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Effect of heating conditions on physical and chemical characteristics of sugar syrup

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Abstract

There has been an interest in the development of Thai local products, including palm sugar. The main raw material of palm sugar in Thailand is the sap of Palmyra palm. The heating process to produce sugar syrup leads to physical and chemical changes. A limitation of 5-hydroxymethyl furfural (HMF) content has been set according to the regulation by Codex Alimentarius (CA) due to its carcinogenic potency. The degree of physical and chemical changes depends on the heating rate and time. It is important to study the effect of slow and quick heating rate for syrup production. Palmyra palm sap had the L*, a*, b* values of 69.21, -0.21 and 8.17. The total and reducing sugar contents were 10.10% and 0.89%, respectively. This sap contained 227.13 mg/L GAE of phenolic compounds and exhibited 0.28 µmol TE/g sample of antioxidant activity. The sap with initial soluble solids of 12.40°Brix was then heated with small flame (slow heating) and big flame (quick heating) to obtain the syrup with the minimum soluble solids of 65°Brix. The slow-heated syrup exhibited significantly higher antioxidant activity and phenolic compounds in comparison to quick-heated syrup ($p < 0.05$). Quick heated syrup had lighter color, while slow heating produced dark, reddish brown color ($p < 0.05$). Quick-heated syrup (HMF 17.54 mg/kg) was in agreement with CA (< 80.00 mg/kg). However, the slow-heated syrup exceeded the maximum standard (HMF 56.34 mg/L). Quick heating is suggested over slow heating for Palmyra palm syrup production to prevent the HMF content from exceeding the CA recommendation during storage.

Keywords: *Borassus flabellifer*, Palmyra palm, Palm sap, Palm sugar syrup, Heating

1. Introduction

Palmyra palm (*Borassus flabellifer*) is a species of tropical plants from Palmae family which mainly takes habitat in the coasts of Southeast Asia and India (Figure 1) [1]. Parts of the plant are used in a wide range of utilization, including the field of food and beverage, medicine and construction [2,3]. Palmyra palm sap is one of the main sources of the raw material for palm sugar production in Thailand, which is one of the increasingly demanded alternative sweetener due to its minimal processing and health effect [4,5]. The sugar is produced through the boiling process of palm syrup for several h. Palm sap with less than 500 colony-forming unit/gram (cfu/g) total viable count and 100 cfu/g mold and yeasts count will be qualified as the raw material [6].

This heating process results in some changes in the physical and chemical characteristics of the sap. The degree of physical and chemical changes itself depends on the heating temperature, time and initial characteristics of the raw material [7]. Evaporation will occur and lead to an increase in total soluble solids. Browning reactions such as Maillard and caramelization reaction take place at boiling temperature, causing the color of raw material to change [4]. High temperature also accelerates the rate of sucrose inversion, in which sucrose is hydrolyzed into glucose and fructose and thus modifies sugar composition of the solution [7,8].

Ho *et al.* [7] reported that long heating time of approximately 150 min triggered more occurrence of Maillard reaction. A report by Apriyantono *et al.* claimed that 90 min heating of coconut sap can reduce sucrose, glucose and fructose content by 16.2%, 72.3% and 64.3% of the raw material, respectively [9].

Browning reactions also form side products such as 5-hydroxymethyl furfural (HMF), which is a remarkable indicator for over-processing. Some publications reported the increase in browning products and flavor compounds [10] which followed the increase of temperature and time. Due to the potential of genotoxicity as

shown in some reports on in vitro assessment [11], Codex Alimentarius set a maximum recommended HMF content of 80 mg/kg for honey and syrup products in tropical countries and 40 mg/kg for other climates [12]. HMF content also increases during storage, which leads to the importance of minimizing HMF production during processing [13].

Characteristics of sugar syrup is regulated in some standards. The National Standard of Indonesia set a minimum sugar content of 65% or higher [14]. However, the traditional production method of sugar syrup makes it difficult to control the heating parameters and the characteristics of the outcome. Therefore, this raises an interest to study the effect of slow and rapid heating by traditionally varying the flame size on the characteristic of sugar syrup. This study is conducted to determine the appropriate method of palm sugar syrup processing based on the change of physical and chemical characteristics during heating.

