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ORIGINAL ARTICLE

Proximate, Chemical and Minerals Characterization of *Nypa fruticans* Palm Sugar Produced by Reverse Osmosis-Pan Boiling (RO-PB) Method

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ABSTRACT - Reverse osmosis is a pressure-driven membrane technology with vast applications and is currently available as a pre-concentration option in the preparation of food or beverage substances. This study was carried out to characterize the proximate, chemical, and mineral attributes of *Nypa fruticans* palm sugar produced by the reverse osmosis-pan boiling (RO-PB) method. RO-PB and control (commercial) sugar were compared statistically to check for significant differences. The proximate value of RO-PB sugar in terms of moisture, crude protein, crude fat, ash, carbohydrate, and energy content is 5.19%, 2.04%, 0.13%, 0.54%, 93.10%, and 381.73 kcal, respectively. As for chemical properties, the pH, total acidity, and reducing sugar of RO-PB sugar are significantly different ($p<0.05$) with control sugar with measurements of 4.87, 0.42%, and 4.29%, respectively. For minerals analysis, both RO-PB and control sugar is high in potassium elements followed by calcium, sodium, phosphorus, and iron with reading of 488.3, 85.00, 44.10, 35.00, and 43.00 ppm, respectively. Overall, integration of reverse osmosis into the pan boiling process for *Nypa fruticans* sugar production has no significant changes or impact on proximate and minerals attributes.

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INTRODUCTION

Palm sugars have been a generational alternative sweetener for decades in Malaysia. In Sarawak, nypa palm sugar or known as gula apong is one of the common palm sugar items produced for both the domestic and export markets. Nypa palm sugar is produced from the sap harvested from stem of nipah palm (*Nypa fruticans Wurmb*) [1; 2]. The structure of the nipah palm is unique, lacks an upright trunk, and has a stem that forms horizontally with dichotomous branching underground [3]. The sap collected from the stalk of the tree is the main ingredient used to make various sugary product forms such as syrup, molasses, and granulated apong. The sweet and aromatic sap is evaporated and boiled until the desired texture is reached and conventionally without any purification phase or any addition of artificial chemicals in its production [4].

Production of gula apong involved extensive boiling and evaporation for a few hours and stirring continuously until caramelized and thickened paste formed. Due to time-consuming, laborious and costly process, there is a need and consideration for a sugar production process alternative. Reverse osmosis (RO) is a pressure-driven membrane process operated by a pressure source to extract permeate of pure water from a solution that contains high levels of soluble solids, dissolved minerals, and other impurities [5; 6]. In the context of palm sugar production, reverse osmosis can be used to concentrate the sap collected from palm trees before it is boiled and crystallized to produce palm sugar. By using RO, the need for prolonged boiling can be reduced, leading to energy savings and potentially a better-quality product, as prolonged boiling can cause caramelization and alter the flavor of the palm sugar.

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Palm sugar in general is a healthier choice of sweetener because of its low glycemic index (GI) [7]. The role of low GI foods is imperative, especially in diabetes control, weight loss, maximum sports performance, and risk reduction of diseases related to heart and hypertension [8; 9]. Like other sugar, nypa palm sugar is rich in nutrients and major carbohydrates mainly sucrose, glucose, and fructose [10]. However, there are variations of sugar-to-sugar composition depending on the tapping of nypa sap practices by the harvester, climates, and geographical surroundings where the nipah plants are located [11].

The natural state of the nipa sap heavily influences the composition of the nipa palm sugar produced in terms of proximate, chemical, and minerals. There are few publications on the physicochemical and proximate attributes studies on nipa sap reported. However, not much documentation on the aforementioned attributes studies on the nipa palm sugar produced by conventional full-pan boiling and also using the integration process, reverse osmosis-pan boiling (RO-PB).

This paper objectively determines and characterizes the proximate, chemical, and mineral properties of the nypa palm sugar produced using conventional full-pan boiling and the reverse osmosis-pan boiling (RO-PB) method. The RO-PB produced is compared statistically with the commercial nypa palm sugar (gula apong) which is produced using conventional full-pan boiling or evaporation method.

