, such as *Saccharomyces cerevisiae*, is commonly found in nypa sap (Limtong *et al*., 2020). Yeast cells are oval-shaped, and their reproduction results in clusters of growing cells (Liu *et al*., 2018; Gunawan *et al*., 2020). Yeast reproduces through budding, an asexual reproduction method where a daughter cell is formed by pinching off a part of the parent cell (Monroy Salazar *et al*., 2016). These characteristics are evident in Samples 1 to 10. Nypa sap contains sugar and other compounds essential for microbial growth. Moreover, the high temperatures of the tropical climate in Sarawak, Malaysia, are conducive to microbial development (Chongkhong & Puangpee, 2018). As a result, yeast can grow rapidly, leading to the natural sugar fermentation of nypa sap.

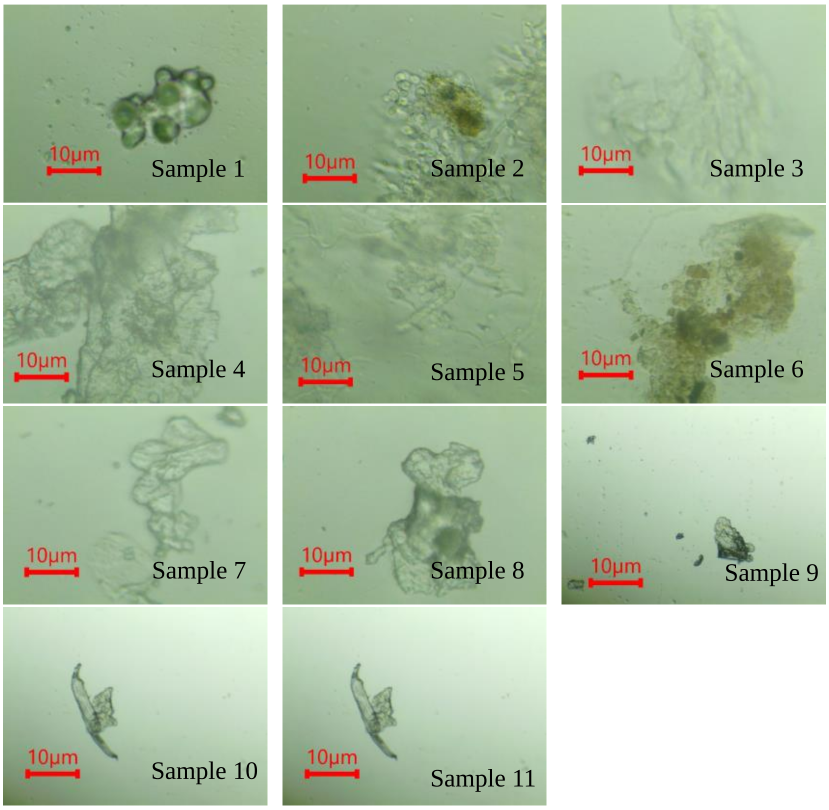

Figure 8. Microscopic observation of impurities in nypa sap samples (40× magnification)

The nypa sap collected using the developed device showed lower contamination compared to bamboo. Foreign particles, namely insects, maggots, and rat droppings, were evident in the sap collected using the traditional method, as shown in Figure 9(a). The wide open-top bamboo can be easily accessed by insects and rats. A sieve was used to filter the collected nypa sap. The accumulated foreign particles covered 1.6% of the total sieve area for the traditional method. Only a small spot (0.05%) was found on the sieve used to filter sap from the device, as shown in Figure 9(b). The tiny maggots found in the developed device were likely due to a small gap between the gripper and the nypa stalk. This occurred only in cases where the stalk had an uneven shape. Therefore, the developed device can effectively prevent insects and pests from contaminating the sap, with the exception of smaller insects measuring 2 mm and below.

(a)

(b)

Figure 9. Images of impurities collected from (a) the traditional method and (b) the developed device using a 0.08mm strainer.

Chemical Properties

Chemical properties are summarised in Table 6, 7 and 8 based on the experiment date, while comparisons between the experiments are shown in Table 9.

