

Improvement of Physico-chemical Properties, Antioxidant Capacity and Acceptability of Carrot Cake by Partially Substituting Sugar with Concentrated *Nypa fruticans* Sap

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ABSTRACT

Nypa fruticans sap (NFS) or neera juice is a sugar-rich palm sap beverage widely consumed among the Malay community in Malaysia. The processed NFS has long been used as a traditional medicine to treat diabetes. Being a natural sweetener, NFS could potentially be used as a novel source to replace added sugar in bakery product. This study aimed to determine the effects of nutritional, textural, antioxidant properties and acceptability of carrot cake formulated with concentrated NFS as partial replacement (0, 5, 10, 15 and 20%) for table sugar. Results indicated that incorporation of concentrated NFS at 20% level significantly ($p < 0.05$) increased the nutritional compositions of carrot cakes which recorded a higher moisture (33.31%), ash (1.47%) and dietary fiber (10.75%) content as compared with control carrot cake. In texture profile analysis, a slight decrease in hardness, springiness, chewiness and resilience were reported with increasing levels of concentrated NFS formulated in the cake. For antioxidative activities, methanolic extract of 20% concentrated NFS-incorporated carrot cake showed the highest total phenolic content (TPC) among other treatments but did not significantly differ ($p > 0.05$) with TPC of 15% NFS-incorporated cake. A significantly higher ($p < 0.05$) free radical scavenging effects on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and reducing power (RP) were also observed in methanolic extract of 20 % NFS carrot cake compared to control cake without NFS substitution. Sensory acceptability test revealed that carrot cake formulated with 20% concentrated NFS, though not

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significant ($p > 0.05$) compared with other treatments (0 to 15%), received the highest scores for colour, texture, appearance and overall acceptability. Overall, the present study suggested that incorporation of concentrated NFS up to 20% in carrot cake to replace sugar could be an effective way to develop nutritious carrot cake with desirable physical and sensorial acceptability.

Keywords: Bakery product, functional food, natural sweetener, *Nypa fruticans*, palm sap

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder commonly presenting with episodes of high blood glucose level (hyperglycaemia) and glucose intolerance, as a result of lack of insulin, defective insulin action, or both (Sicree et al., 2006). In Malaysia, the prevalence of DM has experienced an upward trend as it remains the second most common chronic illness in the country. In a span of just a decade, there has been an 80% increase in the prevalence of diabetes. This number has exceeded the estimated prevalence of DM for the year 2025 (Letchuman et al., 2010). The increasing trend of DM seems to have linked to the continued escalation of the availability of added sugar and sweeteners (kg per capita per year) in Malaysia which has risen from 28.8 kg to 48.7 kg, or almost 70% between 1967 and 2007, according to the data from the Malaysian Food Balance Sheet (Food and Agriculture Organization [FAO], 2017). The common added sugars which include table sugar, sweeteners and jam are widely used in food industry for

preparation of commercially processed products such as breads, biscuits, cakes, carbonated drinks, ice cream and local *kuih*. The main types of added sugar that are frequently used in processed foods are white refined sugars which are virtually 100 percent sucrose without additional nutrients (Insel et al., 2018).

Consumption of artificial sweeteners such as aspartame, sucralose, saccharine and neotame on the other hand has been promoted as a prevention strategy to replace added sugar. However, the health risks of artificial sweeteners consumptions are still a highly controversial topic, which have allegedly been linked to adverse effects such as cancer, weight gain and metabolic disorders (Gupta et al. 2012; Harpaz et al., 2018; Marinovich et al., 2013). Therefore, discovery of novel alternative nutrient-rich natural sweetener to replace added sugar in processed foods is currently of major interest.

The nipa palm (*N. fruticans*) is a high sugar-yielding mangrove palm tree that produces palm sap from the cut stalks of inflorescences after the removal of the floral or fruit heads (Fong, 1992; Hamilton & Murphy, 1988; Päiväke, 1996). NFS composed mainly of sucrose, glucose and fructose and it is also an abundant source of inorganic minerals, amino acids and vitamins (Aimi et al., 2013; Nguyen et al., 2016). Due to this high sugar content, the NFS has been utilised as a material of treacle, amorphous and for producing vinegar alcohol or fermented beverage called “tuba” or “soom” in the Philippines,

“arak” or “tuak” in Indonesia, “toddy” in Malaysia, India and Bangladesh (Hamilton & Murphy, 1988; Päiväke, 1996).

