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# Sustainable Processes and Chemical Characterization of Natural Food Additives: Palmyra Palm (*Borassus Flabellifer* Linn.) Granulated Sugar

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**Abstract:** Palmyra palm (*Borassus flabellifer* Linn.) is an important sugar-producing plant that is widely distributed in tropical Asian countries. Its jaggery and sweet sap are prevalent in Cambodia as a substitute for table sugar. They contain essential minerals, vitamins, and biological compounds. We investigated the changes in the nutritional composition, antioxidant properties, and biological activity of palm granulated sugar prepared by using three different drying–solidification processes under vacuum conditions: the drying temperature was controlled at 80 °C, 90 °C, and 100 °C, and the drying time was 60, 75, and 90 min, respectively. Palm granulated sugar contains 10 kinds of vitamins (mainly vitamin E 52.15–55.12 mg/100 g), 5-hydroxymethylfurfural (2.18 to 41.92 mg/100 g), and 38 volatile compounds that belong to the alcohol, ketones, pyrazines, acids, and phenols groups, and an aldehyde group. Moreover, palm granulated sugar exhibits a high total phenolic content (2.77–8.94 mg gallic acid equivalent/100 g), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (20.15%–37.88%), and ferric reducing antioxidant power (FRAP) value (322.68–378.23  $\mu\text{mol Fe}^{2+}/\text{mL}$ ). Furthermore, palm granulated sugar-treated NIH3T3 cells showed a higher cell viability of 18.10% to 23.68%. This study confirmed that palm granulated sugar prepared at 90 °C for 75 min can have a better product quality with increased vitamin and mineral contents, antioxidant properties, and biological activity, while also being low in 5-hydroxymethylfurfural (HMF) content.

**Keywords:** palmyra palm; granulated sugar; antioxidant properties; biological activity

## 1. Introduction

*Borassus Flabellifer*, also called palmyra palm, is a popular material used to produce palm sugar in Asian countries [1]. Palm trees play an important role in agriculture in Cambodia. *B. flabellifer* sap is used for wine, palm jaggery, and palm sugar, which are all rich in vitamins and minerals [2]. Many pharmacological advantages of *B. flabellifer* have also been reported [3,4]. *B. flabellifer* sap extract reduced the growth of serum glucose levels in sucrose-loaded rats [5] and has anti-inflammatory activity [6] and analgesic effects [7]. In addition, 2,3,4-trihydroxy-5-methylacetophenone extract from palm syrup exhibited 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity [8].

Palmyra palm sugar in Cambodia is known by other names such as palm jaggery, palmyra jaggery, Neera (India), and Gur (South Asia). It has a unique flavor and produces less energy than cane sugar [9]. The palm sugar industry is an unorganized rural industry in Asian countries. The clean sap of *Borassus flabellifer* is heated to 110 °C to 120 °C and blended until it is brown and thickened [10]. This process is mainly based on the traditional practices of village producers and was transferred down from older generations without accompanying scientific data. The disadvantages of palm sugar developed using this method are its unstable product quality with low dry matter content (under 80%) [11], dark color [12], and uncontrollable bacteria.

According to the report titled “Palm Sugar Market: Global Industry Analysis (2012–2016) and Forecast (2017–2025)”, the value of the worldwide palm sugar market was US\$ 1684.2 million in 2017 and was estimated to reach US\$ 2205.8 million in 2025. Income from trade is anticipated to gain at a compounded annual growth rate of 3.4% in the prediction time (2017–2025) [13]. Recently, the commercialization potential of palm sugar as an important alternate sweetener has become an attractive prospect. However, these products are not currently popular. The price of palm sugar is often decided by its quality, as well as by its color, flavor, and texture. The amount of palm sugar available depends on the genetic and metabolite characteristics of the plant, environmental factors, soil, harvest, and production process. Moreover, the aroma, texture, color, and taste of palm sugar are affected by the dominance of assorted physical and chemical changes occurring during the method of concentration [12].

The palmyra jaggery industry faces the problem of determining the correct quantification of lime to be used to prevent the fermentation of palm sugar as well as the tapping, heating temperature, and heating time variables required for maintaining proper quality, yield, and nutritional properties [14]. Vacuum-drying allows materials dried in a reduced-pressure atmosphere to counter any undesirable effects and enhances a product’s quality and nutritional value [15]. Therefore, the vacuum-drying method could improve palm sugar’s features [16,17].

Some published studies on *B. flabellifer* sugar only focused on palm sugar cake made from palm trees grown in Thailand [16], India [18], and Indonesia [19]. Several studies have also concentrated on the physicochemical, thermo-physical, and antioxidant properties of cake palm sugar [12,16,17]. However, no report is currently available regarding the changes in vitamin content, 5-hydroxymethylfurfural (HMF) content, volatile compounds, and biological activity of palm granulated sugar produced using a vacuum-drying process.

Consequently, we evaluated the chemical composition (physicochemical properties, nutritional components, vitamin, HMF content, and volatile compounds) and biological activities (total phenolic content, DPPH, ferric reducing antioxidant power, and cytoprotective activity) of palm granulated sugar produced using concentrated palm syrup of *B. flabellifer* flower sap from Cambodia under different drying–solidification conditions.

## 2. Materials and Methods

### 2.1. Preparation of Palmyra Palm Granulated Sugar

Concentrated palmyra palm syrup ( $86 \pm 2^\circ$  Brix) was provided by LU SHU Health Co., Phnom Penh, Cambodia (collected during November 2018, from many palmyra palm trees in the Kampot countryside). The syrup was heated to 40 °C to 50 °C and poured into an evaporating flask. Drying–solidification was carried out using a vacuum pan (1 MPa, rotary speed 80 rpm) with different drying temperature (80 °C, 90 °C, and 100 °C) and time (60 min, 75 min, and 90 min) conditions. The samples prepared at 80 °C, 90 °C, and 100 °C for 60, 75, and 90 min were respectively represented by EPS1, EPS2, and EPS3, NPS1, NPS2, and NPS3, and OPS1, OPS2, and OPS3, individually (Figure A1). Then, the granulated sugar was milled and separated by using a laboratory sieve with a diameter of 6.3 mm. All samples (Figure A2) were stored at  $-20^\circ$  C for two months.

## 2.2. Measurement of Color, pH, Moisture Content, and Water Activity

The moisture content was determined by a drying method at 105 °C. Water activity ( $A_w$ ) was measured by a water activity meter (HygroPalm AW, Switzerland). The color was measured by using a color meter (ZE 6000, Japan). The results are expressed according to  $L^*$ ,  $a^*$ , and  $b^*$  values, showing black to white, green to red, and blue to yellow, respectively.

## 2.3. Determination of Total Sugar and Reducing Sugar

The phenol-sulfuric acid method was used to measure total sugar content [20]. The reducing sugar was determined by the dinitrosalicylic acid method [21].