The TSS brix of nypa sap for Experiments 1, 2 and 3 are recorded in Tables 6 to 8, showing a range of 11–18%. Sample 11 served as a control, using bamboo for sap harvesting in Experiments 2 and 3. The TSS reading from Experiment 1 showed a lower average brix compared to Experiments 2 and 3. This is likely due to the rain that occurred on 5th April 2022, between 6 pm and 6 am in Kampung Tambirat, Sarawak, as recorded by the World Weather Online website. The sucrose content of the sap can be affected by weather conditions, with low TSS brix observed in sap collected after rainy days due to higher water content (Matsui *et al*., 2014). The results obtained in this research are mostly within the range reported in other studies from Indonesia, which ranged from 15-17 °brix, but lower than that of Thailand, which reported 22.9 °brix (Rachman & Sudarto, 1995; Phetrit *et al*., 2020). It is worth noting that Thai practitioners add kiam (*Cotylelobium lanceolatum Craib*) as a preservative in nypa sap to retard the fermentation process.

Tables 6 to 8 show the pH values for each sample collected throughout the experiments. There is no significant difference in pH between nypa sap collected using bamboo and the developed device. All samples were acidic, with pH values ranging between 3.35 and 4.34. These readings are within the range of 3.18 - 4.20 reported in another study (Semjonovs *et al*., 2014). The acidic pH indicates that the nypa sap has undergone fermentation. This is likely due to the long harvesting duration, which took overnight (about 17 to 18 hours) before undergoing the cooking process. Initially, the pH of fresh nypa sap is neutral, but gradually the acidity increases and the pH decreases as fermentation occurs (Tamunaidu & Saka, 2012).

Table 6. Chemical properties of nypa sap from Experiment 1 (15th April 2022).

Sample	brix (%)	pH	Glucose (mmol/L)	TDS (PPM)	EC (Us/cm)
1	12	3.44	21.60	2468	4936
2	12	3.44	28.60	2401	4802
3	13	3.89	27.20	2531	5062
4	12	3.44	29.70	2292	4584
5	11	3.35	26.90	2105	4210
6	16	4.34	32.60	2844	5688
7	16	4.07	31.40	2633	5266
8	12	3.44	27.10	2186	4372
9	13	3.80	28.40	2468	4936
10	12	3.44	27.10	2468	4936
11	13	3.70	28.00	2374	4748

Table 7. Chemical properties of nypa sap from Experiment 2 (18th May 2022).

Sample	brix (%)	pH	Glucose (mmol/L)	TDS (PPM)	EC (Us/cm)
1	13	3.53	31.10	2842	5684
2	15	3.71	H1	2330	4660
3	12	3.53	30.20	2478	4956
4	13	3.44	H1	2472	4944
5	16	3.89	H1	2872	5744
6	14	3.53	32.10	2419	4838
7	15	3.35	H1	2707	5414
8	16	3.71	32.90	2504	5008
9	13	3.44	H1	2201	4402
10	13	3.53	31.60	2671	5342
11	15	3.62	30.10	2726	5472

*H1 indicates that the value is greater than 33.30 mmol/L
(The instrument is unable to read values greater than 33.30 mmol/L)

Table 8. Chemical properties of nypa sap from Experiment 3 (24th May 2022).

Sample	brix (%)	pH	Glucose (mmol/L)	TDS (PPM)	EC (Us/cm)
1	16	3.89	H1	1943	3886
2	16	3.89	30.60	2511	5023
3	18	3.80	H1	2511	5023
4	14	3.71	H1	2282	4564
5	13	3.89	26.30	2282	4564
6	15	3.62	H1	2282	4564
7	15	3.98	H1	2651	5302
8	15	3.89	H1	2522	5044
9	13	3.71	H1	2225	4451
10	14	3.98	H1	2186	4372
11	14	3.97	25.9	2606	5213

*H1 indicates that the value is greater than 33.30 mmol/L
(The instrument is unable to read values greater than 33.30 mmol/L)

Table 9. Summary of chemical properties of nypa sap from all three experiments.

Experiment	Sample	brix (%)	pH	Glucose (mmol/L)	TDS (PPM)	EC (Us/cm)
1 (15th April 2022)	1-10	12	3.32	25.4	2193	4386
2 (18th May 2022)	1-10	14	3.57	32.4	2550	5099
	11 (control)	15	3.62	30.1	2726	5472
3 (24th May 2022)	1-10	15	3.84	32.3	2340	4679
	11 (control)	14	3.97	25.9	2606	5213