Previous study by Sukairi et al. (2018) regarding NFS had reported that the nipa sap contained significant amount of bioactive compounds and showed a great potential in the antidiabetic properties, suitable to be used as an alternative to produce natural sugar that gave a lot of benefits. These bioactive compounds present as food components have an influence on the body, specific tissues or cells. Bioactive compounds such as polyphenols, carotenoids, tocopherols and phytosterols are distinct from nutrients in which they are not essential and, currently, no daily intake value is recommended (Gibney et al., 2009). However, they establish beneficial effects such as antioxidant activity, inhibition or induction of enzymes and gene expressions (Correia et al., 2012). The majority of dietary antioxidant compound such as polyphenols potentially retard carbohydrate digestion by delaying the activities of the enzymes α -amylase and α -glucosidase which are responsible for the digestion of carbohydrate and absorption of glucose in the digestive tract, respectively (Ali et al., 2006; Bhandari et al., 2008). The most common polyphenols that exhibit α -amylase inhibition characteristics are flavonoids. Flavonoids act as potent non-competitive inhibitors to the enzyme α -amylase by hydrophobic interactions (Yuan et al., 2014). This inhibition causes the delaying digestion of the monosaccharides in the intestine, lowering blood glucose level and reducing the potential of DM.

According to Malaysian Adult Nutrition Survey (MANS), consumption of bakery products such as biscuits, breads, local *kuih* has appeared in the list of top ten daily consumed foods (Norimah et al., 2008). Therefore, being a popular, easily available and convenient food, bakery products have a wide potential in developing functional foods by incorporation or partial replacement for sweetener to improve the overall nutritional quality of the bakery products. In this study, plain carrot cake was chosen as a baked-based product model for its healthier content, to partially substitute the table sugar for concentrated NFS up to 20% as a reduction of 20% sugar is the most acceptable and could be achieved without difficulty as reported by Shukla (1995). The objective of this study was to determine the effect of partial replacement of sugar with concentrated NFS on nutritional compositions, textural properties, antioxidative activities and sensorial acceptability of carrot cakes.

MATERIALS AND METHODS

Materials

Four batches of fresh NFS sample were collected and supplied from the same nipa farm at different months (May, July, September and November in the year 2018) from Pak Su Kemumin Enterprise, Kampung Kemumin, Kota Bharu, Kelantan state of Malaysia. The sap was acquired by cutting the stalk of matured nipa fruit and sap was collected four times daily to avoid spontaneous fermentation by minimising the chance of contamination through direct

contact with the environment. The end cut of the stalk was wrapped with sterile plastic bags to maintain hygiene. The collected sap was pooled together and immediately delivered in a cooler box with ice at 0°C to the laboratory located at School of Health Sciences, Universiti Sains Malaysia, Kelantan. Fresh NFS was then filtered using cream separator (Motor Sich-100-18 Separator, Ukraine) to remove the sedimentation.

Experimental Design

The filtration, processing and chemical analyses of fresh NFS sample were done on the same day of sample delivery to maintain the quality of fresh NFS sample. Concentrated NFS sample was obtained after the processing of NFS and to be substituted readily in the carrot cake formulations.

There were control and four experimental carrot cake formulations. The control formulation was the carrot cake without substitution of concentrated NFS (0%), whilst the experimental formulations were the carrot cakes incorporated with 5, 10, 15, 20% concentrated NFS to partially replace sugar. This study compared the different carrot cake formulations in terms of the nutritional values, textural properties, antioxidant capacities and sensorial acceptability.

Processing of NFS

The dehydration technique and storage of neera were adapted with modifications from the techniques described by Naknean et al. (2013). Forty grams of the filtered sap were dehydrated in a thermal dehydrator (Anywin

FD770, China) at 62°C for an overnight (15 hours) to evaporate the moisture content. After heated, concentrated NFS samples were stored in a chiller at -4°C prior to analysis and further use.

Chemical Analyses of NFS

The measurement of pH was done on NFS samples using HANNA pH 211 microprocessor pH meter (USA) according to Association of Official Analytical Chemists [AOAC] (2012). Calibration was accomplished employing buffer solutions at pH 7.0 and 4.0. The quality of fresh NFS sample was monitored prior to analysis by measuring its pH, if a fresh NFS sample has an initial pH below 4.0, the fresh NFS will be discarded as fermentation has possibly occurred in the sample. According to Aimi et al. (2013), pH at about 3.6 indicates that the palm sap is in the fermented stage in which the fermentation is dominated by yeasts and lactic acid bacteria. Total soluble solids of samples as °Brix were measured using hand refractometer (Atago 3851 PAL-BX/RI, Japan). The sugar content of NFS was analysed with the Boehringer Mannheim/ R Biopharm 10 716 260 035 sugar analysis kit for enzymatic analysis of sucrose, glucose and fructose according to the manufacturer's instructions. Moisture of NFS samples was analysed according to AOAC methods (AOAC, 2000).

