ANTIOXIDANT CAPACITY OF VACUUM EVAPORATION OF PALM SYRUP (*ARENGA PINNATA)*

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ABSTRACT: *The applications of different methods (open pan, freeze drying and vacuum evaporation) of Arenga pinnata saps were evaluated. The palm sugars were determined for chemical properties and antioxidant capacity. The vacuum evaporation method showed that, the total sugar content, total acidity and browning intensity were slightly lowered ($p<0.05$) compared to the other methods. Considering the antioxidant parameters, the results showed that the vacuum evaporation method significantly ($p<0.05$) enhanced the antioxidant equivalent ascorbic acid content (AEAC) and hydrogen peroxide scavenging capacity respectively. Moreover, the vacuum evaporator exhibited the highest antioxidant capacity by phosphomolybdenum method. These present findings showed that the vacuum evaporation method could significantly enhance the antioxidant capacity for production of palm sugar syrup.*

Keywords: Open pan, Freeze drying, Application, Method, Production

1. INTRODUCTION

Arenga pinnata tree which is originated from tropical South Asia and South East Asia such as India and Bangladesh was brought to Malaysia, Indonesia and Philipines [1]. This tree is very suitable in tropical region and humid climate [1]. In Malaysia, this tree is usually found at wet tropical rain forest which also extended its ranging to the dry forests [2]. Furthermore, this tree has been regarded as one of the top multipurpose and economically tree as interpreted by [3]. The vital product of this tree is the fresh palm sap which is produced in the inflorescences and is collected in the process of tapping using bamboo tube. The fresh palm sugar sap was known to rich in sugar content, which mainly sucrose, glucose and fructose which is associated only in small amounts. It is content proteins, mineral elements and phenolic compound [4]. Other author also stated the palm sugar is a source of the energy source due to its properties in slow release energy [5].

The saps yielded by *Arenga pinnata* tree which varies in nutrient content were usually produced into concentrated palm sugar block. The palm sugar is more demanded in application of foods and beverages as a sweetener. The heating processes of palm saps assisted the browning formation of palm sugar, which induced the reaction of non-enzymatic browning reaction. Besides that, the non-enzymatic reaction between the reducing sugars and amino group during heating is important in giving off flavour to the palm sugar. However, the high temperature and longer heating might be reduced the active nutrients content in the palm saps. It is well known that the naturally occurring antioxidants were rapidly reduced when being treated. On the other hand, the possible naturally occurring antioxidants activity that might presents in the palm sugar was also substantially decreased. In fact, many authors have reported the heating process causes the deterioration of nutrients content as well as the organoleptic quality of the products [6].

Many studies shown that the heating application had affected the physical and chemical qualities [7].The treatment processing for food such as drying, cooking, and extrusion may disturb the preservation of antioxidants in food matrices [8]. Moreover, many studies had claimed that the food processing is the major contributor to the degradation and alteration of natural phytochemicals, which may influence the antioxidant capacity in foods. Complementary to this, the application of modern technologies like freeze dry and vacuum evaporator are probably assisted the preservation of the product quality as well as the antioxidant capacity in the palm sugar. Besides that, this implementation in terms of slow and controlled temperature processes could slow down the degradation of antioxidants during processing of palm sugar. Thus, the objective of the present study is to evaluate the chemical compositions and the antioxidant capacity in the palm sugar employed by different methods of production (open pan, freeze drying and vacuum evaporation).

2. MATERIALS AND METHODS

Fresh palm saps were harvested from *Arenga pinnata* tree that grown at Balung Plantation, Sabah. Three different methods (open pan, freeze drying and vacuum evaporation) were applied to the palm saps. The concentrated block palm sugar was prepared using an open pan by continuous heating under wood fire stove. Meanwhile, the productions of palm sugar in powder and syrup forms were prepared using freeze dryer and vacuum evaporator respectively.

The palm sugar samples were prepared with the ratio (1:4) with pure water [9]. The total sugar content was measured using refractometer (Hanna, USA) and the values were expressed as g/ml equivalent of sucrose content. Total acidity was measured by modified method [10] titration method with NaOH and was expressed as citric acid equivalent as an acid factor. Browning intensity was determined using UV-Vis spectrophotometer (Sastec, Germany). at 420 nm. The palm sugar samples were diluted according to (1:9) in ratio using pure water [11].