The acidity of sap is a result of microbial activities that convert sugars into ethanol (Tamunaidu & Saka, 2012; Yanez, 2013; Semjonovs *et al*., 2014).*Pichia* spp. and *Saccharomyces* spp. are among the main bacteria found in nypa sap responsible for fermentation (Yamagata *et al*., 1980). Additionally, other species of microbes such as *Hanseniaspora guilliermondii, Lachancea thermotolerans, Hanseniaspora vineae, Lachancea fermentati,* and *Torulaspora delbrueckii* have been identified in nypa sap (Limtong *et al*., 2020). The presence of these microbes may be due to the cleanliness of the harvester and the device used in the nypa sap harvesting process, as well as the long collecting time and open device that may be exposed to contaminants from the outside (Naknean *et al*., 2010). While the cleanliness of the device has been emphasised in this research, the personal cleanliness of the harvester may vary, which could explain the variability in pH readings observed in this research, even within the same experimental setting.

Fermentation of nypa sap typically begins a few hours after tapping and becomes more rapid over time. This justifies the acidic condition observed in the samples, as the local setting for sap collection in this research was set at 17 to 18 hours after tapping. A few samples collected using the developed device showed a higher pH value (pH = 4.34), indicating that proper cleanliness procedures are required to get a reliable quality of sap acidity. Furthermore, shortening the collecting period before temperatures get too hot (i.e., before sunrise) could be beneficial, as the ideal temperature for active yeast fermentation is reported to be 32 $^{\circ}$C (Chongkhong & Puangpee, 2018).

The conductivity of a liquid is an indicator of the number of dissolved substances, chemicals, and minerals present in it. Higher amounts of these impurities will result in higher conductivity. During fermentation, the chemical compounds in nypa sap are broken down, leading to changes in electrical conductivity. Active fermentation activity can result in higher readings of TDS and EC at specific times. TDS and EC values are proportional, as described in Equation 2.

Low readings of TDS and EC were observed in the samples collected on rainy days. Regardless of weather conditions, on average, higher TDS and EC values were observed in samples collected using the traditional method compared to those collected using the collecting device. The use of bamboo, which has been repeatedly smoked for sterilization, may contribute to impurities in the sap. The bamboo is exposed to smoke from the high-temperature burning of firewood, which can result in the formation of polycyclic aromatic hydrocarbons (PAHs). PAHs are a class of chemicals and carcinogens that naturally occur as a result of burning coal, oil, gas, wood, garbage and tobacco. PAHs are often found attached to stable particles in water, which can settle in sediment (Nwachukwu *et al*., 2019). In the case of nypa sap, the PAHs formed by the smoked bamboo may also be attracted to stable particles in the sap, which can settle in sediment. The deposited PAHs in the sediments can then remobilize into nypa sap, leading to an increase in conductivity and total dissolved solids. Therefore, a reduction of 7% to 11% in TDS and EC significantly improves sap purity.

Tables 6 to 8 present the glucose content of the collected nypa sap. Experiment 1 shows the lowest glucose content of the sap compared to Experiments 2 and 3. This is attributed to rain in Kampung Tambirat, Sarawak on 5th May 2022, between 6 p.m. and 6 a.m. (during Experiment 1) as recorded by the World Weather Online website. Plant photosynthesis rate is influenced by sunlight, and low sunlight intensity during rainy days can result in a decrease in photosynthesis rate (Lantemona *et al.,* 2013). This condition becomes a limiting factor for sugar production through metabolism. Additionally, the metabolic heat generated by microbial growth during fermentation is found to be proportional to oxygen consumption, with an average of 3.38 kcal per gram of O_2 (Minkevich & Eroshin, 1973). Since these mesophilic microbes grow best between 20 °C and 40 °C, proper sap storage requires ventilation space to disperse the heat produced by microbial respiration activities (Manpreet *et al*., 2005; Slaa & Gnode, 2009). This justifies why Experiment 1 and Sample 11 (control) from Experiments 2 and 3, which used traditional bamboo, showed lower glucose content compared to sap collected using the developed device. The traditional bamboo, which has an open top, provides good ventilation, thereby lowering the temperature inside the bamboo compared to the closed chamber of the device.