## 2.4. Determination of Mineral Content

The levels of minerals (potassium, iron, sodium) were analyzed by using a flame atomic absorption spectrophotometer (PinAAcle 500, PerkinElmer, Waltham, MA, USA) [22].

## 2.5. Determination of Vitamin Content

Determination of water and fat-soluble vitamins (A, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>5</sub>, B<sub>6</sub>, C, D<sub>2</sub>, E) and folic acid was performed with high-performance liquid chromatography with UV-PDA (Photo diode array) detection [23,24].

## 2.6. Determination of Total Phenolic Content

The determination of total phenolic content involved the use of a Folin–Ciocalteu reagent method with the gallic acid standard [25,26]. Briefly, 20 µL of 10% palm granulated sugar solution was mixed with 100 µL Folin–Ciocalteu reagent. Then, 80 µL of 10% sodium carbonate compound was added to the blend after 5 min. The mixture was set at room temperature for 1 h. Finally, the absorbance was measured at 765 nm.

## 2.7. Determination of DPPH Radical Scavenging Activity

The method of Payet and Asikin was used to estimate the DPPH free radical scavenging activity of palm granulated sugar with minor modification [27,28]. Briefly, 50 µL of sugar solution (5 mg/mL concentration) was added to 50 µL DPPH solution 0.1 mM and 100 µL MES buffer (200 mM) (pH 6.0). Absorbance was measured at 517 nm by using an Epoch microplate spectrophotometer (BioTek Instruments, Winooski, VT, USA) after 15 min. The percentage of DPPH radical scavenging ability was determined using Equation (1):

$$\text{DPPH (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of the sample}}{\text{Absorbance of control}} \times 100 \quad (1)$$

## 2.8. Determination of Ferric Reducing Antioxidant Power (FRAP)

The method of Phillips was used to determine the FRAP value [29]. Briefly, 100 µL sugar solution (5 mg/mL concentration) was blended with 100 µL of 0.2M sodium phosphate buffer and 100 µL of 1% (w/v) K<sub>3</sub>[Fe(CN)<sub>6</sub>] solution. The blend was incubated at 50 °C for 20 min, mixed with 100 µL of 10% (w/v) trichloroacetic acid and centrifuged at 5000 rpm for 10 min. Next, 200 µL of the supernatant was combined with 200 µL distilled water. An amount of 200 µL of the above blend was transferred to a 96-well plate (Costar 3599, Corning Inc., Corning, NY, USA). Each well contained 10 µL of 0.1% (w/v) iron (III) chloride. The absorbance was measured at 700 nm.

## 2.9. Determination of 5-Hydroxymethylfurfural (HMF) Content

HMF content was detected as described in [30] with some modifications. Firstly, 10 g sugar was dissolved and made up to 50 mL with deionized water. Then, the mixture was centrifuged at 5000 rpm

for 15 min. The supernatant was used to measure HMF content. Next, 2 mL supernatant was imported into a tube. An amount of 2 mL of 12% trichloroacetic acid and 2 mL of 0.025 M thiobarbituric acid was added and mixed completely. The tube was placed in a water bath at 40 °C for 50 min. After incubating, the tube was immediately cooled with water to room temperature. The absorbance was measured at 445 nm. A standardization curve for HMF was used to quantify the HMF concentration.

### 2.10. Determination of Volatile Compositions

The analysis of volatile components proceeded according to Asikin [28] with some modifications. Briefly, the volatile components of palm granulated sugar were extracted using an organic solvent. The sample (50 g) was mixed with 200 mL diethyl ether and cyclohexanol 1% and put into a flask. The mixture was kept at 5 °C for 24 h. Then, the solution was filtered by filter paper, and the volatile components were separated by solvent-assisted flavor evaporation using a vacuum at 40 °C. Next, the extract was dried by 10 g anhydrous sodium sulfate for 12 h at 5 °C and concentrated in a vacuum by a gentle nitrogen stream. The extract was stored at −20 °C.

All samples were analyzed by using gas chromatography (GC) combined with an aflame ionization detector (FID) and mass spectrophotometry. In the process, 1 mL sample was injected into a DB-Wax column (60 m × 0.25 mm i.d., film thickness 0.25 mm) at a split ratio of 1:10, and the linear velocity of the helium carrier gas was determined. The temperatures of the GC injector and FID were set at 250 °C. The oven was set initially at 40 °C for 2 min, increased up to 200 °C at a rate of 2 °C/min, and kept constant at 20 °C for 38 min.

### 2.11. Odor Description and Detection Analyses

The odor of volatile components of palm granulated sugar was determined by using gas chromatography (GC)-olfactometry with an Agilent system connected to an FID and olfactory detection port [28].

### 2.12. Cytoprotective Effect of Palm Granulated Sugar against *tert*-Butyl Hydroperoxide (*t*BuOOH)

NIH3T3 fibroblast cells were bought from BCRC (BCRC60008, Hsinchu, Taiwan) and maintained in our laboratory ( $1 \times 10^6$  cells/mL, kept at 37 °C under 5% CO<sub>2</sub> and 95% air in the RPMI 1640 medium) were used for the examination. The cells were subjected to oxidative stress as described in [31,32]. Briefly, cells were cultivated with or without sugar (20 µL, 20 mg/mL) dissolved in phosphate-buffered saline in a 96-well microplate for 30 min, then treated with 500 µM *t*BuOOH and incubated for 3 h. Cell viability was evaluated by the microculture tetrazolium assay [33]. Next, 25 µL of MTT solution (5 mg/mL) was added to wells and kept at 37 °C for 4 h. Then, 100 µL of lysis buffer was added to wells and incubated at 37 °C for 16 h to dissolve dark blue formazan crystals. The absorption of formazan solution was measured at 570 nm and shown as cell viability by using a microplate spectrophotometer (BioTek Instruments, Winooski, VT, USA).

### 2.13. Data Analysis

All analyses were repeated three times, and the data were expressed as mean ± standard deviation. The data were analyzed by ANOVA followed by the Tukey test for comparing means. A value of  $p < 0.05$  was considered statistically significant. Statistical analysis involved using Minitab 17 software.

## 3. Results

### 3.1. Physicochemical Characteristics

The physical parameters (water content,  $a_w$ , pH, color) of palm granulated sugar are presented in Table 1. The water content of tested palms ranged from 2.91% to 5.12%. The water content of drying samples at 100 °C was higher than at 80 °C and 90 °C and was also similar to previous reports for

palm sugar (0.98%–2.47%) [17]. The water content variation of sugars is created by differences in the manufacturing process [34].

The  $A_w$  values of the palm granulated sugar ranged from 0.48 to 0.30. The lowest and highest  $A_w$  values were for samples OPS1 (100 °C, 90 min) and EPS1 (80 °C, 60 min). The high  $A_w$  values quickly promote microbial growth and biochemical degradation reactions, all of which shorten the storage of sugars [22]. These results showed that palm granulated sugars could extend the storage time, and the OPS1 sample was the most stable. This result agreed with an earlier report on water activity [17].

