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PHYSICOCHEMICAL PROPERTIES AND ENHANCED ANTIOXIDANT ACTIVITY OF *ARENGA PINNATA* SAP THROUGH DIFFERENT PROCESSING METHODS

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ABSTRACT

Palm saps harvested from *Arenga pinnata* were applied to open pan cooking, freeze drying and vacuum evaporating methods and the effects on the physicochemical properties and antioxidant activity were determined. Results showed that, vacuum evaporation method significantly ($p<0.05$) enhanced the antioxidant activity and moisture content as well as the pH of the sample. The other qualities such as brix, carbohydrate, protein and HMF were slightly lower compared to the other methods might be due to induction of non-enzymatic browning between reducing sugars and amino acids in the palm sap at the processing stage.

Keywords: Open pan; Freeze dry; Vacuum evaporation; Non-enzymatic browning; Antioxidant

INTRODUCTION

Palm saps harvested from *Arenga pinnata* is regarded as one of the nourishment drinks, which supplies energy for rural communities in certain parts of Malaysia, Thailand and Indonesia. The palm saps also being concentrated into palm sugar blocks and normally sold for direct consumption or for food flavouring. The saps are claimed rich with nutrients which may be attributed to the phytochemical activities that activated the antioxidant activity in the palm sugar. It has been extensively studied that consuming the antioxidant will help to fight free radicals in the body. Besides, the utilization of natural antioxidants has been emphasized in order to fight the free radicals that initiated the various diseases, carcinogenesis, and aging [1]. Complementary to this, the literature described by [2,3] has been examined the contribution of antioxidants in foods that protecting from free radical scavenging activity.

However, in normal preparation method (open pan cooking), the active compounds presence in the saps that represented the antioxidant might be substantially destroyed due to persistent and incompatible processing method that causes the high temperature effects [4]. At the same time, the effect from the overheating method had altered the

unique flavour and colour of the palm sugar which also accelerated the production of the non-enzymatic browning reaction (Maillard reaction) [5]. Many authors have reported that natural antioxidants could be demolished during drying treatment as it delicate to oxidation, radiation, etc. [6]. Then again, the depletion of the present or added antioxidants was derived from heating processes and increasing temperature [7]. Therefore, a slow and controlled temperature cooking process such as freeze drying and vacuum evaporation were predicted to have better antioxidant preservation.

There is study scrutinised the development of modern technologies for commercial application and evaporation method is acknowledged to be the best in term of enhancement, economical as well as widely used method [8]. Moreover, this approach is intended to avoid the direct and prolong heating process of palm saps. Thus the main objective of this study was to evaluate the physicochemical properties as well as the antioxidant activity of different palm sugar production methods.

EXPERIMENTAL

Sample collection and preparation
Arenga pinnata sap was harvested from Balung Plantation, Sabah. The palm saps were processed using three different methods. The open pan cooking method was used to prepare concentrated block palm sugar by continuous heating under firewood. Whereas slow and controlled temperature using freeze dryer and vacuum evaporator were applied to prepare palm sugar in powder and syrup forms, respectively.

Determination of physicochemical properties pH
The pH value was measured by pH meter (Crison, Spain). 1 g of palm sugar samples were diluted with 20 mL of pure water at ambient temperature.

Brix content
The Brix content was performed by refractometer (Hanna, USA). The brix values were expressed as percentage of sucrose content. The palm sugar samples were weighed about 0.50 g and let to be dissolved in 10 mL of pure water the analysis was made for triplicates (Amin et al., 2010).

Moisture content
The moisture content was determined using method prescribed by Association of Analytical Communities (AOAC). The palm sugar samples were weighed about 5 g respectively. The samples were left into an oven for 24 hours at 105ºC. The residual moisture was calculated according to the equation as follows:

$$\text{Moisture content (\%)} = \frac{\text{Weight of wet sample - Weight of dry sample}}{\text{Weight of dry sample}} \times 100 \qquad (1)$$

Protein content
Folin–Ciocalteu reagent was used to determine the total protein content as described by [9]. The bovine albumin serum was used as an external standard at 0.05 to 1.00 mg/mL [10]. 1g of palm sugar samples were diluted with 10 mL of pure water. The 0.2 ml of samples was added to 2 mL of alkaline copper sulfate reagent. The solution was mixed thoroughly for about 10 minutes. After that, the 0.2 mL of Folin-Ciocalteu reagent solution was added to the mixed solution and let to be incubated for 30 minutes. The absorbance reading was measured at 660 nm.

Carbohydrate content
The total carbohydrate content was carried out by Phenol-Sulphuric Acid assay. The proposed method is a major improvement over widely used Phenol–Sulfuric Acid Method developed by [11]. 1.0 g of palm sugar samples were diluted using 10 mL of pure water. According to reference method from [12], 3 mL of concentrated sulphuric acid was mixed rapidly in 1 mL of samples and vortex for about 30 s. The mixture was left to stand in ice bath for about 2 min and the absorbance of the mixture was measured at 315 nm.