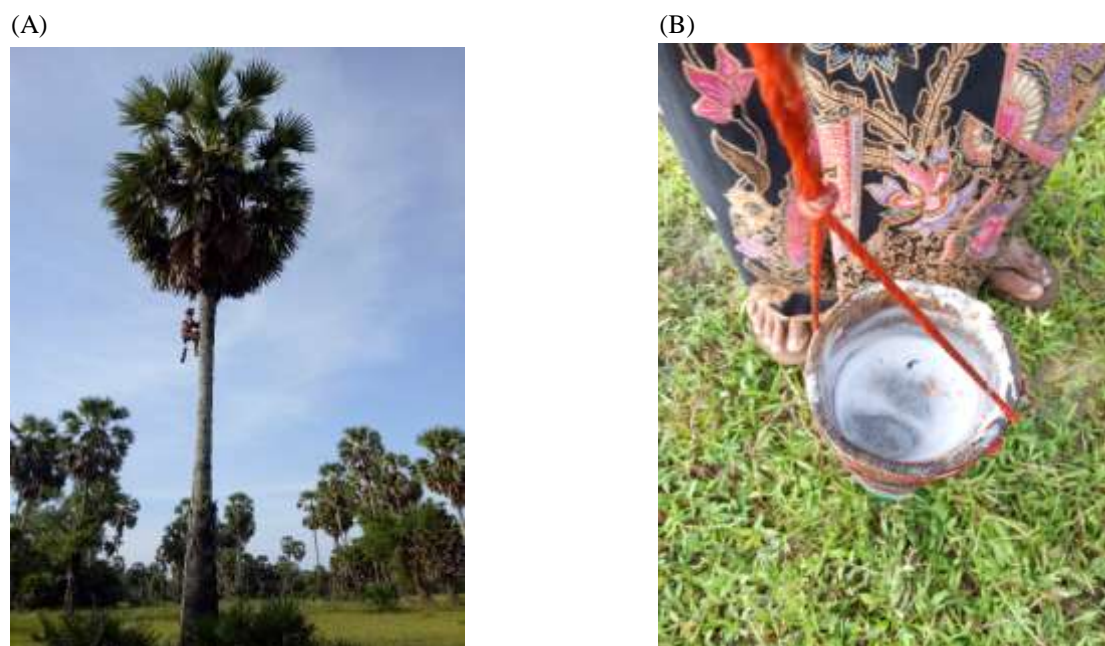


Figure 1 Palmyra palm tree (A) and sap (B).

2. Materials and methods

2.1 Materials

The fresh sap of Palmyra palm was obtained from the producer in Songkhla province, Thailand. The addition of Kiam wood as an antimicrobial agent was applied. Fresh sap was delivered through approximately 2 hours of transportation to the Department of Food Technology, Prince of Songkla University, Hat Yai campus in a cool storage box (4 °C) without any heating pretreatment. Then, the sample was used for further analysis.

2.2 Syrup preparation

Two methods of heat treatment were applied to the samples, slow heating with a small flame and quick heating with a big flame. The changes in temperature and total soluble solid during heating were recorded. Palmyra palm sap was heated in an open pan with temperature approximate to 110 °C to mimic traditional production method until a minimum final total soluble solid of 65°Brix based on Thai industrial standard [6]. The syrup was stirred continuously during heating.

2.3 Physical characterization

2.3.1. Color analysis

The measurement of color was done with a colorimeter (Colorflex, hunterlab, Virginia, USA). The measurement system used was Commission International de l'Eclairage (CIE) system, in which colors were stated in L (lightness), a* (redness and greenness) and b* (yellowness and blueness) [15].

2.3.2. Clarity

The measurement method was taken from Naknean [15]. The clarity of samples was measured through spectrophotometric analysis based on the transmittance at 650 nm. The results were stated in percentage.

2.4 Chemical characterization

2.4.1 pH

pH was measured at ambient temperature using pH meter (Mettler Toledo, K.S.P. OctaTech Co. Ltd., Songkhla, Thailand). Calibration was done with pH 4 and pH 7 standards.