The pH values were modified slightly, from 6.90 to 6.99. Our data were higher than the results of previous studies in which granulated jaggery had pH values of 5.26 and 6.60 [35,36]. The pH value change may be explained by chemical reactions occurring during the palm jaggery heating process [37] and by the appearance of Maillard reaction products (MRPs) [38].

The food product color is the most important factor affecting acceptance by the customer and also expresses the natural transformation of the product (ripeness, processing, and storage) [16]. Table 1 shows the color change of palm granulated sugar with increasing drying temperature and time. All these samples displayed similar trends: the palm granulated sugar color changed from a green-yellow (low  $-a^*$ , L and high  $+b^*$ -values) to a red-golden yellow (high  $+a^*$ , L and low  $+b^*$ -values) [39]. The result showed a slight change of L-value (lightness–darkness) from 115.69 (NPS1) to 122.57 (OPS2). The  $a^*$  values ranged from  $-0.21$  (EPS1) to  $0.39$  (OPS3). The  $b^*$  values tended to decrease from 3.18 (EPS1) to 1.57 (OPS3). The  $a^*$  and L values at 100 °C were higher than at 80 °C and 90 °C. The different colors of all samples might have been due to the difference in drying conditions used in our study. A previous study used the boiling and drying process to lead to the increased  $a^*$  value that may result from non-enzymatic browning, including MRPs and caramelization products [16]. However, the caramelization could not occur here because this reaction effectively takes place at temperatures  $> 120$  °C. Therefore, only the Maillard reaction can lead to the browning of palm granulated sugar [40,41].

**Table 1.** Color, moisture content, pH, and water activity ( $A_w$ ) of palm granulated sugar.

Properties	EPS1	EPS2	EPS3	NPS1	NPS2	NPS3	OPS1	OPS2	OPS3
Moisture content (%)	5.12 ± 0.18 <sup>a</sup>	4.24 ± 0.25 <sup>b</sup>	3.88 ± 0.09 <sup>c</sup>	5.08 ± 0.06 <sup>a</sup>	3.31 ± 0.18 <sup>d</sup>	3.26 ± 0.08 <sup>d</sup>	4.97 ± 0.07 <sup>a</sup>	3.00 ± 0.06 <sup>de</sup>	2.91 ± 0.02 <sup>e</sup>
Water activity ( $A_w$ )	0.48 ± 0.01 <sup>a</sup>	0.45 ± 0.02 <sup>b</sup>	0.35 ± 0.03 <sup>de</sup>	0.40 ± 0.01 <sup>c</sup>	0.33 ± 0.01 <sup>def</sup>	0.31 ± 0.01 <sup>f</sup>	0.35 ± 0.00 <sup>d</sup>	0.32 ± 0.01 <sup>ef</sup>	0.30 ± 0.02 <sup>f</sup>
pH	6.90 ± 0.04 <sup>a</sup>	6.96 ± 0.03 <sup>a</sup>	6.99 ± 0.01 <sup>a</sup>	6.92 ± 0.04 <sup>a</sup>	6.95 ± 0.18 <sup>a</sup>	6.99 ± 0.08 <sup>a</sup>	6.86 ± 0.11 <sup>a</sup>	6.98 ± 0.05 <sup>a</sup>	6.99 ± 0.03 <sup>a</sup>
L* value	116.24 ± 0.28 <sup>cd</sup>	117.13 ± 0.84 <sup>cd</sup>	119.14 ± 0.72 <sup>b</sup>	115.69 ± 0.39 <sup>d</sup>	116.25 ± 2.02 <sup>cd</sup>	121.04 ± 0.22 <sup>a</sup>	117.83 ± 0.39 <sup>bc</sup>	122.57 ± 0.62 <sup>a</sup>	121.54 ± 1.05 <sup>a</sup>
a* value	−0.21 ± 0.02 <sup>e</sup>	−0.17 ± 0.03 <sup>e</sup>	−0.21 ± 0.02 <sup>e</sup>	−0.11 ± 0.02 <sup>e</sup>	0.05 ± 0.02 <sup>d</sup>	0.15 ± 0.02 <sup>cd</sup>	0.26 ± 0.04 <sup>bc</sup>	0.31 ± 0.02 <sup>ab</sup>	0.39 ± 0.09 <sup>a</sup>
b* value	3.18 ± 0.24 <sup>ab</sup>	2.85 ± 0.21 <sup>abc</sup>	2.38 ± 0.20 <sup>cd</sup>	2.86 ± 0.35 <sup>abc</sup>	1.84 ± 0.42 <sup>de</sup>	1.83 ± 0.31 <sup>de</sup>	3.27 ± 0.18 <sup>a</sup>	2.39 ± 0.24 <sup>bcd</sup>	1.57 ± 0.27 <sup>e</sup>

Values are mean ± standard deviation of triplicates. The same letters within a row are not significantly different ( $p < 0.05$ ).

### 3.2. Chemical Composition

One of the standard characteristics of palm granulated sugar compared to white sugar and refined sugar is its nutritional rate and mineral load. The chemical composition of palm granulated sugar is shown in Table 2.

According to the composition, sugar was a major component of palm granulated sugar, at 91.04% to 93.28%. The total sugar content of palm granulated sugar at 100 °C (0.35%–2.24%) was higher than at 80 °C and 90 °C. This was also higher than in a previous study of palm sugar powder [11]. The reducing sugar content was 5.55% to 6.61%, and this decreased slightly with increasing drying temperature and time. The reason for this could be that reducing sugars participate in the Maillard reaction and increase the browning of palm granulated sugar (increasing the HMF content). Besides, previous studies have demonstrated the slow degradation over time of reducing sugar (fructose, glucose) into various intermediates and advanced MRPs, including  $\alpha$ -dicarbonyl compounds, organic acids, and melanoidins [42]. Additionally, some authors noted that the difference between total sugar and reducing sugar content may explain the contamination of lactic acid bacteria in sugar. Microorganisms can convert sucrose to glucose and fructose and to organic acids or alcohols [11].

The mineral composition of fresh inflorescence sap has a high concentration of potassium, sodium, iron, and phosphorus [43]. We also detected all components at high levels in palm granulated sugar. Potassium had the highest content among all other minerals (688.45–705.27 mg/100 g). This result was consistent with granulated panelas [22] but higher than brown sugar (10.30 mg/L) [44]. Potassium is a vital mineral to balance fluids, different minerals within the body and maintain blood pressure [45]. The other elements found were sodium and iron. The sodium content ranged from 23.10 to 24.50 mg/100 g, which was much higher than that of the granulated non-centrifugal sugars (1.94–5.60 mg/100 g) [46]. Moreover, the iron content in palm granulated sugar was also higher than that of brown sugar (1.88–2.05 mg/kg vs. 2.3 mg/kg) [44]. In general, the mineral content varied in samples, but these differences were not statistically significant.