Furthermore, natural fermentation begins with an increase in lactic acid, followed by ethanol, and finally acetic acid (Somashekaraiah *et al*., 2019). *Saccharomyces* sp. is one of the bacteria found in nypa sap that is responsible for fermentation, as glucose is predominant in nypa sap, followed by fructose and sucrose (Phetrit *et al*., 2020). An increase in glucose content indicates an increase in fermentation rate. This is because, under aerobic conditions, the microbes ferment sucrose, with the yeast enzyme invertase breaking down the sugar into glucose and fructose as the first step (Marques *et al*., 2015). Fructose and glucose are then metabolised to carbon dioxide and several organic acids, with acetic and gluconic acids being formed through the conversion of glucose via gluconolactone (Lynch *et al*., 2019). The measurement principle of the glucometer is based on enzymatic reaction, which also measures the oxidation of glucose to gluconic acid. Therefore, an increase in glucometer reading also indicates the oxidation of glucose to gluconic acid or the conversion of sugar into acetic acid.

CONCLUSION

The chemical properties of nypa sap are measured by TSS and pH level. Initially, fresh sap has a neutral pH and high sucrose content. Gradually, it turns acidic due to the activities of microorganisms that convert sugars into ethanol (Nguyen *et al*., 2016). As sucrose is converted to simple sugars, such as glucose, the sucrose content of the sap decreases, which is indicated by a lower brix reading. However, the glucose content shows an adverse trend compared to the other two parameters. As time passes, the glucometer reading also increases, representing the oxidation of glucose into gluconic acid. The presence of gluconic acid imparts a sour taste to the nypa sap, indicating the level of fermentation. The freshness of the sap can be extended by controlling the fermentation process, such as by adding preservatives, which is a common practice in other countries like Thailand and Indonesia.

In conclusion, the physicochemical properties of the collected sap show no significant difference regardless of the collecting method used. However, the sap collected using the developed device improves the cleanliness condition of the sap compared to traditional bamboo. Physically, the new device has improved the cleanliness of the collected sap, with a reduction of about 97% in foreign particles found in the sap compared to the traditional method. Furthermore, the chemical impurities of the sap are significantly improved as shown by the reduced TDS and EC readings. In terms of practicality, the device is easy to use, as indicated by a 40% reduction in the time taken to assemble the device compared to the first trial. The device is also versatile, as it can flexibly cope with various stalk conditions, such as vertical and curving orientations. Moreover, the performance of the developed device is expected to improve further by optimizing the standard operating procedures and incorporating preservatives in future experiments.

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REFERENCES

Ballerini, T., Chaudon, N., Fournier, M., Coulomb, J.-P., Dumontet, B., Matuszak, E. & Poncet, J. (2022). Plastic pollution on Durance riverbank: First quantification and possible environmental measures to reduce it. *Frontiers in Sustainability*, 3:866982. DOI: 10.3389/frsus.2022.866982

Benyathiar, P., Kumar, P., Carpenter, G., Brace, J., & Mishra, D. K. (2022). Polyethylene terephthalate (PET) bottle-to-bottle recycling for the beverage industry: A review. *Polymers*, 14(12): 2366. DOI: 10.3390/polym14122366

Biron, M. (2017). Recycling: The first source of renewable plastics. In Biron, M. (ed.) *Industrial Applications of Renewable Plastics*, Oxford: William Andrew Publishing. pp. 67-114. DOI: 10.1016/B978-0-323-48065-9.00003-0

Chongkhong, S. & Puangpee, S. (2018). Alternative energy under the royal initiative of his majesty the king: ethanol from nipa sap using isolated yeast. *Songklanakarin Journal Science Technology,* 40(3): 648-658.

Elewa, M., El-Saady, G., Ibrahim, K., Tawfek, M. & Elhossieny, H. (2020). A novel method for brix measuring in raw sugar solution. *Egyptian Sugar Journal*, 15(0): 69-86. DOI: 10.21608/esugj.2020.209517

Fundador, E.O. & Calumba, K.F. (2020). Using a glucometer for home-based quantification of sugar in baked goods. *Philippine Journal of Science*, 149(4). DOI:10.56899/149.04.10

Gunawan, W., Maulani, R.R., Hati, E.P., Awaliyah, F., Afif, A.H. & Albab, R.G. (2020). Evaluation of palm sap (*Neera*) quality (*Arenga pinnata Merr*) in processing of house hold palm sugar (case study on Aren farmers in Gunung Halu Village, Gunung Halu district, West Bandung regency). *IOP Conference Series: Earth and Environmental Science*, 466. DOI: 10.1088/1755-1315/466/1/012001

Lantemona, H., Abadi, A.L., Rachmansyah, A. & Pontoh, J. (2013). Impact of altitude and seasons to volume, brix content, and chemical composition of aren sap in north sulawesi. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 4(2): 42-48. DOI: 10.9790/2402-0424248