MATERIALS AND METHODOLOGY

Sap Sample Collection

The nypa fruticans saps were purchased from the local harvester and seller in the Pusa area, an active site for palm sugar production. The saps were harvested overnight, collected, frozen, stored in a low temperature containment and transported to the University of Technology Sarawak, UTS food technology laboratory facility. The saps are further pre-analyzed for screening and clarification purposes before being thermally treated for sterilization to eliminate potential fermentation microorganisms.

Preparation of Nypa Palm Sugar

Clarified and sterilized nypa saps were concentrated with a reverse osmosis (RO) process at a transmembrane pressure of 80 Bar and a temperature range of 20-25°C [12]. Once the total soluble solids reached 30°Brix, the saps were further cooked using an open pan boiling technique at the temperature of 105°C for few hours until dark-browny sugar paste texture was formed and the total soluble solids reading is around 70-80°Brix. The sugar is kept cool and stored in a tightly sealed container for at least overnight before further analysis.

The pH, Total acidity (TA), and Total Soluble Solids (TSS)

The AOAC method 1990 was used to determine the pH, titratable acidity (TA), and total soluble solids of the sugar samples [13]. A calibrated pH meter (HACH®: USA) using buffers with pH 4 and 7 was used to determine the pH of the sugar. The measurement was done in triplicate, and the average result was calculated. The total acidity was measured using the titration method without using pH meter. 10 grams of sugar sample dissolved with 100 ml of distilled water in a conical flask and added with 1ml of phenolphthalein indicator. The homogenate is titrated to a definite pink endpoint with O.1N NaOH and the amount of NaOH used is recorded. The result was calculated as a percentage of lactic acid equivalent. Meanwhile, the total soluble solids content of the sugar product was measured using a handheld refractometer Atago (Japan) with a measurement spectrum of 51° to 100°Brix. and the result was expressed in terms of Brix percentage.

Reducing Sugar

The reducing sugar was determined by the dinitrosalicylic acid method and the results were expressed in terms of percentage [14].

Determination of Moisture Content

Determination of total moisture content was determined by the evaporation method using drying oven. Initially, the aluminum pans were treated for 2 hours at 100°C before use to eliminate any possible moisture that may contribute to the result or experimental error. The pans were kept cooled in desiccator for approximately 20 minutes and then weighed. The exact weight of the pans is determined and then 2 grams of sugar sample were added and recorded. The experimental runs were repeated in triplicate with the addition of two blanks as control. All the aluminum pans and the controls were transferred into pre-heated oven, at 100°C and dried for 24 hours. After 24 hours, the pan and blanks were cooled down for 20 minutes and each of the pan is weighed and the results were recorded. The actual total moisture content in terms of percentage was determined according to equation (1).

$$Moisture\ (\%) = \frac{Weight\ of\ moisture\ (g)}{Weight\ of\ sample\ (g)} \times 100\% \quad (1)$$

Determination of Ash Content

The ash content of the sugar samples was determined by the dry ash method by using furnace. The ash crucibles were heated to 100°C, cooled down to room temperature in the desiccator, and reweighed before used. Approximately 2.0 grams of sugar samples were put into the crucible and the weight was recorded. All crucibles containing samples were placed into the furnace or muffle oven at 550°C for 12 hours, followed by cooling time for few hours before weighing again. Complete ash process is indicated when all the samples turn into ash with a light gray color. The ash contents left in the crucibles were weighed and recorded. The actual ash contents in terms of percentage were calculated according to equation (2).

$$Ash\ (\%) = \frac{Weight\ of\ ash\ (g)}{Weight\ of\ sample\ (g)} \times 100\% \quad (2)$$