**Table 2.** Chemical composition of palm granulated sugar.

Compositions	EPS1	EPS2	EPS3	NPS1	NPS2	NPS3	OPS1	OPS2	OPS3
Total sugar (%)	91.04 ± 0.75 <sup>d</sup>	91.65 ± 0.72 <sup>abcd</sup>	92.01 ± 0.16 <sup>abcd</sup>	91.23 ± 0.78 <sup>cd</sup>	92.87 ± 0.64 <sup>abcd</sup>	92.93 ± 0.93 <sup>abc</sup>	91.36 ± 0.44 <sup>bcd</sup>	93.11 ± 0.13 <sup>ab</sup>	93.28 ± 0.71 <sup>a</sup>
Reducing sugars (%)	6.61 ± 0.30 <sup>a</sup>	5.87 ± 0.22 <sup>bcd</sup>	5.81 ± 0.21 <sup>bcd</sup>	6.48 ± 0.22 <sup>ab</sup>	5.73 ± 0.36 <sup>cd</sup>	5.63 ± 0.16 <sup>d</sup>	6.34 ± 0.17 <sup>abc</sup>	5.62 ± 0.27 <sup>d</sup>	5.55 ± 0.13 <sup>d</sup>
Sodium (mg)	24.12 ± 4.85 <sup>a</sup>	24.39 ± 1.62 <sup>a</sup>	23.52 ± 3.93 <sup>a</sup>	24.16 ± 3.84 <sup>a</sup>	23.73 ± 5.59 <sup>a</sup>	24.50 ± 2.1 <sup>a</sup>	23.32 ± 8.7 <sup>a</sup>	23.07 ± 8.9 <sup>a</sup>	22.72 ± 1.3 <sup>a</sup>
Potassium (mg)	688.45 ± 8.43 <sup>a</sup>	689.63 ± 5.07 <sup>a</sup>	690.76 ± 3.14 <sup>a</sup>	702.13 ± 10.47 <sup>a</sup>	699.93 ± 7.48 <sup>a</sup>	698.45 ± 4.67 <sup>a</sup>	701.49 ± 3.65 <sup>a</sup>	705.27 ± 5.85 <sup>a</sup>	699.73 ± 6.95 <sup>a</sup>
Iron (mg)	1.99 ± 0.14 <sup>a</sup>	2.02 ± 0.07 <sup>a</sup>	2.03 ± 0.08 <sup>a</sup>	1.88 ± 0.08 <sup>a</sup>	1.92 ± 0.04 <sup>a</sup>	1.98 ± 0.03 <sup>a</sup>	2.05 ± 0.07 <sup>a</sup>	2.04 ± 0.05 <sup>a</sup>	2.01 ± 0.04 <sup>a</sup>

Values are mean ± standard deviation of triplicates. The same letters within a row are not significantly different ( $p < 0.05$ ).



### 3.3. Vitamin Content

Vitamins are essential for maintaining the physiological state of humans, and the lack of a sufficient intake will result in severe diseases. Water-soluble vitamins act mainly as coenzymes, whereas fat-soluble ones act in numerous and more advanced ways [47]. The palm sap collected from the inflorescence of palmyra palm is a good source of vitamins such as vitamin A, niacin, thiamin and riboflavin [48,49]. Our study is the first to present the vitamin content of palm granulated sugar. The results of the vitamin composition are in Table 3. The 10 vitamins found included vitamins A, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>5</sub>, B<sub>6</sub>, C, D<sub>2</sub>, E, and folic acid. Vitamin E content was the highest, from 52.15 to 55.12 mg/100 g, and vitamin B<sub>2</sub> content was the lowest, from 0.04 to 0.07 mg/100 g. Moreover, palm granulated sugar contained vitamin C, with a value from 2.78 to 4.01 mg/100 g, and vitamin D<sub>2</sub>, with a value from 2.11 to 2.23 mg/100 g. Palm granulated sugar also contained vitamin B groups including B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>5</sub>, and B<sub>6</sub> with values that ranged from 0.04 to 2.15 mg/100 g. The result was similar to a previous examination which showed that fresh sap of *B. flabellifer* was a good source of vitamin B complex and ascorbic acid [50]. Moreover, the levels of folic acid ranged from 2.51 to 3.33 µg/100 g in this study.