2.4.2 Total acidity

Acidity was determined using a titrimetric method. One gram of samples was diluted in 10 mL distilled water. The solution was titrated with 0.01 N sodium hydroxide as the titrant and 1% phenolphthalein as the indicator. Results were calculated and recorded in percentage as lactic acid. For syrup samples, 2.5 g of sample was diluted into 100 mL of distilled water prior to analysis.

2.4.3 Total and reducing sugar content

Sugar content was assessed through Lane-Eynon method, in which total sugar and reducing sugar were analyzed separately. Lane-Eynon method was a titrimetric analysis with Fehling solution as the titrant and clarified samples as the titrate. An appropriate weight of the sample (50 g of Palmyra palm sap, 20 g of the syrup) was diluted to 100 mL of distilled water and added with approximately 2.5 mL of lead (II) acetate and potassium oxalate until precipitate was formed. Water was added to the solution to obtain a final volume of 250 mL and filtered with Whatman 1 filter paper. 50 mL of the filtrate was taken as the sample of total sugar analysis. This sample was pretreated with 5 mL of 37% hydrochloric acid addition, heat treatment for 10 minutes and pH adjustment with 5 M sodium hydroxide and 2 M hydrochloric acid. The rest of the filtrate was subjected to reducing sugar analysis [16].

2.4.4 Antioxidant activity

Antioxidant activity was determined as scavenging activity against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical. 1.5 mL of the sample solution in appropriate concentration (20% w/w of Palmyra palm sap or 2 g syrup/12 g solution, diluted in distilled water) were added into test tubes containing 1.5 mL of 0.15 μ M DPPH diluted in methanol. The mixture was homogenized and kept in the dark for 60 min. The absorbance was later observed with UV-Vis spectrophotometer (Shimadzu, Bara Scientific Co. Ltd., Bangkok, Thailand) at 517 nm. The blank of DPPH mixed with distilled water in the same manner as the sample was used. The antioxidant activity was measured in μ mol Trolox equivalent based on the standard curve of Trolox ranged from 10 to 60 μ M [17].

2.4.5 Phenolic content

The sample was diluted into an appropriate concentration (50% w/w for sap samples, 2.5% w/w for syrup samples) with distilled water. 1 mL of this solution was added into 0.5 mL of 50% v/v Folin-Ciocalteu reagent and 2.5 mL of sodium carbonate. The mixture was homogenized and kept in dark for 40 min. The absorbance was observed at 725 nm with UV-Vis spectrophotometer. The blank solution was prepared by replacing Folin-Ciocalteu reagent with distilled water. The phenolic content or total reducing capacity was measured in mg gallic acid per grams of the dry sample based on the standard curve of gallic acid.

2.4.6 Total soluble solid

Total soluble solid was measured using hand refractometer (Atago, Japan). The results were recorded in Brix.

2.4.7 Moisture content

Water content was analyzed through gravimetric analysis with vacuum oven as the drying equipment. 3 grams of the samples were put into aluminum pans and heated in the vacuum oven at 60 °C, 70 mmHg for 6 h. Dried samples were weighed after being cooled in the desiccator for 30 min. This process will be repeated until a constant weight is obtained. Water content was determined as the percentage of the mass of lost water to the initial mass of the sample. This determination only applies to syrup samples [18].

2.4.8 Water activity

Measurement was done in the temperature range of 25-28 °C by water activity analyzer (Aqua Lab, Decagon Devices, Inc., Washington, USA). This determination only applies to syrup samples [15].

2.4.9 Hydroxymethyl furfural (HMF) content

HMF content was determined through spectrophotometric analysis. Syrup sample (5-10 g) was diluted in 50 mL deionized water. The solution was centrifuged with Hitachi centrifuge (High Speed Refrigerated Centrifuge CR 226III with R202A rotor, Bio-Active Co. Ltd., Thailand) at 5000 rpm for 15 min. The supernatant (2 mL) was taken and added to a tube containing 2 mL of 12% trichloroacetic acid and 2 mL 0.025 M thiobarbituric acid. The mixture was incubated in a water bath at 40 °C for 50 min. After being cooled down, the absorbance of the mixture was analyzed with UV-Vis Spectrophotometer at 443 nm. HMF content was measured based on standard curve ranged from 0.005 to 0.03 mg/kg. This determination only applies to syrup samples [15].