Limtong, S., Am-In, S., Kaewwichian, R., Kaewkrajay, C. & Jindamorakot, S. (2020). Exploration of yeast communities in fresh coconut, palmyra, and nipa palm saps and ethanol-fermenting ability of isolated yeasts. *Antonie Van Leeuwenhoek*, 113(12): 2077-2095. DOI: 10.1007/s10482-020-01479-2

Liu, T., Huang, A., Geng, L.H., Lian, X.H., Chen, B.Y., Hsiao, B.S., Kuang, T.R. & Peng, X.F. (2018). Ultra-strong, tough and high wear resistance high-density polyethylene for structural engineering application: a facile strategy towards using the combination of extensional dynamic oscillatory shear flow and ultra-high-molecular-weight polyethylene. *Composites Science and Technology*, 167: 301-312. DOI: 10.1016/j.compscitech.2018.08.004

Lynch, K.M., Zannini, E., Wilkinson, S., Daenen, L. & Arendt, E.K. (2019). Physiology of acetic acid bacteria and their role in vinegar and fermented beverages. *Comprehensive Reviews in Food Science and Food Safety*, 18(3): 587-625. DOI: 10.1111/1541-4337.12440

Madigal, J.P.T., Cariaga, J.F., Suarez, T.C.E. & Agrupis, S.C. (2020). Assessment of the physico-chemico and microbiological changes during nipa (*Nypa fruticans*) sap fermentation collected from different sites in cagayan province, Philippines. *Indian Journal of Science and Technology*, 13(32): 3230-3236. DOI: 10.17485/IJST/v1 3i32.1251

Madigal, J.P.T., Calixto, B.G.S.C.G.S., Duldulao, M., Ubiña, T. D. & Agrupis, S. C. (2020). Development of nipa (*Nypa fruticans*) sap closed collection vessel. *Agro Bali: Agricultural Journal*, 3(2): 108-117. DOI: 10.37637/ab.v3i2.611

Manpreet, S., Sawraj, S., Sachin, D., Pankaj, S. & Banerjee, U.C. (2005). Influence of process parameters on the production of metabolites in solid-state fermentation. *Malaysian Journal of Microbiology*, 1(2): 1-9. DOI: 10.21161/mjm.120501

Marques, W.L., Raghavendran, V., Stambuk, B.U. & Gombert, A.K. (2015). Sucrose and *Saccharomyces cerevisiae*: a relationship most sweet. *FEMS Yeast Research*, 16(1): 1-16. DOI: 10.1093/femsyr/fov107

Matsui, N., Okimori, Y., Takahashi, F., Matsumura, K. & Bamroongrugsa, N. (2014). Nipa (*Nypa fruticans Wurmb*) sap collection in Southern Thailand I. Sap production and farm management. *Environment and Natural Resources Research*, 4(4): 75-88. DOI: 10.5539/enrr.v4n4p75

Minkevich, I.G. & Eroshin, V.K. (1973). Productivity and heat generation of fermentation under oxygen limitation. *Folia Microbiologica*, 18(5): 376-385. DOI: 10.1007/BF02875932

Monroy Salazar, H., Salem, A.Z.M., Kholif, A., Monroy, H., Pérez, L.S., Zamora, J.L. & Gutiérez, A. (2016). Yeast: description and structure. In Salem, A.Z.M., Kholif, A.E. & Puniya, A.K. (eds.) *Yeast Additive and Animal Production*, Delhi: Research Publishing Services. pp. 4-13.

Naknean, P., Meenune, M. & Roudaut, G. (2010). Characterization of palm sap harvested in Songkhla province, Southern Thailand. *International Food Research Journal,* 17(14): 977-986.

Nguyen, D. Van, Sethapokin, P., Rabemanolontsoa, H., Minami, E., Kawamoto, H. & Saka, S. (2016). Efficient production of acetic acid from nipa (*Nypa fruticans*) sap by Moorella thermoacetica (*f. Clostridium thermoaceticum*). *International Journal of Green Technology*, 2: 1-12.

Nistico, R. (2020). Polyethylene terephthalate (PET) in the packaging industry. *Polymer Testing*, 90: 106707 DOI: 10.1016/j.polymertesting.2020.106707

Nwachukwu, C., Hieu, M.V., Chládková, H. & Fadeyi, O. (2019). Strategy implementation drivers in correlation with strategic performance. *Management and Marketing Journal,* 17(1): 19-38.