Determination of Crude Protein Content

Total protein in the sugar sample was determined by using the Kjeldahl method with the FOSS brand system. Approximately 2.0 grams of sample were weighed using an electronic balance and mixed with 12 mL of sulphuric acid in the digestion tube. 2 tablets of Kjeltabs Cu 3.5 catalyst were added and the tube was swirled gently to homogenate the mixture. The tube was attached to a digestion chamber that has been pre-heated up to 420°C. The sample was digested for 1 hour and 15 minutes until a blueish or greenish solution was formed. After digestion, the tube containing samples was kept cooled for 10-20 minutes and attached to the distillation unit where 80mL of deionized water was added. The distillation outlet was submerged into the receiver solution (25mL of 4% boric acid) in the conical flask and during the distillation process, 50mL of 40% NaOH was dispensed into the tube. The presence of an alkali-ammonia bonding in the conical flask is indicated by the color changes of the receiver solution into light green. The receiver solution containing alkali-ammonia was then titrated with standardized 0.1N HCl with the addition of three drops of methyl red and bromocresol green as an indicator. The end of titration is marked by the color changes from light green to light pink and the volume of HCl used was recorded. The total percentage of nitrogen in the samples was calculated using equation (3) and the total of crude protein percentage was determined according to equation (4) below.

$$Total\ Nitrogen\ (\%) = \frac{(V_S - V_B) \times N \times 0.014}{Weight\ of\ sample(g)} \times 100\% \quad (3)$$

$$Crude\ protein\ (\%) = Percentage\ of\ nitrogen(\%) \times F \quad (4)$$

Where,

V_S = Volume of HCl used in titration, mL for sample
V_B = Volume of HCl used in titration, mL for blank
N = Normality of HCl
F = Protein factor, 6.25

Determination of Crude Fat Content

The fat content of nypa sugar was determined by using the Soxhlet method through the FOSS Soxtec extraction system of ST 255. Apparatus such as extraction cup was dried overnight at 103°C and kept cool in a desiccator chamber for 30 minutes before use. Approximately 2.0 grams of pre-dried sugar sample were weighed on electronic balance, transferred into cellulose-based thimble and covered with cotton at the mouth of thimble. A magnetic ring was inserted into the thimble and attached to the Soxtec system at each magnetic terminal or adapter which holds the thimble vertically firmly. The cooled extraction cup was filled with fat solvent, 120mL of petroleum ether, and placed below the thimble containing samples. The samples underwent automated processes of boiling, rinsing, evaporation, and cooling for roughly 1 hour and 20 minutes. Extraction cups containing extracted crude fats and lipids were dried for 30 minutes and kept cooled down in the desiccator for another 20 minutes before being weighed. The fat content was calculated according to equation (5) and the results were expressed in terms of percentage.

$$Crude\ Fat\ (\%) = \frac{Weight\ of\ fat}{Weight\ of\ sample} \times 100\% \tag{5}$$

Determination of Total Carbohydrate

The total carbohydrate of nypa sugar was determined using calculation methods according to equation (6) and expressed in terms of percentage.

$$Total\ Carbohydrate\ (\%) = 100\% - A - B - C - D \tag{6}$$

Where,

A = Crude Protein Content (%)
B = Crude Fat Content (%)
C = Moisture Content (%)
D = Ash Content (%)

Determination of Solubility

The solubility of each sugar sample was determined method modified by Naknean et al., 2010 [15]. Approximately 1 gram of sugar sample and 100 ml of distilled water were mixed in a beaker. The mixture underwent centrifugation at designated setting of 3500 rpm for 20 minutes at temperature of 24°. After centrifugation, the solution was screened using a pre-weighed filter paper (Whatman, No. 1) and then left for drying in the oven at temperature of 105°C for 7 hours. The filter paper was weighed to determine the value of total sugar residue and the percentage of solubility was calculated based on equation (7).

$$Solubility\ (\%) = \frac{Total\ sugar\ sample - total\ sugar\ residue}{Total\ sugar\ sample} \times 100\% \tag{7}$$

Determination of Water Activity

The water activity (a_w), of the sample was determined and measured using a water activity meter at room temperature (Aqualab Water Activity Meter, Decagon, Washington, USA).