5-hydroxymethylfurural (HMF) content
The determination of 5-hydroxymethylfurural (HMF) method was modified from [13, 14]. Briefly, the palm sugar samples were diluted into 1:10 ratio using pure water. The standard of HMF was used as a reference standard and ranged between 2 to 20 mg/L. The absorbance was determined at 235 nm and the results were expressed as mg/kg.

Antioxidant activity
2,2-Diphenyl-1-picrylhydrazyl (DPPH) reagent was used to monitor the free radical scavenging effect of the palm sugars at 517 nm using UV-Vis spectrophotometer (Sastec, Germany). According to modified method from [15], the palm sugar samples were diluted with 5 mL of pure water and Whatman filter paper was used to filter the samples. After that, the samples were diluted again to reach at 4º brix. The 100 µl of the samples was homogenized with 1.5 mL of 0.1mM DPPH solution in methanol. The mixtures were left for 30 minutes in the dark. The radical scavenging activity was calculated using the following equation [16]:

$$\text{DPPH (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \qquad (2)$$

Statistical analysis
All analysis was conducted in triplicate and results were expressed as mean ± standard deviation. The statistical analysis ($p<0.05$) was determined by one-way analysis of variance (ANOVA) followed by Tuk ’ u S P k S S (SPSS v 19.0).

RESULTS AND DISCUSSION

Results in Table 1 showed all palm sugar samples were prone to be acidic. The pH value of vacuum evaporated was less acidic than the open pan and freeze dried palm sugar. The values of palm sugars obtained were similar with the freshness of honey that ranged between pH 3.4 to 6.1 [17]. High in acidity could promote the fermentation of sugar into organic acid in the honey that gives off flavour and stability against microbes [18]. Also, the formation of non-enzymatic products during heating affluences the changes in the pH value [19, 20]. Maillard reaction or known as non- enzymatic products were formed through the condensation processes between reducing sugars with free amino group of amino acids or proteins via a formation of a schiff's and the Amadori arrangement [21][22]. The effect of Amadori product was affluences by the pH of the system respectively [23]. At pH 7 or below, the Amadori product will form the HMF, the intermediate product in the Maillard reaction and sugars will degrade at high temperatures [24]. Other study reported that the formation of organic acids like formic and acetic acids in Maillard reaction was responsible for pH declination due to the declension of fructose and glucose [21]. With respect to the results obtained, the slow and controlled temperature by vacuum evaporation method could control the pH of palm sugar from getting more acidic.

Table 1: pH value of palm sugar samples

Samples	pH
Open pan	3.67 ± 0.01^{a}
Freeze dried	4.12 ± 0.05^{b}
Vacuum evaporated	4.62 ± 0.02^{b}

Data presented as mean ± SD of triplicate results. The different superscripts represented the significant differences ($p<0.05$).

Table 2 showed the brix content of all palm sugar samples were falling within the value stated by United States Department of Education (USDOE) Standard at >65°Brix . Other study reported that, the brix content must be ensured to be >65°Brix to avoid any microbial growth in maple syrup [22].

Vacuum evaporation method had significantly ($p<0.05$) lowest whereas freeze drying and open pan cooking exhibited significantly ($p<0.05$) higher in brix content. The present results were related to the conversion of sugar into organic acid during the heating process which affected the total of brix content [9]. The conversion of sugars in palm sugars was much dependent with temperature and time heating during the process. Due to longer heating and inconsistent temperature, it increased the sugar concentration of palm sugar, however the slow and controlled temperature of vacuum evaporation method could slow down the conversion of sugars into the other products. From the foregoing, HMF will be formed from the sugars due to long heating process [23].

Table 2: Brix content of palm sugar samples

Samples	Brix°
Open pan	80.25 ± 0.05^{a}
Freeze dried	80.69 ± 0.32^{a}
Vacuum evaporated	70.05 ± 0.47^{b}

Data presented as mean ± SD of triplicate results. The different superscripts represented the significant differences ($p<0.05$).

Table 3 showed the vacuum evaporation method contained significantly ($p<0.05$) highest of moisture content among all palm sugar samples. There were no significant differences ($p<0.05$) were observed in open pan cooking and freeze drying method. The higher in moisture content indicated higher in viscosity due to low in concentration of sugar content. However, low in moisture content predicted to have more storage stability and was favorable for longer shelf life. However, the high in moisture content was unavoidable and is the limitation of the technology. The previous research stated that the moisture content that exhibited more than 20% or 200 g/kg, the microbial content (*Osmophile* yeast) will presence to yield the carbon dioxide and ethanol [23].