Carrot Cake Preparation

Formulations of plain carrot cake for this study were adapted from a well-known baking site (Jaworski, n.d.) with modification

and listed in Table 1. Concentrated NFS was incorporated into the formulation to substitute sugar by 5, 10, 15 and 20% based on sugar weight (g) as in control formulation (0%). To begin with, the dry ingredients: wheat flour, cinammon ground, baking powder, baking soda were filtered and mixed evenly. In another bowl which contained the dry ingredients, eggs were mixed together with the sugar or concentrated NFS at speed 3 using electric hand mixer (KHIND HM 200, Malaysia) for approximately 5 minutes until the mixture turned pale and foamy. Next, corn oil was added into the egg mixture together with vanilla essence. Mixing was continued until the mixture was uniformly mixed. Next the dry ingredients were added into the mixture by gently folding the former into the latter using a spatula. Grated carrot was then added to the mixture. The combined batter was poured into a 6 inch pan. The cake was baked at 150°C for 40 minutes in the oven (ELBA 30L, Italy).

Nutritional Analysis

Proximate analysis was analysed according to AOAC methods (AOAC, 2000) to determine moisture, ash, protein, fat and total dietary fiber contents of control and NFS carrot cakes. Total carbohydrate was calculated by difference using the formula:

$$\% \text{ Carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ fat} + \% \text{ ash} + \% \text{ protein})$$

For mineral analysis, carrot cake samples were sent to Biochem Laboratories Sdn. Bhd., Penang to determine the sodium and potassium content of cake samples using Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES). Generally, ICP-OES application is a tool to detect metals and non-metals especially in solution. This tool utilises Argon gas to move the sample vapour into a chamber under high vacuum condition where both the samples and gas are heated such that elements will give off a characteristic wavelength of light.

Table 1
The raw ingredients used and incorporation level (%) of concentrated NFS

Ingredients (g)	Formulation (% concentrated NFS used to replace sugar) g /100 g flour				
	0% NFS (control)	5% NFS	10% NFS	15% NFS	20% NFS
Sugar	80.0	76.0	72.0	68.0	64.0
Concentrated NFS	0.0	4.0	8.0	12.0	16.0
Wheat flour	100.0	100.0	100.0	100.0	100.0
Corn oil	50.0	50.0	50.0	50.0	50.0
Egg	60.0	60.0	60.0	60.0	60.0
Ground cinnamon	2.1	2.1	2.1	2.1	2.1
Baking powder	4.2	4.2	4.2	4.2	4.2
Baking soda	2.1	2.1	2.1	2.1	2.1
Carrot	150.0	150.0	150.0	150.0	150.0
Orange zest	1.0	1.0	1.0	1.0	1.0
Vanilla essence	2.1	2.1	2.1	2.1	2.1

Physical Analysis

Textural analysis of carrot cakes was conducted instrumentally using TA.XTPlus Texture Analyser (Stable Micro Systems Ltd., Surrey, UK) which was driven by Exponent software package. The soft inner portion of cake was evaluated. Each cake was cut into 2.5 cm sided cubes, where the upper and lower crusts were eliminated. A 75-mm diameter aluminium plate (P/75) was used for compression. Probe height was initially calibrated to ensure that the travel distance of the probe can be recorded. The test was carried out under the following condition, test speed 1 mm s⁻¹, strain 50%, double cycles, 5 sec interval between cycles and trigger force 5 g. The hardness (N), springiness, cohesiveness, resilience and chewiness attributes of cakes were calculated from the curves obtained.

Sample Extraction for Total Phenolic Content (TPC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay

Carrot cake sample (1 g) was extracted with 100 mL methanol 80% (v/v) in conical flask (250 mL) wrapped with aluminium foil according to method suggested by Uthumporn et al. (2015) with slight modification. The mixture was then shaken overnight in an orbital shaker (Thermo Scientific Forma Incubated Benchtop Orbital Shaker Model 420, United States) at 160 rpm at 27°C. Next, the mixture was poured into centrifuge tubes and centrifuged (Eppendorf MiniSpin Plus, Germany) at 420 × g for 30 minutes to get a clear solution. Light exposure was prevented

throughout the extraction process. The extract was directly used to determine total phenolic content (TPC) and antioxidant value (2,2-diphenyl-1-picrylhydrazyl, DPPH).