Determination of Minerals Content

The mineral content of sugar samples such as potassium (K), calcium (Ca), sodium (Na), phosphorus (P), and iron (Fe) was determined by using atomic absorption spectroscopy (AAS) method according to AOAC. The result was expressed in unit of parts per million (ppm).

Statistical Test

All the sugar samples were analyzed in triplicate and the sugar comparison study produced by the RO-PB method and commercially available sugar was tested using t-test at $p \leq 0.05$ to check for significance differences. All statistical analysis was carried out using SPSS version 23.0 (Statistical Package for the Social Science, IBM: United States) software.

RESULTS AND DISCUSSION

Proximate compositions of both control (commercial nypa sugar) and RO-PB processed nypa sugar are shown in Table 1. The constituent analysis of RO-PB sugar revealed the content of moisture, crude protein, crude fat, ash, and carbohydrate as 5.19%, 2.04%, 0.13%, 0.54%, and 93.10%, respectively, with an energy value of 381.73 kcal.

Table 1. Proximate composition of control (commercial) and RO-PB sugar

Compositions	Sugar (control)	RO-PB sugar
Moisture (%)	5.03±1.70 [a]	5.19±1.12 [a]
Crude protein (%)	2.67±1.77 [a]	2.04±0.91 [a]
Crude fat (%)	0.25±0.05 [a]	0.13±0.07 [a]
Ash (%)	0.48±0.08 [a]	0.54±0.04 [a]
Carbohydrate (%)	92.57±3.22 [a]	93.10±2.51 [a]
Energy Content (kcal)	383.21±1.02 [a]	381.73±2.28 [a]

* Means ± SD in the same row with the same letters are not significantly different.

Moisture Content

Stability of the sugar product normally associated with moisture content and water activity indicator. Both indicators commonly used to oversee the storage life of sugar as water or moisture potentially empower microbial growth and biochemical degradation activity [16]. Also, controlling the moisture levels in the sugar could be a preventive mechanism towards osmophilic type bacteria growth. The moisture content of both control and RP-PB sugar is in an acceptable percentage range 5.03%±1.70 - 5.19%±1.12. No significant differences between both results suggesting that RO-PB method for moisture content reduction are almost as effective with full evaporation process.

Crude Protein

Originally, the crude protein in nypa sap content is high but due to heat sensitivity during processing, most proteins are disintegrated or used for other biochemical reaction. Maillard reaction is a normal occurrence during the production of palm sugar or syrup and protein acts as a substrate [17]. High evaporation temperature and long processing time catalysed Maillard reaction and these two factors are responsible for low crude protein in sugar [18]. Crude protein for RO-PB sugar lower than control but the values were not statistically significant in term of differences. This observation is likely because of microorganism interference during sap harvesting and storing before being processed into sugar. Some microorganisms excluding fermentative bacteria use proteins as a carbon or nitrogen source for metabolism and genetic composition [19].

Crude Fat

Aside from low glycaemic properties, Nypa sugar is also low in fat and cholesterol which makes it favorable as a replacement for typical white sugar [20]. Naturally, nypa sap crude fat contents are made up of a total of less than 1% of proximate characteristics. The conversion of the sap into the sugar by thermal and pressure-driven process, RO showed no sign of crude fat content increment. These findings support the fact that nypa sugar is a free or low-fat sweetener either as a natural or processed food ingredient.

Ash

The ash content of the sugar reflects the measure of total mineral content. The ash determination of both control and RO-PB showed a low percentage of ash with less than 1% (0.48±0.08 and 0.54±0.04). Statistically, there were no significant differences of ash percentage between RO-PB and control. However, the ash percentage for RO-PB is slightly higher due to some of the retained minerals during the production process. Pressure-driven process such as reverse osmosis, RO, has a retention capability of some minerals but is partially loss during the committing steps of evaporation. Compared to control sugar produced by full evaporation, diminished and loss effects of the minerals are more severe.