**Table 3.** Vitamin content of palm granulated sugar.

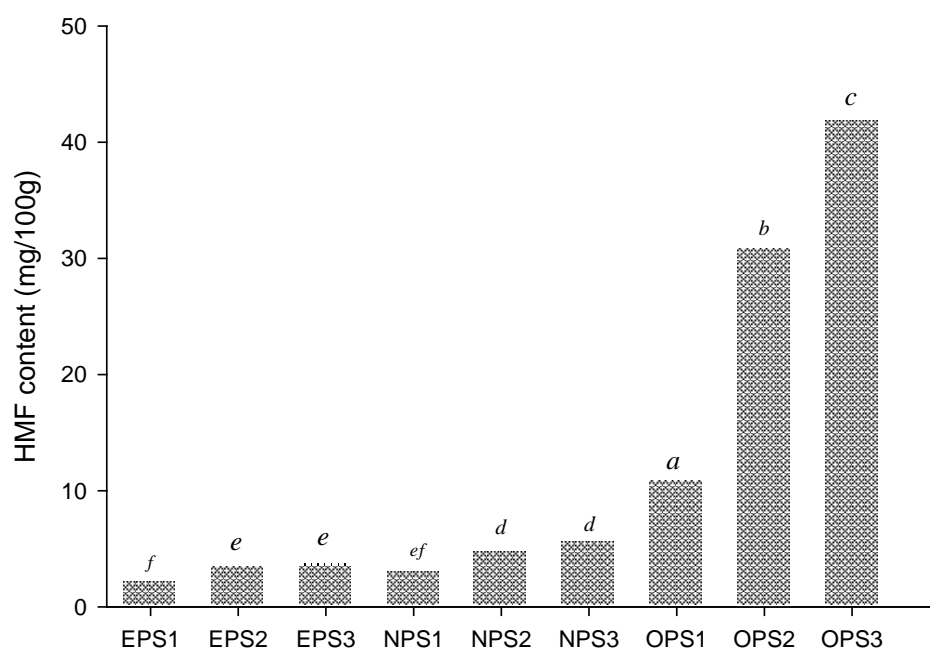
Vitamin (Per 100 g)	EPS1	EPS2	EPS3	NPS1	NPS2	NPS3	OPS1	OPS2	OPS3
Vit A (mg)	1.86 ± 0.09 <sup>a</sup>	1.84 ± 0.06 <sup>a</sup>	1.74 ± 0.06 <sup>ab</sup>	1.84 ± 1.11 <sup>a</sup>	1.76 ± 0.04 <sup>ab</sup>	1.65 ± 0.05 <sup>b</sup>	1.76 ± 0.04 <sup>ab</sup>	1.72 ± 0.04 <sup>ab</sup>	1.60 ± 0.04 <sup>ab</sup>
B <sub>1</sub> (mg)	0.97 ± 0.09 <sup>a</sup>	0.92 ± 0.05 <sup>ab</sup>	0.91 ± 0.09 <sup>ab</sup>	0.90 ± 0.02 <sup>abc</sup>	0.83 ± 0.05 <sup>abcd</sup>	0.72 ± 0.04 <sup>cd</sup>	0.83 ± 0.10 <sup>abcd</sup>	0.77 ± 0.05 <sup>bcd</sup>	0.70 ± 0.04 <sup>d</sup>
B <sub>2</sub> (mg)	0.07 ± 0.01 <sup>a</sup>	0.06 ± 0.01 <sup>abc</sup>	0.06 ± 0.01 <sup>ab</sup>	0.06 ± 0.01 <sup>ab</sup>	0.05 ± 0.01 <sup>abc</sup>	0.05 ± 0.00 <sup>abc</sup>	0.05 ± 0.01 <sup>bc</sup>	0.05 ± 0.01 <sup>abc</sup>	0.04 ± 0.01 <sup>c</sup>
B <sub>3</sub> (mg)	2.15 ± 0.04 <sup>a</sup>	2.15 ± 0.02 <sup>a</sup>	2.14 ± 0.07 <sup>a</sup>	2.1 ± 0.12 <sup>a</sup>	2.09 ± 0.11 <sup>a</sup>	2.08 ± 0.07 <sup>a</sup>	2.01 ± 0.04 <sup>a</sup>	1.98 ± 0.12 <sup>a</sup>	1.95 ± 0.07 <sup>a</sup>
B <sub>5</sub> (mg)	0.66 ± 0.08 <sup>ab</sup>	0.58 ± 0.05 <sup>abc</sup>	0.50 ± 0.02 <sup>cd</sup>	0.67 ± 0.02 <sup>a</sup>	0.56 ± 0.03 <sup>bc</sup>	0.5 ± 0.02 <sup>cd</sup>	0.61 ± 0.04 <sup>abc</sup>	0.56 ± 0.02 <sup>bc</sup>	0.44 ± 0.04 <sup>d</sup>
B <sub>6</sub> (mg)	0.19 ± 0.02 <sup>a</sup>	0.14 ± 0.02 <sup>bc</sup>	0.11 ± 0.02 <sup>cd</sup>	0.16 ± 0.01 <sup>ab</sup>	0.13 ± 0.02 <sup>bc</sup>	0.12 ± 0.01 <sup>cd</sup>	0.15 ± 0.02 <sup>abc</sup>	0.12 ± 0.03 <sup>bcd</sup>	0.09 ± 0.01 <sup>d</sup>
Folic acid (µg)	3.12 ± 0.15 <sup>a</sup>	3.08 ± 0.04 <sup>ab</sup>	2.95 ± 0.11 <sup>abc</sup>	3.01 ± 0.06 <sup>abc</sup>	2.85 ± 0.10 <sup>abc</sup>	2.72 ± 0.11 <sup>bc</sup>	2.89 ± 0.09 <sup>abc</sup>	2.72 ± 0.17 <sup>bc</sup>	2.64 ± 0.24 <sup>c</sup>
Vit C (mg)	4.01 ± 0.14 <sup>a</sup>	3.45 ± 0.17 <sup>bcd</sup>	3.15 ± 0.16 <sup>de</sup>	3.85 ± 0.07 <sup>ab</sup>	3.41 ± 0.21 <sup>bcd</sup>	3.07 ± 0.14 <sup>de</sup>	3.66 ± 0.23 <sup>abc</sup>	3.21 ± 0.16 <sup>cde</sup>	2.78 ± 0.09 <sup>e</sup>
Vit D <sub>2</sub> (mg)	2.15 ± 0.04 <sup>abc</sup>	2.23 ± 0.02 <sup>a</sup>	2.11 ± 0.05 <sup>c</sup>	2.17 ± 0.03 <sup>abc</sup>	2.21 ± 0.03 <sup>ab</sup>	2.14 ± 0.02 <sup>bc</sup>	2.17 ± 0.02 <sup>abc</sup>	2.11 ± 0.05 <sup>c</sup>	2.15 ± 0.02 <sup>abc</sup>
Vit E (mg)	55.12 ± 0.88 <sup>a</sup>	54.68 ± 1.11 <sup>a</sup>	54.34 ± 0.69 <sup>ab</sup>	55.01 ± 0.18 <sup>a</sup>	54.98 ± 1.07 <sup>a</sup>	54.23 ± 1.06 <sup>ab</sup>	54.23 ± 0.75 <sup>ab</sup>	54.12 ± 1.10 <sup>ab</sup>	52.15 ± 0.60 <sup>b</sup>

Values are mean ± standard deviation of triplicates. The same letters within a row are not significantly different ( $p < 0.05$ ).

### 3.4. HMF content

HMF is a heterocyclic compound and forms from reducing sugars through the Maillard reaction and caramelization [51]. HMF is formed as products or intermediates in heat-induced reactions and significantly adds to the sensory properties of heated foods [52]. HMF used to be a top-quality indicator of thermally processed foods until its toxic properties were discovered. HMF was purported to induce genotoxic and mutagenic effects in microorganisms and human cells and promote carcinoma in rats [53].

The HMF content in palm granulated sugar ranged from 2.18 to 41.92 mg/100 g (Figure 1). The HMF contents of EPS1, EPS2, and EPS3 were rather low, at 2.18, 3.39, and 3.77 mg/100 g, respectively. However, this increased quickly and was highest at 100 °C (10.09–41.92 mg/100 g). The HMF content of palm granulated sugars at 80 °C and 90 °C was much lower than at 100 °C. The HMF content is based greatly on the processing technique, heating level, pH, total acidity and storage condition [54,55]. With increasing drying temperature, the reaction speed between sugar and amino acid groups increased, leading to an exponentially increasing Maillard reaction rate [56]. The same research has not yet shown HMF contents; however, Naknean's study showed that the HMF content of palm sugar cake samples in Songkhla province ranged from 21.81 to 341.80 mg/kg [16]. Moreover, a palm sugar syrup study reported that HMF content varied from 20.13 to 185.39 mg/kg [57]. For the Codex Alimentarius on sugars, the HMF content of honey should not be more than 40 mg/kg. However, the HMF content can be up to 80 mg/kg in honey from regions with tropical ambient temperatures. The HMF content of samples dried at 100 °C was higher than the maximum limit recommended by Codex Alimentarius.



**Figure 1.** 5-hydroxymethylfurfural (HMF) content in palm granulated sugar under different drying–solidification conditions.