2.4.10 Statistical analysis

All data from the analysis were taken in triplicates. The experimental design was carried out using complete randomized design (CRD). Physical and chemical characteristics of the sap and syrup samples were analyzed statistically with one-way analysis of variance (ANOVA) with heating treatment as the independent variable (IV). The multiple comparison method selected was Duncan's multiple range test with a confidence level of 95%.

3. Results and discussion

The changes in temperature and total soluble solid during heating are displayed on Figure 1. During heating process, an increase in total soluble solid of each sample was observed. The final total soluble solid was 66°Brix for slow-heated syrup and 67°Brix for quick-heated syrup, which suits the standard of minimum 65°Brix [6]. To reach the desired total soluble solid, slow heating took 105 min, while quick heating only needed 45 minutes. The durations of slow heating and quick heating were significantly different ($p < 0.05$). The final temperatures of slow and quick-heated syrup were 105 °C and 104.4 °C, respectively.

The trend of increasing soluble solid was not directly proportional to the heating time. As can be seen in Figure 2, the soluble solid increased slowly at the beginning and accelerated once the solids reached around 30°Brix. This "jump" phase occurred earlier in quickly heated samples, as the turning point was recorded around 10-15 min while it was reached around 15-20 min for slowly heated samples. This trend associates with the plot curve of water evaporation as described by Lahsasni *et al.* [19].

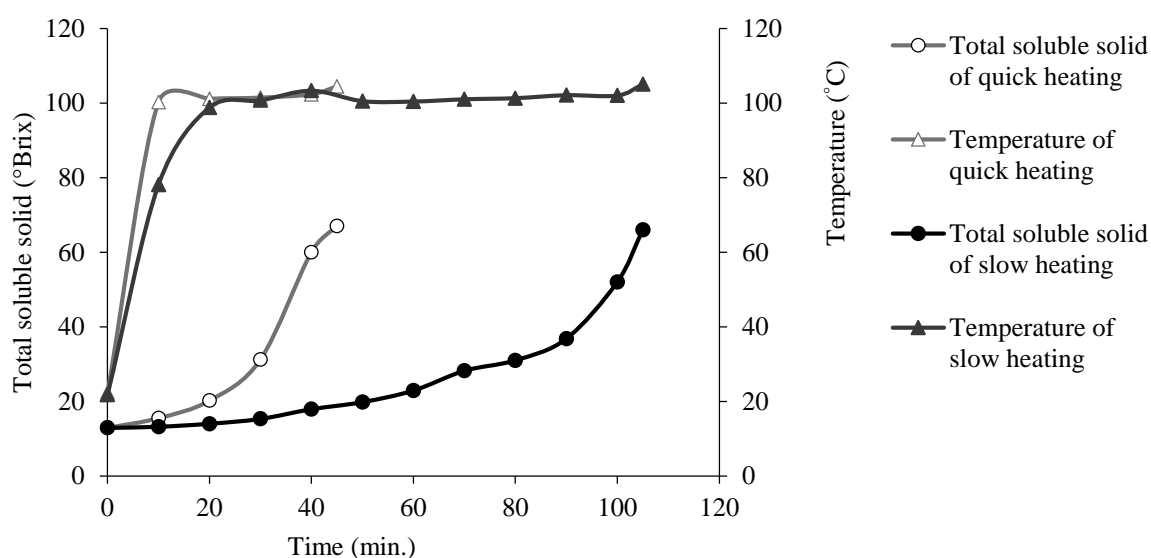


Figure 2 Correlations among total soluble solid, heating temperature and time of Palmyra palm sap processing to obtain syrup.

3.1 Physical characterization

3.1.1 Color analysis

The results of the physical characterization are displayed in Table 1. L^* values of the sap and sugar syrup ranged from 17.42 to 69.41. The mean differences between the sap and the syrup from both slow heating and quick heating treatments were observed on 95% confidence level. The a^* values of the samples ranged from -0.21 to 21.40 with significant mean differences between the sap and both treatments of the syrup. Meanwhile, the values of b^* were ranged from 8.17 to 47.14. The different result was also shown between slow and quick treatments of the samples. Samples of each treatment were significantly different in terms of chroma ($p < 0.05$).