The antioxidant equivalent ascorbic acid content (AEAC) was measured as described by [12]. The palm sugar samples were diluted in methanol and 0.75 mL of the dilution was then mixed with 1.50 mL of a 0.02 mg/mL DPPH solution in methanol. The mixture was then let to stand for 15 min

and the absorbance reading was measured at 517 nm. The ascorbic acid was used as a standard calibration curve and expressed as mg of ascorbic acid equivalent antioxidant content per 100g of palm sugar. The hydrogen peroxide scavenging capacity was determine according to the method by [13]. Hydrogen peroxide solution was prepared in phosphate buffer (pH 7.4). The 1 mL dilution sample of palm sugar was mixed with 2 mL of hydrogen peroxide solution. After 10 min, the absorbance reading was measured at 230 nm against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide was calculated as scavenged (%) = (Absorbance of the control – absorbance of the sample/ Absorbance of the control) x 100.

The total antioxidant capacity of sugar sap was assayed by phosphomolybdate method using ascorbic acid as a standard [14]. The 0.1 ml of sample solution was added with 1 ml of reagent solution of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate and was incubated in a water bath at 95°C for 90 min. The absorbance reading at 695 nm against a blank that contained 1 ml of the reagent solution along with an appropriate volume of the solvent and incubated under similar conditions. The total antioxidant capacity was expressed as mg/100g of ascorbic acid equivalent.

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 Tukey's using Statistical Package for Social Science (SPSS version 19.

3. RESULTS AND DISCUSSION

Table 1. Chemical properties of different methods of palm sugar. Data presented as mean ± SD of triplicate results. The different superscripts represented the significant different ($p<0.05$).

Parameter	Open pan	Freeze dryer	Vacuum evaporator
Total sugar (%)	67.60 ± 0.35^a	71.47 ± 0.53^b	49.60 ± 0.35^c
Total acidity (%)	0.05 ± 0.05^a	0.06 ± 0.00^a	0.03 ± 0.01^a
Browning intensity	3.56 ± 0.01^a	2.32 ± 0.01^b	1.56 ± 0.03^c

From the table 1 the total sugar equivalent to sucrose content showed all the palm sugar samples were significantly different ($p<0.05$) and vacuum evaporated palm sugar exhibited the lowest total sugar content at 49.60%. The other two palm sugars (open pan cooked and freeze dried) had higher total sugar content at 67.60% and 71.47%, respectively. The slow and controlled temperature in vacuum evaporation process might promote the slow conversion of sucrose in the palm sugars into organic acid. Other author revealed that the conversion of sucrose to glucose and fructose and also organic acids and alcohol in palm sugars were under the invertase reaction [15].

While this is the case, the vacuum evaporator could minimize the decreases of sucrose content to reducing sugars during the processing method of palm syrup. In fact, reducing sugars content play as a substrate for Maillard (browning reaction) that vital for determination quality of the palm syrup from degraded and formed into other compounds such as HMF.

The percentage of total acidity was determined as the citric acid equivalence and no significant different ($p<0.05$) for all palm sugar samples were observed. Result showed that, the vacuum evaporator method was found to have the lowest acidity (0.03%) of palm syrup meanwhile the freeze dryer method appeared the highest acidity (0.06%). The initial pH for the fresh palm saps was around 6.10 that still have a sweet taste and pleasant smell [16]. Other author reported that the high percentage of total acidity indicated that the sugar fermentation process had occurred [17]. In this respect, [18] obtained the *Arenga pinnata* saps has a degree of acidity with pH 5.5 to 6.0. Due to palm saps rich in sugars content, the microorganisms such as lactic acid bacteria and *Saccharomyces cerevisiae* utilised as an energy source that induced the fermentation process to occur. Besides, these microorganisms promoted the conversion reactions to acids and alcohol [19]. Besides that, the phenomena of Maillard reaction or known as non-enzymatic browning that resulted from sugars and amino acids interaction tend to decrease the pH values. This is because, the higher pH was prone the Maillard reaction to take place since the sugar was in open chain form and the amino group was unprotonated [20].

The study revealed by [21, 22] stated that, the pH was constantly droppin as the heating time getting higher during the reaction of Maillard. From this explanations denoted that the vacuum evaporator method could avoid the rapid process of sugars fermentation in the palm sugar.

The table 1 showed that the browning intensity of all palm sugar samples were significantly different ($p<0.05$). The vacuum evaporated of palm syrup showed the lowest browning intensity (1.56) followed by freeze dried palm sugar (2.32) and open pan (3.56). The highest absorbance at 420 nm remarked that more non-enzymatic browning reactions had a presence at higher rate of reactions. Complementary to this, other researcher propounded that factors that significantly affect the rate of the Maillard reaction were the pH, amino compounds and reducing sugar content [23].High in browning intensity denoted that the palm sugar might contain high in HMF amount. It was stated the non-enzymatic browning reaction yielded the intermediate product (HMF) which increased with sugar content and baking time [24]. Due to toxicological status in having carcinogenic potential, the Codex Alimentarius limited the HMF content to only 40 mg/kg [25].