### 3.5. Composition of Volatiles

The best-known character of palm granulated sugar is its unique flavor, which is achieved by the Maillard reaction. The volatile compositions of palm granulated sugar are displayed in Table 4. A total of seven volatile groups was analyzed, including alcohol, ketones, pyrazines, acids, phenols, and aldehyde groups. The maximum and minimum values of volatile compounds were found in NPS1 (1.45 mg/100 g) and OPS3 (1.17 mg/100 g). The dried palm granulated sugar at 80 °C and 90 °C presented a higher number of volatile components than at 100 °C (3.98 and 4.01 vs. 3.87 mg/100 g).

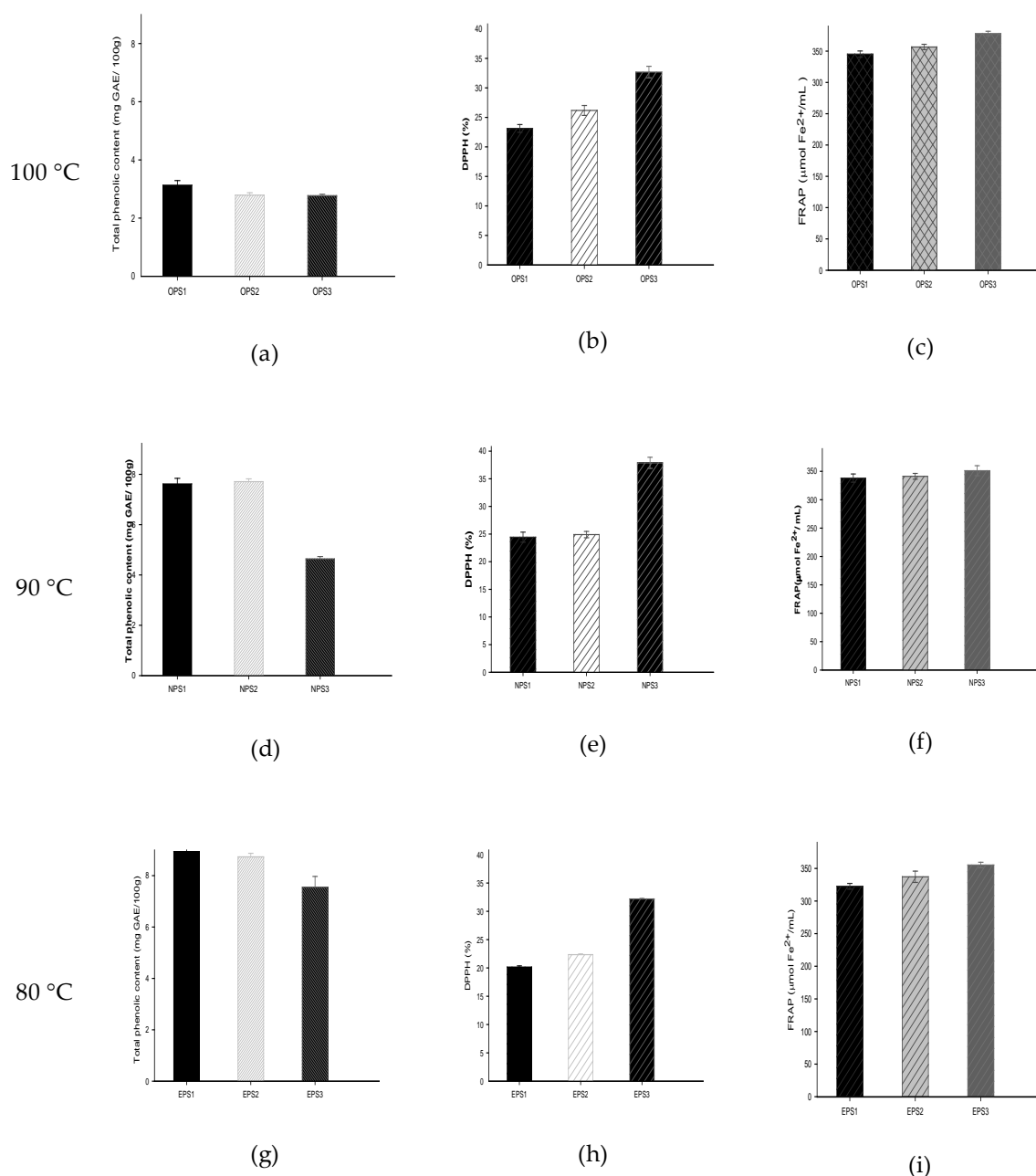
This result agreed with other studies [58,59]. Flavor compound formation in the Maillard reaction involves the reaction temperature and time, pH, and water content, together with the types of sugar and amino acids. Both the quantity and quality of volatile compounds affect the precursors, thermal process parameters, pH, and ratio of amino nitrogen to reducing sugar [60].

Palm granulated sugar extract contains mainly volatile fractions such as alcohols (0.48–0.56 mg/100 g, six compounds), ketones (0.34–0.39 mg/100 g, 12 compounds) and acids (0.49–0.64 mg/100 g, nine compounds). Moreover, palm granulated sugars included two sulfurs, two phenols, six pyrazines, and one aldehyde. The rate and quantity of those compounds represent the volatile profile with different forms of odors [28]. Our results showed the major compounds of ketones to be 2,3-dihydro-3,5-dihydroxy-6-methyl-4-pyran-4-one, 1-hydroxy-2-propanone, 2,5-pyrrolidinedione and pantolactone, which could create a pleasantly sweet, cotton candy-like, caramel, and coffee-like characteristic [28]. MRPs can range from a pleasant, flowery, and fragrant aroma to a burnt, pungent, nutty, and caramel-like odor [61], based on the amino acid and sugar composition in foods and also their reaction pathways [62]. Likewise, the pyrazines are Maillard reaction-derived flavor compounds that display associated flavors of cooked, roasted, toasted, and baked cereals [61]. The number of pyrazines of palm granulated sugar was reduced (from 0.06 to 0.08 mg/100 g) as the production of pyrazines was encouraged at pH values from 8.00 to 9.55 [59]. The presence of 2,5-dimethyl-pyrazine, 2,6-dimethyl-pyrazine, and 2,3,5-trimethyl-pyrazine provides a nutty, roasted, coffee-like and earthy odor for palm granulated sugar [63]. However, aldehyde and phenol groups such as 2-methoxy-phenol, 2,6-dimethoxy-phenol, and vanillin may provide sweet, herbaceous, maple-like, caramel, and cotton candy-like odors. The alcohol groups contained six compounds that were reported to have volatile aroma components in non-centrifugal cane brown sugar [28]. Amounts of S-(R',R')-2,3-butanediol (0.24 mg/100 g), R-(R',R')-2,3-butanediol (0.09 mg/100 g), and ethanol (0.21 mg/100 g) in EPS1 were higher than OPS3, at 0.03, 0.02, and 0.03 mg/100 g, respectively. Additionally, 2-propenoic acid, 2-hydroxy-propanoic acid, and benzoic acid showed high quantities in the acid group. Besides originating from tissue, the acid compounds arise in the sap collection process [18,64] and the clear levels of sulfur compounds were identified as dimethyl sulfoxide (0.10–0.15 mg/100 g) and dimethyl sulfone (0.01–0.02 mg/100 g). Altogether, sulfur-containing Maillard odorants represent the most dominant aroma compounds and provide the flavor of cooked meats. These volatile compounds are stable and present the flavor and aroma of stewed beef juice, bread crust, roasted chicken, cocoa powder, peanuts, roasted beef, popcorn, and coffee [65].