Carbohydrate and Energy Content

Carbohydrates determined through calculation showed no significant difference ($p>0.05$) between control and RO-PB sugar with 92.57%±3.22 and 93.10%±2.51, respectively. Total carbohydrates in sap form are quantitively low but they increased substantially during production due to the reduction of moisture component. Palm sugar carbohydrate content in palm sugar is mainly composed of sucrose followed by glucose or fructose [21]. Palm sugar is considered rich in carbohydrates and suitable to be incorporated as an ingredient for high-energy source foods and beverages.

Distinct physicochemical and proximate attributes of sugar produced are observed through the natural state of the sap obtained. There are a few considerable factors that may influence the abundance of the composition of proximate and chemical of the sap, for instance, harvesting periods, geographical location of the plant, time, and year-to-year growing period [22; 23]. The composition of some attributes also changes when subjected to downstream processing such as thermal and membrane-filtration treatment which significantly underwent fortification or degradation. The reverse osmosis process, for instance, has a retention capability on some minerals, particularly in food concentration applications [12]. The concentration by RO discards only the unwanted water or moisture component of food while increasing the value of soluble solids and some minerals by folds to a certain extent. However, high-temperature treatment by boiling after RO pre-concentration diminishes the retention effect and hence explains why the value of most proximate and chemical composition between control sugar and RO-PB are not significantly different. Statistical t-tests between control and RO-PB sugar showed no significant differences across each one of the proximate attributes. Therefore, integrating the RO process as a pre-concentration step has negligible impact on the outcome of the proximate results.

Table 2. Chemical properties of control (commercial) and RO-PB sugar

Properties	Sugar (control)	RO-PB sugar
pH	5.36±0.04^{a}	4.87±0.06^{b}
Total acidity (%)	0.21±0.03^{a}	0.42±0.01^{b}
Total soluble solid (°Brix)	82.5±0.00^{a}	80.5±0.00^{b}
Water activity (a_w)	0.55±0.02^{a}	0.58±0.03^{a}
Reducing sugar (%)	5.27±0.02^{a}	4.29±0.02^{b}
Solubility (%)	99.56±0.09^{a}	99.12±0.06^{a}

*Means ± SD in the same row with the same letters are not significantly different.

Chemical properties of both control (commercial nypa sugar) and RO-PB processed nypa sugar in terms of pH, total acidity (TA), total soluble solids (TSS), water activity, reducing sugar and solubility are shown in Table 2.

PH, Total Acidity, and Total Soluble Solids

There are few organic acids noticeable in the nypa sap, for instance, tartaric, formic, acetic, and lactic acid. The total acidity of nypa sugar was calculated based on lactic acid equivalence as it is the predominant organic acid present [24]. There comparison between the control and palm revealed a significant distinction in terms of pH value. The disparity of acidity is likely impacted by the post-harvest practices and the preservation of the sap. For the control sugar sample, the sap used is in excellent condition as indicated with a natural pH value in the range of 5 to 6 [25]. Moreover, the fermentative oxidation by the microorganisms is none to minimal as the sap is immediately processed after being harvested from the nipah plant stalks.

For RO-PB sugar, the sap used is obtained from the same harvesting area as the control sugar sap, but it is subjected to a few days of storage and transportation to the UTS research facility. Generally, fresh nypa sap has a sweet and fruity odor and does not contain higher alcohols and acetic acid. On the other hand, the fermented sap contains a large amount of ethanol, alcohol, esters, diacetyl, and higher acetate [26]. The gap in-between harvesting and processing is sufficient for fermentation to occur as the phenomenon can take place rapidly. Fermentation causes changes in pH towards acidity attributes and also increases the total acidity. The acidity properties of palm sugar are clearly indicated by the significant differences when compared with commercial sugar in terms of both total acidity and pH value.

Total Soluble Solids (TSS) refer to the total amount of soluble substances present in a solution and typically measure the concentrations of sugars and other soluble substances. TSS values of control and PB-RO sugar are 82.5±0.00 and 80.5±0.00, respectively. The sugar concentrations for control were slightly higher and the result has a relation with the sap used before being processed into sugar. Sap used in PB-RO is more acidic (lower pH) than sap used for control because of pre-fermentation which converts some sugar into alcohol byproduct and generally reduces total sugar count and also affects the total soluble solids content.