Table 3: Moisture content of palm sugar samples

Samples	Moisture (%)
Open pan	5.25 ± 1.70^{a}
Freeze dried	7.65 ± 1.65^{a}
Vacuum evaporated	23.78 ± 1.77^{b}

Data presented as mean ± SD of triplicate results. The different superscripts represented the significant differences ($p<0.05$).

Carbohydrate content in table 4 showed no significant differences ($p<0.05$) between vacuum evaporated and freeze dried palm sugar. In comparison, the open pan palm sugar exhibited slightly higher the carbohydrate content due to high temperature process that urged the chemical reaction of the sugars (glucose, fructose and sucrose). While this is the case, [25] $\geq 88\%$ of the carbohydrate content of date fruits was defined by the formation of digestible sugar. Research reported by [26] described that major carbohydrates found in the agaves syrup were fructose, glucose, inositol and mannitol. However, the conversion of the long heating sugar will lead to the conversion of HMF.

Table 4: Carbohydrate content of palm sugar samples

Samples	Carbohydrate (%)
Open pan	8.90 ± 0.36^{a}
Freeze dried	8.53 ± 0.22^{b}
Vacuum evaporated	8.31 ± 0.09^{b}

Data presented as mean ± SD of triplicate results. The different superscripts represented the significant differences ($p<0.05$).

Table 5 showed there were significance differences ($p<0.05$) in protein content among palm sugar samples. Vacuum evaporation method had significantly ($p<0.05$) low in protein content compared to other methods. A slow and controlled temperature during the evaporation process had influenced the low concentration of the palm sugar. The high temperature seems to promote the increases of the protein content during the open pan cooking of palm sugars. In this respect, the protein was involved in Maillard reaction that interacts with reducing sugars that contributed to flavour and colour apparent.

Table 5: Protein content of palm sugar samples

Samples	Protein (%)
Open pan	13.52 ± 0.91^{a}
Freeze dried	5.41 ± 0.53^{b}
Vacuum evaporated	3.62 ± 0.06^{c}

Data presented as mean ± SD of triplicate results. The different superscripts represented the significant differences ($p<0.05$).

The different superscripts represented the significant differences ($p<0.05$). Figure 1 showed HMF in vacuum evaporated palm sugar appeared the significantly ($p<0.05$) lowest. Open pan cooking method was observed to have high in HMF content compared to the other methods. These results signify that high in temperature and longer heating processing time were the major contributor in HMF content. [27] reported that the induction of HMF formation in honey was heated at 50–100 ºC. The previous research demonstrated that non-enzymatic browning reaction yielded the intermediate product (HMF) which increased with sugar content and baking time [13]. HMF, or chemically known as 5-hydroxymethyl- 2-furaldehyde, is a water-soluble heterocyclic organic compound from sugars derived. HMF is created during food pasteurization and cooking as consequences from sugars dehydration like glucose and fructose.

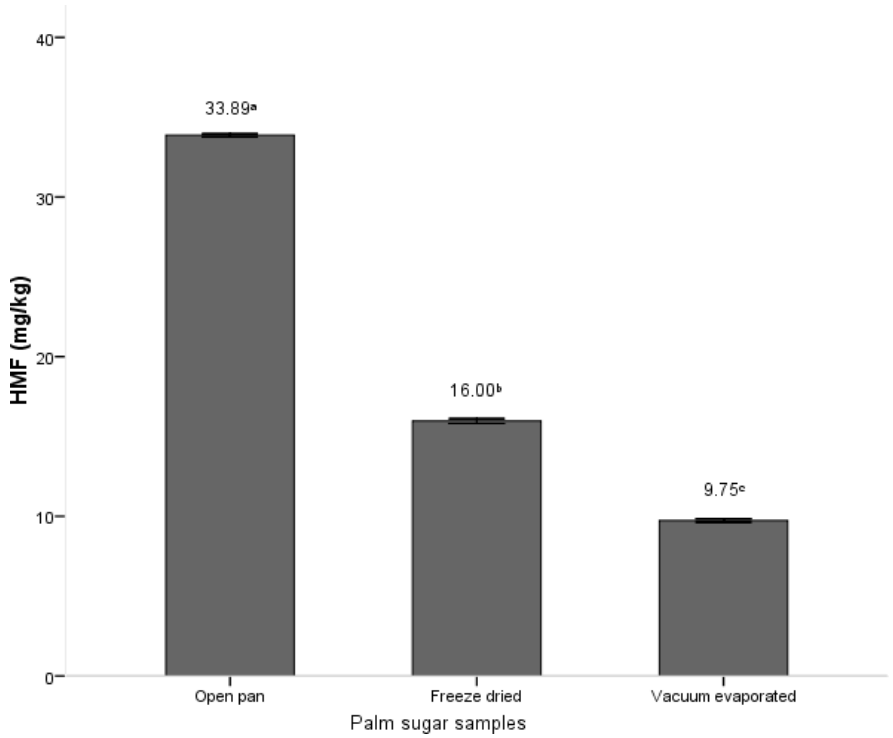

Figure 1: HMF content of palm sugar samples. Data presented as mean ± SD of triplicate results