### 3.6. Total Phenolic Content and Antioxidant Properties

Phenolic compounds contain the hydroxylated aromatic ring and have redox properties—properties that allow them to be antioxidants [66]. Antioxidants are exogenous or endogenous molecules that mitigate any form of oxidative/nitrosative stress or its consequences [67]. Figure 2 shows the results of the determination of total phenolic content and the antioxidant capacity of palm granulated sugar.



**Figure 2.** The total phenolic content and antioxidant activity of palm granulated sugar under different drying–solidification conditions. (a,d,g) Total phenolic content, (b,e,h) DPPH, and (c,f,i) FRAP.

The amount of total phenolic contents differed among samples from 2.77 to 8.94 mg/100 g. At 80 °C, the total phenolic content was the highest from 7.55 to 8.94 mg/100 g. When the temperature increased to 90 °C and 100 °C, the total phenolic contents were significantly reduced from 4.64 to 7.62 mg/100 g and from 2.77 to 3.13 mg/100 g, respectively. Phenolic content is easily destroyed during the heating

process. Similar findings reported that the total phenolic content of palm sugar ranged from 2.14 to 16.29 mg/100 g [17] and 0.48 µg of GAE/mg [26].

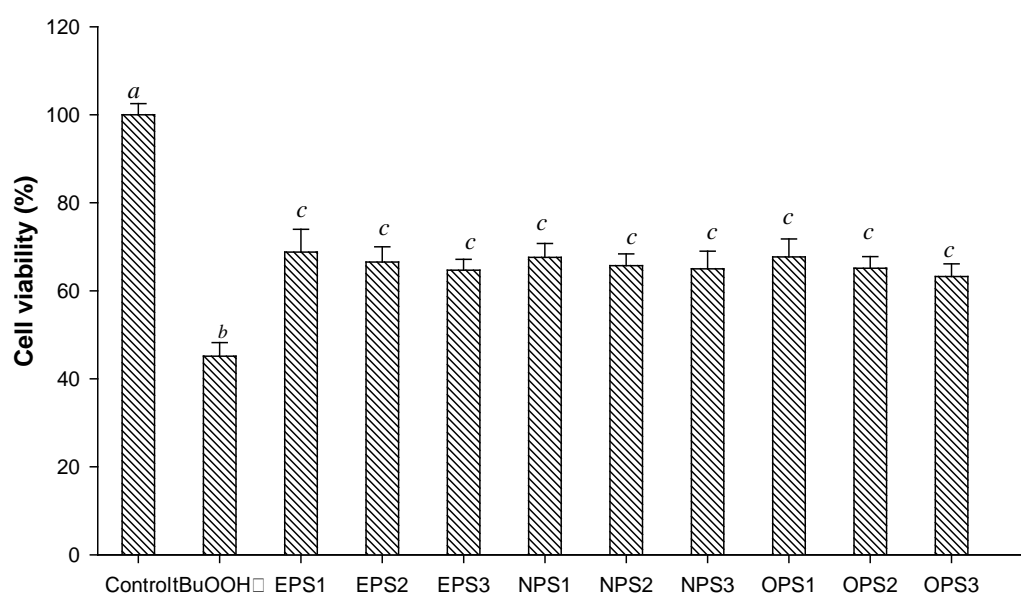
The relationship between phenolic content and antioxidant activities was reported in several studies [68,69]. Phenolic content affects antioxidant potential by various mechanisms related to the scavenging of free radicals [68,70]. The DPPH content was about 20.15% to 37.88%. The highest and the lowest DPPH percentages were for NPS3 (100 °C, 90 min) and EPS1 (80 °C, 60 min). The scavenging effect of studied samples with the DPPH radical was in the following order: NPS3 > OPS3 > EPS3 > OPS2 > NPS2 > NPS1 > OPS1 > EPS2 > EPS1. A similar trend was observed in a study of the DPPH radical scavenging activity of cane brown sugar, from 14.5% to 26.90% [27], and granulated non-centrifugal sugars, from 38.04% to 71.08% [46]. The increasing DPPH radical scavenging activity could be a result of grown MRPs and caramelization products. Previous studies have reported that MRPs possessed the ability to donate hydrogen and had the potential for free radical reaction [71,72].

The possible antioxidant property of palm granulated sugar was predicted by its ability to reduce the TPTZ-Fe(III) complex to the TPTZ-Fe(II) complex [67,73]. All samples displayed reducing power to various degrees. The reducing power of all samples was in the order of OPS1 > OPS2 > EPS3 > NPS3 > OPS1 > NPS2 > NPS1 > EPS2 > EPS1. Samples prepared at high temperatures, long periods or both showed higher FRAP values, such as OPS3 (100 °C, 90 min), OPS2 (100 °C, 75 min), EPS3 (80 °C, 90 min), and NPS3 (90 °C, 90 min), corresponding to 378.23, 356.71, 355.29, and 351.16 µmol Fe<sup>2+</sup>/mL. Similar results were found previously: the FRAP value of raw cane sugar was 0.17 to 0.33 mmol/100 g, that of dark brown sugar was 0.69 mmol/100 g and that of granulated white sugar was 0.01–0.02 mmol/100 g, which were lower values than the present results [29]. The alterations in the antioxidant potential of unrefined sugars were determined by factors such as the methods used for preventing antioxidant effectiveness [74]; the ratio of inverted sugars; the amount of phenolics, flavonoids [35] and MRPs [27]; and processing methods [28]. Another element is the concomitant production of Fe(II), which is a known pro-oxidant and can bring about the production of additional radicals in the reaction medium (such as OH• from the Fenton reaction). Then, the absorbance of these compounds was measured, leading to falsely high results for the FRAP value [52].