Water Activity and Solubility

Water activity is associated with the availability of moisture content in the sugars. The water activity is also co-related with microbial activities in directly proportional trends. The higher the water activity (a_w) values, the higher the chance of microbial growth and potential biochemical degradation reactions [27]. Semi or crystalline solid sugars are in nature very hygroscopic [20], in which they may readily retain moisture from surroundings and disrupt the balance between water activity and shelf-life of the sugar. The properties of the sugar are controllable by storing it in a cool dry environment, proper packaging material containing a moisture absorber and bulking agent for preservation. In terms of solubility, both control and RO-PB sugar are highly soluble at room temperature with percentages of 99.56%±0.09 and 99.12%±0.06, respectively. Sugar in nature dissolves readily and is very soluble in water at room temperature [28]. The alteration of the sugar production process in this case unaffected the outcome of solubility to a significant extent.

Reducing Sugar

Palm sugar is mostly composed of non-reducing sugar, sucrose. Sucrose is converted into reducing sugar through hydrolysis which is accelerated by high temperature and long heating time of processing [21]. The intensity of the brown color of the sugar is indicated by the presence of a high reducing sugar content that promotes the Maillard reaction [29]. The determination of reducing sugar for control and RO-PB sugar are 5.27%±0.02 and 4.29%±0.02, respectively. The percentage of control reducing sugar are significantly higher which reflects the effective yield of conversion from sucrose to reducing sugar through full evaporation process. Long and continuous heating process can limit the intervention from some micro-organisms that can cause variation to total sugar and reducing sugar content percentage. As for RO-PB sugar, there is a chance of micro-organisms contamination during RO process especially, that can convert sucrose to other invert sugar such as glucose and fructose, and subsequently to organic acids and alcohol byproducts [30].

Table 3. Minerals profile of control (commercial) and RO-PB sugar

Minerals	Sugar (control)	RO-PB sugar
Potassium, K (mg)	344.10	488.3
Calcium, Ca (mg)	77.9	85.00
Sodium, Na (mg)	31.5	44.10
Phosphorus, P (mg)	37.2	35.00
Iron, Fe (mg)	2.56	3.00

The mineral composition of both sugars was analyzed using the AAS detection method and the results are presented in Table 3. Overall, both sugar has a high concentration of potassium, sodium, and phosphorus. Among all, Potassium (K) is the most abundant mineral and the observation is in line with the studies by other researchers. Reid and Hayes (2003) [31] on nypa palm syrup, Hebbar et al., (2015) [32] on coconut sugar, and recently, Thi Le et al., (2020) [33] on Palmyra palm sugar has a similarity where potassium is the most abundant mineral when detected for analysis. Rich in potassium enable nypa sugar as a selection of vital vitamin source for fluid equilibrium within the human body and also stabilize blood pressure level [34].

Every mineral element detected is in appropriate proportion including heavy metals such as Iron, (Fe) which are very crucial in small quantities for human bodily and biological function [35]. Overall, there are no significant differences for all minerals detected for both control and RO-PB sugar. However, the content variation of nutrients and minerals are not only influenced by the plant varieties but also by other factor such as soil condition and climate [36]. Even for nypa sap harvested from the same area, the nutrients can be varied due to environmental variables.

CONCLUSION

Reverse osmosis-pan boiling (RO-PB) method has shown a promising potential as an alternative with the production of sugar equivalent to the one produced by conventional full pan boiling or evaporation method. In this study, the proximate and minerals analysis of RO-PB sugar when compared with control (commercial) sugar are not significantly different ($p>0.05$). However, major minerals such as potassium (K) and calcium (Ca) is slightly higher in RO-PB due to retention effect of reverse osmosis process. As for chemical properties, higher acidity for RO-PB sugar were observed indicated by lower pH value and two folds of total acidity than control sugar. Low pH affects the outcome of other attributes and resulting to lower total soluble solids and reducing sugar content. Variation of pH detected in this study is likely contributed by early fermentation of nypa sap used and therefore, a fresher sap is recommended.

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