The study evaluated by [11] showed that the highest concentration of sucrose formulation in palm sugar-like flavoring yielded in high browning intensity. With respect to the results obtained, vacuum evaporator seems to have lowest in a sucrose inversion reaction which slows down the conversion of sugars into other products (Amadori rearrangement products) and HMF. The decomposition of Amadori will produce many compounds such as reductones that may be precursor for browning pigment, melanoidins [26].

Table 2. Antioxidant capacity of different methods of palm sugar. Data presented as mean ± SD of triplicate results. The different superscripts represented the significant different ($p<0.05$).

Parameter	Open pan	Freeze dryer	Vacuum evaporator
AEAC (mg/100g)	0.03 ± 0.00[a]	0.05 ± 0.00[b]	0.07 ± 0.00[c]
Hydrogen peroxide scavenging (%)	21.80 ± 1.0[a]	36.78 ± 5.61[b]	47.51 ± 5.81[c]
Phosphomolybdenum (mg/100g)	0.56 ± 0.170[a]	0.81 ± 0.03[b]	1.25 ± 0.02[c]

The table 2 results showed that the antioxidant content was determined by antioxidant equivalent ascorbic acid (AEAC). The AEAC content of the palm sugar samples was measured in mg/kg using the stable radical DPPH. With respect to the results obtained, there were significant differences ($p<0.05$) in antioxidant content of all palm sugar samples respectively. As it can be observed, the vacuum evaporator showed slightly highert (0.07 mg/kg) than the other methods. As expected the open pan exhibited the lowest (0.03 mg/kg) of antioxidant content compared to freeze dryer (0.05 mg/kg). Other researcher [29] denoted that high syrup concentration contributed in low concentration of DPPH which indicated with high antioxidant activity. However, the present findings were in contrast where the open pan method exhibited the highest browning intensity had contained low free radical scavenging effect meanwhile the lowest browning intensity by vacuum evaporator method had contained highest free radical scavenging effect. These data elucidated that the palm syrup by vacuum evaporation might preserve the natural occurring antioxidants better than the other two methods. Besides that, the non-enzymatic browning product might not become the main reason that responsible for antioxidant activity.

From the results obtained, the percentage of hydrogen peroxide scavenging activity showed that there were significant different ($p<0.05$) in all palm sugar samples. Then again, the vacuum evaporator exhibited the stronger inhibition effect to the hydrogen peroxide (47.51%) compared to freeze dryer (36.78%) and open pan (21.80%). These findings showed that both methods freeze dryer and open pan showed less efficacy in scavenging ability of hydrogen peroxide. During the propagation period the hydrogen peroxide and hydrogen peroxide free radicals that deduced from alkyl free radicals contributed as the major products [30]. The research reported by [31] about the properties of hydrogen peroxide that could be a toxic to cell though it is not much proactive but could trigger the hydroxyl radical in the cell body.

Meanwhile, the total antioxidant capacity which is expressed as ascorbic acid equivalents was determined using the spectrophotometric method. This method involves in thermally generating auto-oxidation during prolonged incubation period at higher temperature [32]. As it can be observed, all the palm sugar samples showed significantly different ($p<0.05$) and vacuum evaporator showed the highest antioxidant capacity (1.25 mg/g). With respect to the results obtained, the slow and controlled temperature processes could reduce the deterioration of naturally occurring antioxidants during processing of the palm sugar saps. Complementary to this, the results indicated that, the vacuum evaporated palm sugar was able to act as reducing agent to reduce Mo (VI) to Mo (V). As interpreted by [33], the reducing power is regarded as one of the indicators in determining the antioxidant capability for medicinal herb. It was clearly shown that, the controlled and slow temperature process by vacuum evaporator performed the positive results in enhancing the antioxidant capacity for production of palm sugar compared to other methods.

4. CONCLUSION

There were significant differences in total sugar, browning intensity and antioxidant capacity among the three methods of processing of palm sugar. This study concluded that the vacuum evaporation method was able to preserve the higher antioxidant capacity than the other methods, while maintaining the other chemicals properties. The slow and controlled temperature process applied in vacuum evaporator might be the main reason to the retention of antioxidant capacity in the palm sugar. The present data research regarding the capability in antioxidant effects may be useful for optimizing the significant temperature during processing of palm sugars by using the experimental of design which may probably useful for the large scale production. Thus, this present study strongly recommended that using vacuum evaporator is the most promising processing method for production of palm sugars. As the vacuum evaporation method is proven to be highly preserved the antioxidant activity, the further study regarding the specific compounds that resemble the antioxidant effect of palm sugars should be studied in depth.

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