The samples display similar color profile with the sap and syrup as reported in previous research, except for quick-heated samples which has lighter and less brownish color than commercial Palmyra palm syrup [15].

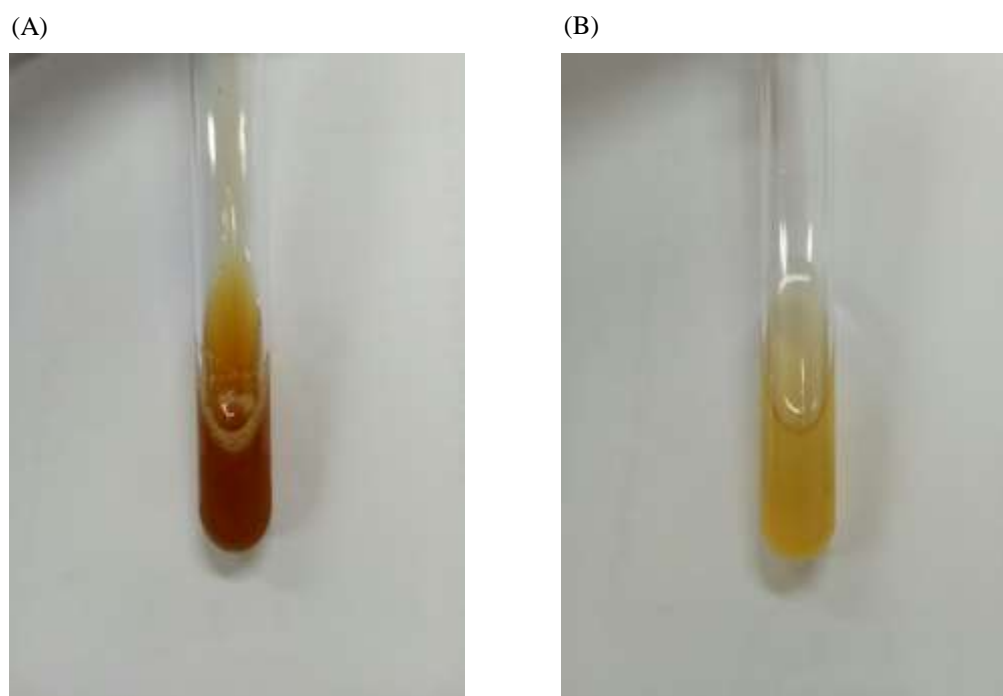


Figure 3 Palmyra palm sugar syrup obtained from slow heating (A) and quick heating (B).

In comparison to the sap, the color of the syrup showed higher intensity in redness and yellowness, while the lightness was found to be lower. These color changes are caused by the formation of HMF through non-enzymatic browning reactions. Brown color comes from the polymerization of HMF, furfural and hydroxyacetyl furan (HAF) which are produced through rearrangements of inversion products [20]. Heating process of Palmyra palm sap created optimum condition for browning reactions. Maillard reaction occurs effectively at temperature above 50 °C. Caramelization takes place under the temperature of 100 °C and above, as well as pH of 3-9 [21]. The study also mentioned that the kinetic of HMF formation was constant along the temperature range of 100-160 °C.

3.1.2 Clarity

The results for clarity analysis are shown on Table 1. Clarity ranged from 1.16% to 9.10%. The difference in means was observed between the sap and syrup ($p < 0.05$). However, the mean difference between heating treatments did not show any significance on the same level of confidence. The clarity of syrup, which was between 1.16% and 1.31%, was within the range of result Naknean's [15], but the sap much lower transmittance than Naknean's reported result of 39.56% to 79.95%.