Due to toxicological status in having carcinogenic potential, the Codex Alimentarius was limited the HMF content to only 40 mg/kg [14]. The previous study of HMF towards honey bee using high fructose corn syrup (HFCS) showed that 150 ppm of HMF treatment cause mortality to the honey bees [28]. High in store honey about 4 to 8 years that highly contained of HMF, had caused the mortality towards the bees [29]. Based on the results obtained, the vacuum evaporation method seems to have low inversion reactions and non-enzymatic reaction which slows down the conversion of sugar into HMF. Furthermore, the non-enzymatic product is unfavourable to be exist in any food production process due to its effect in shortening the shelf life of the product [25].

Figure 2 showed that the scavenging of the stable DPPH activity and there were significance differences ($p<0.05$) was observed in antioxidant activity among all samples. In previous study, the DPPH method was used to determine the free radical scavenging activity in foods and beverages as this method is accurate, rapid, simple and inexpensive [30]. Vacuum evaporation method had significantly ($p<0.05$) highest in antioxidant activity compared to freeze drying and open pan cooking method. However, it was observed that heating process of palm saps into concentrated sugars might have given the physicochemical stability, but on the other hand the natural occurring antioxidants were significantly reduced. This could be the best reason of the lowest in antioxidant activity of open pan cooking method. Moreover, the higher temperature process might have destroyed active compounds that resembled the antioxidant effects.

Meanwhile, the consequences of the processing and storage also could losses the naturally occurring antioxidants [31]. The vacuum evaporator process has assisted the formation of palm sugar syrup under slow and controlled temperature which capable to slow down the degradation of antioxidant activity. In term of slow and controlled temperature, it was noteworthy to claim that using vacuum evaporation method might have preserved antioxidant content compared to freeze drying and open pan cooking methods. This is consistence with other study by [32] that the vacuum evaporator was able to reduce the degradation of palm sugar quality. [33] examined the palm dates syrups using rotary evaporator processes had showed that the high percent inhibition of the DPPH radical that ranged between 67% to 94%.

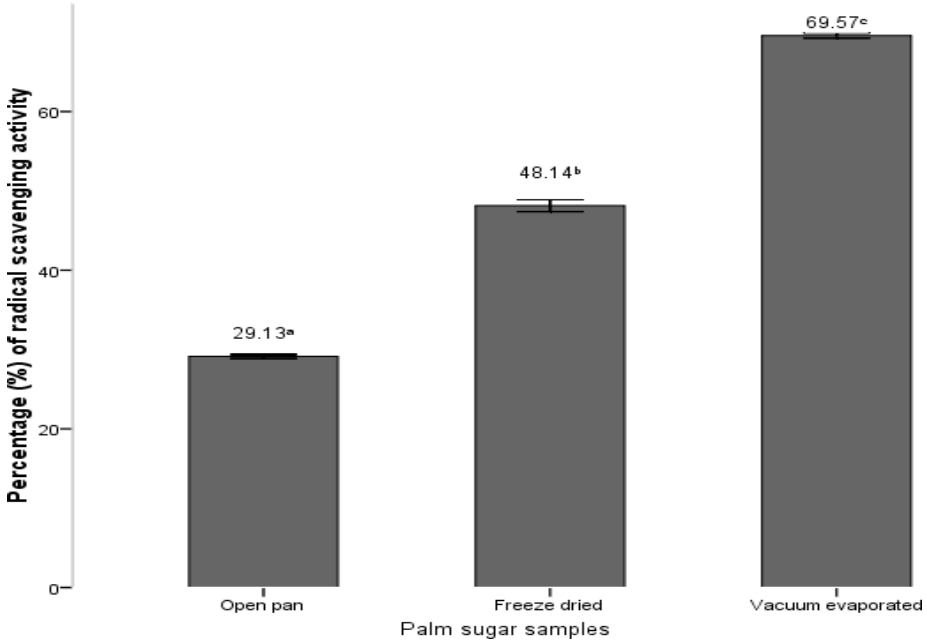

Figure 2: Antioxidant activity of palm sugar samples. Data presented as mean ± SD of triplicate results. The different superscripts represented the significant different ($p<0.05$)

CONCLUSION

This study concluded that vacuum evaporation method was able to preserve higher antioxidant activity than the other processing methods. The high temperature and prolonged heating might be the main factor in the reduction of antioxidant activity in the palm sugar. Thus, this study suggested that the slow and controlled temperature processes using vacuum evaporator is the most promising processing method of palm saps.

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