### 3.7. Cytoprotective Effect

NIH3T3 fibroblast cells were treated with tBuOOH, and the cytoprotective ability of palm granulated sugar was investigated. However, to confirm the safety of palm granulated sugar with NIH3T3 fibroblast cells, the sugar solution (20 mg/mL) was investigated for its toxicity for 24 h under 37 °C and 5% CO<sub>2</sub>. The result showed that the sugar concentration of 20 mg/mL was not the cause of NIH3T3 cell death (data not shown).

tBuOOH is an organic hydroperoxide which is widely used to induce oxidative stress. The impact of palm granulated sugar on cell viability was estimated by microculture tetrazolium assay. NIH3T3 cells incubated with tBuOOH and palm granulated sugar showed high cell viability as compared with tBuOOH individually (Figure 3). EPS1 and OPS3 showed the highest and lowest cell viability. The cell viability of palm granulated sugar-treated cells ranged from 63.23% to 68.81%, and this value was 45.13% for the sample without sugar. Palm granulated sugar-treated cells showed higher cell viability, from 18.10% to 23.684%. Although the cell viability for palm granulated sugar-treated cells varied, this change was not significantly different. A similar study reported the potential for inhibiting cellular reactive oxygen species of unrefined sugar by reduced DCFDA fluorescence signal intensity [26]. Variations in in vivo cell-based antioxidant potentials of unrefined sugars result from many elements, such as the availability of biological compounds, limitations on the take-up of the cells, and the method of activity and metabolism [26]. The cell viability of sugar-treated cells in this study was higher than that of white sugar and brown sugar and lower than that of jaggery sugar [32].



**Figure 3.** Cytoprotective effect of palm granulated sugar with t-butyl hydroperoxide-induced cell damage in NIH3T3 cells. Control, cells without sugar/tert-butyl hydroperoxide (tBuOOH) treatment; tBuOOH, cells treated with 500  $\mu$ M tBuOOH.

#### 4. Conclusions

The current study is the first to investigate the changes in chemical composition, HMF content, volatile compounds and biological activity of palm granulated sugar from *B. flabellifer* L flower sap (Cambodia) under different drying–solidification conditions. The drying–solidification of palm granulated sugars at low temperatures produced less reducing sugar and HMF content but increased the vitamin and total phenolic content. Meanwhile, dried products at high temperatures formed a darker color, lower water content, and water activity, but the antioxidant activity was slightly increased by the functional properties of MRPs. Within these experimental conditions, the pH value, mineral content, and cell viability of palm granulated sugars were not affected. In conclusion, the vacuum drying process at 90 °C for 75 min showed the potential of new industrial methods to shorten the production time and increase the bioactive phytochemical content of palm granulated sugar.

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**Conflicts of Interest:** The authors declare no conflict of interest.



Appendix A

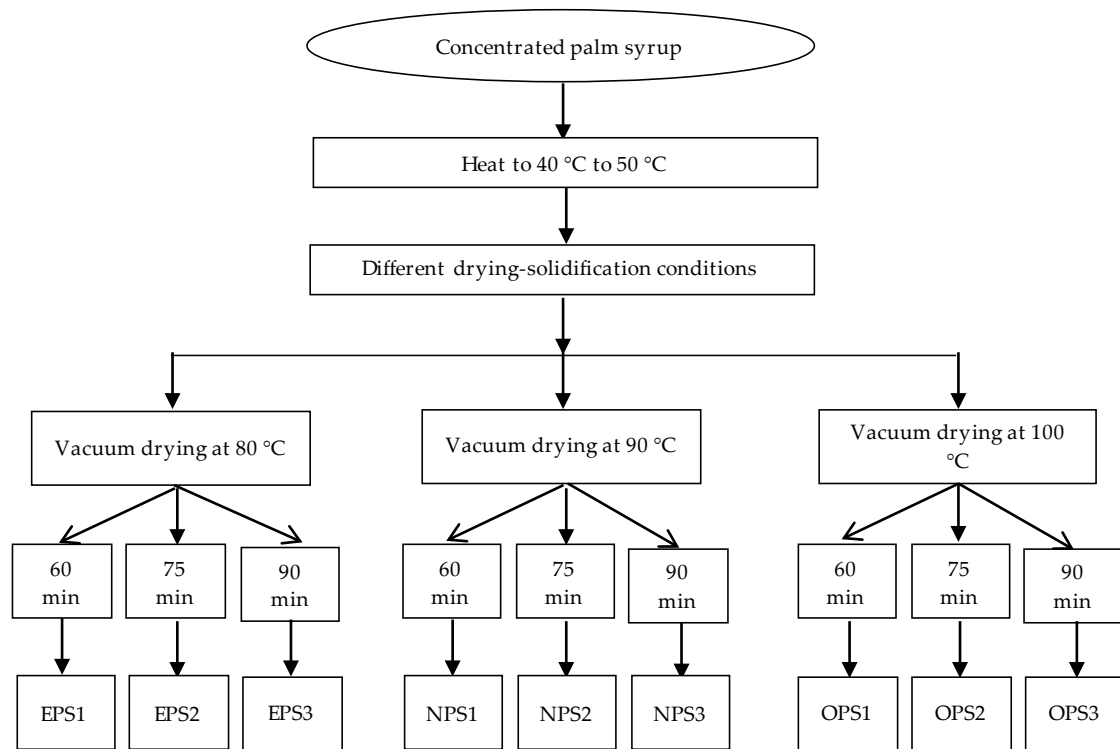


Figure A1. Diagram palm granulated sugar from concentrated palm syrup dried at 80 °C, 90 °C, and 100 °C and at time 60, 75 and 90 min.

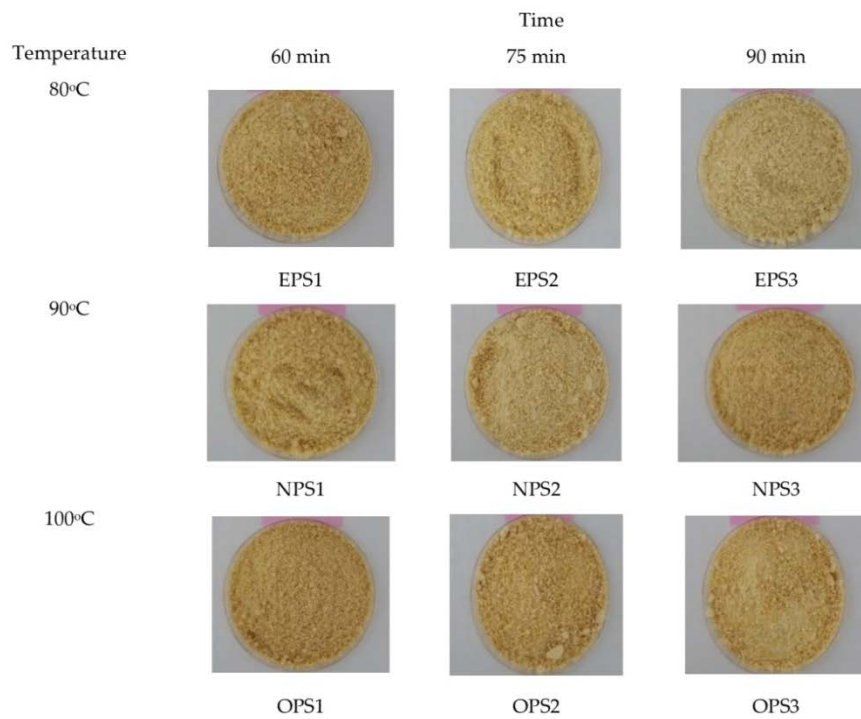


Figure A2. Palm granulated sugar under different drying-solidification conditions.

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