The transmittance of all syrup was found to be lower than the sap. This was due to the formation of colloidal brown pigments during Maillard and caramelization reactions [15]. Melanoidin skeletons, the product of early stages of non-enzymatic browning reactions, tends to form polymers by binding with existing amino acids [7]. In addition, moisture vaporization during heating caused the proteins, polyphenols and insoluble particles of the sap to concentrate, which also contributes to the increase in turbidity of the syrup [4].

Table 1 Physical characteristic of Palmyra palm sap and syrup.

Properties	Sample		
	Sap	Slow-heated syrup	Quick-heated syrup
L*	69.41 ± 0.20 ^c	17.42 ± 0.41 ^a	35.35 ± 0.72 ^b
a*	-0.21 ± 0.02 ^a	21.40 ± 1.14 ^c	16.03 ± 0.50 ^b
b*	8.17 ± 0.05 ^a	27.21 ± 1.24 ^b	47.14 ± 0.79 ^c
Hue	-88.54 ± 0.17 ^a	51.83 ± 0.41 ^b	71.21 ± 0.81 ^c
Chroma	8.17 ± 0.05 ^a	34.62 ± 1.66 ^b	49.79 ± 0.62 ^c
Clarity (%)	9.10 ± 0.26 ^b	1.16 ± 0.03 ^a	1.31 ± 0.02 ^a

Note: Each value is the mean of determinations ± standard deviation.

The different superscripts in the same row denote the significant difference ($p < 0.05$).

3.2 Chemical characterization

3.2.1 pH and total acidity

The results of pH and total acidity analysis are shown in Table 2. pH of the samples valued between 5.32 to 6.94. A significant difference of means was observed among samples, both between the raw material and final products and between treatments of syrup. pH of Palmyra palm sap was neutral, which was consistent with the result by Naknean, Meenune and Roudaut [8]. In addition, pH of Palmyra palm syrup from both heating treatments decreased from their initial values.

Total titratable acids of the samples were ranged from 0.13% to 9.24%. Significantly different means were shown between treatments ($p < 0.05$). This result corresponded to the values of pH. The changes in acidity and pH can be explained by the occurrence of Maillard reaction, in which acidic products such as glyoxal, pyruvaldehyde, furfural and carboxylic acids are obtained [7].

3.2.2 Total sugar and reducing sugar

All total sugar and reducing sugar content of samples are displayed in Table 2. Percentages of total sugar content fell in the range of 10.10% to 57.00%, while reducing sugar percentages were ranged from 0.84% to 6.40%. The difference between total sugar and reducing sugar contents of syrup treated with slow and quick heating was not significant ($p \geq 0.05$). Total sugar content of both treatments were lower than the minimum standard based on the National Standard of Indonesia [14].

Total and reducing sugar content in each sample increased from the initial values. The increase in total sugar content was related to the evaporation of water from the syrup which concentrated the sugar. The effect of evaporation should affect the content of reducing sugar as well. However, reducing sugar is likely to depend more on the occurrence of inversion reaction. The trend of reducing sugar content was found to follow the trend of pH values, which indicates the effect of acidity on sugar inversion [13].

3.3.3 Phenolic content and antioxidant activity

The phenolic content and antioxidant activity of the samples are shown in Table 2. The phenolic content of the sap (227.13 mg/L Gallic acid concentration (GAE)) was significantly lower than both the slow and quick-heated syrup (2147.89 mg/L GAE and 1971.08 mg/L GAE, respectively) ($p < 0.05$). The means of both analyses were significantly different between heating treatments ($p < 0.05$). This might be caused by the increase of polyphenols concentration due to water evaporation and the effect of other reducing agents in the syrup. On the other hand, the samples may also obtain some newly-formed reducing agents, which consist of Maillard reaction products (MRPs), such as melanoidin and caramelization products (CP) through heating process [17,22].

Table 2 Chemical characteristic of Palmyra palm sap and syrup.