Phetrit, R., Chaijan, M., Sorapukdee, S. & Panpipat, W. (2020). Characterization of nipa palm's (*Nypa fruticans Wurmb*.) sap and syrup as functional food ingredients. *Sugar Tech*, 22(1): 191-201. DOI: 10.1007/s12355-019-00756-3

Rachman, A.K., & Sudarto, Y. (1995). Nipah sumber pemanis baru. Yogyakarta: Kanisius.

Radam, R.R., Noor, H., Sari, M. & Lusyani, H. (2014). Chemical compounds of granulated palm sugar made from sap of nipa palm (*Nypa fruticans Wurmb*) growing in three different places. *Wetlands Environmental Management*, 2(1): 108-115.

Reed, D.R., & McDaniel, A.H. (2006). The Human Sweet Tooth. *BMC Oral Health*, 6(1): 17. DOI: 10.1186/1472-6831-6-S1-S17

Rusydi, A.F. (2018). Correlation between conductivity and total dissolved solid in various type of water: A review. *IOP Conference Series: Earth and Environmental*

Science, 118. DOI: 10.1088/1755-1315/118/1/012019

Sainz-Polo, M.A., Ramírez-Escudero, M., Lafraya, A., González, B., Marín-Navarro, J., Polaina, J. & Sanz-Aparicio, J. (2013). Three-dimensional structure of *Saccharomyces invertase*: Role of a non-catalytic domain in oligomerization and substrate specificity. *Journal of Biological Chemistry*, 288(14): 9755-9766. DOI: 10.1074/jbc.M112.446435

Saxena, A., Agarwal, V., Yadav, P., Tripathi, S., Kasliwal, A. & Ansari, H.S. (2022). Impact of single-use plastic on local communities in India. *International Journal of Scientific Research in Science, Engineering and Technology*, 9(5): 168–177. DOI: 10.32628/IJSRSE
T229534

Semjonovs, P., Denina, I., Fomina, A., Patetko, A., Auzina, L., Upite, D., Upitis, A. & Danilevics, A. (2014). Development of birch (*Betula pendula Roth*.) sap based probiotic fermented beverage. *International Food Research Journal*, 21: 1763–1767.

Slaa, J. & Gnode, M. (2009). Yeast and fermentation: the optimal temperature. *Journal of Organic Chemistry: Chem. Dut. Aspects*, 134(1): a-c.

Somashekaraiah, R., Shruthi, B., Deepthi, B.V. & Sreenivasa, M.Y. (2019). Probiotic properties of lactic acid bacteria isolated from neera: A naturally fermenting coconut palm nectar. *Frontiers in Microbiology*, 10: 1–11. DOI: 10.3389/fmicb.2019.01382

Somorin, Y.M., Odeyemi, O.A. & Ateba, C.N. (2021). Salmonella is the most common foodborne pathogen in African food exports to the European Union: analysis of the rapid alert system for food and feed (1999–2019). *Food Control*, 123: 107849. DOI: 10.1016/j.foodcont.2020.107849

Sulaiman, S., Jafarzadeh, S., & Fazilah, A. (2020). Characterization of physicochemical and antioxidant properties of oil palm trunk saps as affected by the storage time in comparison to nipa sap. *Journal of Oil Palm Research*, 32(1): 75-82. DOI: 10.21894/jopr.2020.0014

Tamunaidu, P. & Saka, S. (2012). On-site sugar analysis and pre-treatment of nipa saps. *Green Energy and Technology*, 108: 121-126. DOI: 10.1007/978-4-431-54067-0_12

Thirumalini, S. & Joseph, K. (2009). Correlation between electrical conductivity and total dissolved solids in natural waters. *Malaysian Journal of Science*, 28(1): 55–61. DOI: 10.22452/mjs.vol28no1.7

Toledo, M. (2014). Brix-Sugar determination by density and refractometry. *Mettler Toledo*, 1-8.

Tonyushkina, K. & Nichols, J.H. (2009). Glucose meters: a review of technical challenges to obtaining accurate results. *Journal of Diabetes Science and Technology*, 3(4): 971–980. DOI: 10.1177/19322
9680900300446

Yanez, M.G. (2013). Glucose meter fundamentals and design. *Freescale Semiconductor*, AN4364.

Yamagata, K., Fujita, T., Sanchez, P.C. & Takahashi, R. (1980). Yeasts flora of palm wine in the Philippines. *Research Bulletin,* 13: 56-55.