Properties	Sample		
	Sap	Slow-heated syrup	Quick-heated syrup
pH	6.94 ± 0.01 ^c	5.32 ± 0.01 ^a	5.47 ± 0.00 ^b
Total acidity (% as lactic acid)	0.13 ± 0.01 ^a	9.24 ± 0.36 ^c	5.45 ± 0.54 ^b
Total sugar (%)	10.10 ± 0.36 ^a	57.00 ± 2.42 ^b	54.95 ± 1.47 ^b
Reducing sugar (%)	0.84 ± 0.01 ^a	6.40 ± 0.13 ^b	6.06 ± 0.18 ^b
Phenolic content (mg/ L GAE)	227.13 ± 0.03 ^a	2147.89 ± 86.88 ^c	1971.08 ± 29.28 ^b
Antioxidant activity (µmol TE/g sample)	0.28 ± 0.01 ^b	0.44 ± 0.01 ^a	0.30 ± 0.03 ^b
Total soluble solid (°Brix)	12.40 ± 0.00 ^a	67.00 ± 0.00 ^b	66.00 ± 0.00 ^b
Moisture content (%)	n/a	27.78 ± 0.33 ^a	25.07 ± 4.39 ^a
Water activity	n/a	0.82 ± 0.00 ^a	0.84 ± 0.01 ^a
HMF content (mg/kg)	n/a	56.34 ± 0.02 ^b	17.54 ± 0.41 ^a

Note: n/a: not analysed. Each value is the mean of determinations ± standard deviation.

The different superscripts in the same row denote the significant difference ($p < 0.05$). Antioxidant activity which is expressed in DPPH assay ranged from 0.28 µmol Trolox equivalents/gram (TE/g) sample to 0.44 µmol TE/g sample. The antioxidant activity of the sap was significantly increased when processed with slow heating treatment ($p < 0.05$). However, quick heating treatment did not give observable change on this property ($p \geq 0.05$). This was because the formation of new reducing agents occurred during the later steps of non-enzymatic browning reactions [21, 22]. Shapla et al. [11] mentioned that HMF was also one of the compounds which contribute to the antioxidant activity of sugar syrup.

3.2.4 Moisture content and water activity

Moisture content and water activity of each samples are viewed on Table 2. No significant difference was shown between both moisture content and water activity of syrup from different heating treatments ($p \geq 0.05$). Water activity covers the type-III water, the portion of water which are not bonded to polar molecules and are free to be used [19]. While in sugar syrup, the sample is estimated to have notable amount of type-I water which are bonded to sugar and protein content. This type of water, however, only covers approximately 5% of the moisture content [23], which might explain why sugar content is probably insignificant in determining the water activity.

3.2.5 HMF content

HMF contents of the samples are displayed on Table 2. The HMF contents were ranged widely from 17.54 to 56.34 mg/kg. Significant mean difference was observed both in terms of sap and syrup with different heating treatment ($p < 0.05$), where slowly heated syrup showed noticeably higher overall results compared to quickly heated samples. Referring to Codex Alimentarius of honey, the HMF content of both slow-heated and quick-heated syrup were in agreement with the recommended range for tropical countries (< 80 mg/kg) [12]. Though HMF might be undesired due to its risk of toxicity and carcinogenicity, some studies reported that the

compound exhibited antioxidative activity [17], which was proven by the increase in the HMF content along with the increase in antioxidant activity.

Reaction time affects the formation of HMF as proposed by Quintas, Brandao, and Silva [20]. Slow heating treatment prolonged the exposure of the samples to high temperature, enhancing the occurrence of heat-conducted reactions. Therefore, the color change of the slow-heat treated samples was more apparent and the colors were significantly darker compared to their quick-heated counterparts. HMF level can raise during storage [15]. Quick-heating prevents the HMF level to reach the recommended limit during storage better than slow-heating.

4. Conclusion

The final characteristics of Palmyra palm sugar products highly depend on the properties of the raw material. Higher average temperature decreases the time needed to evaporate moisture content of the sap. Quick-heated Palmyra palm syrup tends to display lighter and yellowish color instead of dark reddish-brown color of slow-heated syrup. Slow heating gives the product higher acidity, phenolic content and antioxidant activity. Quick heating gives the product higher final moisture content and water activity. Both slow-heated and quick-heated syrup met the standard of HMF according to Codex Alimentarius. However, both treatments did not meet the National Standard of Indonesia for coconut syrup in which the sugar content should be more than 65%. Quick-heated syrup is recommended over slow-heated syrup to avoid the rise of HMF level during storage.

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