Sample Extraction for Reducing Power Assay

Carrot cake sample (0.25 g) was extracted triplicate according to method described by Szawara-Nowak et al. (2017) with 5 mL methanol 67% (v/v) in conical flask wrapped with aluminium foil and shaken overnight in an orbital shaker (Thermo Scientific Forma Incubated Benchtop Orbital Shaker Model 420, United States) at 160 rpm at 27°C. Next, the mixture was poured into centrifuge tubes and centrifuged (Eppendorf MiniSpin Plus, Germany) at 420 × g for 30 minutes to get a clear solution. The extract was dried in a fume hood overnight. Then the dried extract was dissolved in 0.2 M, pH 6.6 phosphate buffer (20 mg/mL) and was used immediately for the measurement of reducing power of cake extracts.

Total Phenolic Content (TPC)

TPC of carrot cakes were determined by using Folin–Ciocalteu's (FC) assay which was described by Singleton and Rossi (1965) with slight modification. Result obtained was expressed as gallic acid equivalent (mg GAE/ g extract).

DPPH Activity

The antioxidant capacity of the cake extracts was studied through the evaluation of the free radical-scavenging effect on the DPPH

radical. The determination was based on the method proposed by Ancos et al. (2002) with modification. This method was also called as DPPH free radical scavenging assay. The DPPH radical scavenging activity was expressed as percentage of inhibition (% of inhibition).

Reducing Power (RP)

The reducing power of cake extracts was determined by the method of Oyaizu (1986) with slight modification according to Liyana-Pathirana and Shahidi (2006). Result obtained was expressed as ascorbic acid equivalent (mg ascorbic acid/ g extract).

Sensory Evaluation

The sensory evaluation of carrot cakes was carried out by 60 untrained panellists consisting of staff and students of the School of Health Sciences, Universiti Sains Malaysia. Panellists received five different formulations of carrot cakes for evaluation. The tested samples were coded with a three-digit randomly permuted number. All samples were rated according to the seven-point hedonic scales (1 = dislike extremely, and 7 = like extremely). Sensory attributes such as colour, appearance, texture, taste, flavour and overall acceptance were evaluated based on their degree of overall likeness for carrot cake.

Data Analysis

All data were subjected to one-way repeated measure analysis of variance (ANOVA) followed by Tukey HSD test to compare mean differences among the samples.

Data analysis was done using IBM SPSS Statistics Version 24 (SPSS Software, Armonk, New York). Three replicates were performed for all batches of fresh and concentrated NFS samples. Also, three batches of carrot cakes were produced in this study for all measurements. Results were expressed as mean values of three replicates \pm standard deviation (SD) except for dietary fiber (n=2), mineral analysis determination (n=2) and sensory evaluation (n=60). All tests were two-tailed and significance level was established at $p < 0.05$.

RESULTS AND DISCUSSION

Chemical Quality of NFS

NFS sample batches were chosen to be collected on May, July, September and November in the same year in 2018 as these months represented a monsoon change in the East Coast of Malaysia. Being one of the East Coast states in Malaysia, Kelantan has faced two monsoon seasons, the Southwest Monsoon from April to September which leads to a drier weather and the Northeast Monsoon from October to March, bringing to a higher rainfall (Malaysian Meteorological Department [MetMalaysia], 2019). The monsoon change has led to the variation of chemical quality of NFS collected as can be seen in Table 2. The pH values of fresh NFS samples ranged from 4.33 on November to 4.82 on May. Preliminary studies on NFS also found that unprocessed fresh NFS has an initial pH of 4.5 - 6.5 (Aimi et al., 2013; Minh, 2014). According to Aimi et al. (2013), pH at about 3.6 indicates palm sap is in the fermented

stage in which the fermenting organisms are dominated by yeasts, particularly *Saccharomyces cerevisiae* and lactic acid bacteria. According to the average rainfall monthly report by Jabatan Pengairan dan Saliran Negeri Kelantan (JPS Negeri Kelantan) (2018), the rainfall station located in Kota Bharu, Kelantan had collected the highest record of average rainfall (30.9 mm) on the month of November. This had resulted in the yield of palm sap having a significantly ($p < 0.05$) higher moisture content (83.86%) on November compared to other months. According to Matsui et al. (2014), the production of palm sap from *N. fruticans* fluctuates in a year in which sugar production tends to decrease in the rainy season. Generally, when the volume of sap produced is high (high moisture content), the sugar content in sap (TSS) tends to decrease. The major carbohydrates found in fresh NFS samples analysed in this study were sucrose (115.45 to 127.03 g/L), followed by fructose (15.25 to 21.92 g/L) and glucose (5.86 to 6.71 g/L). Differences in sucrose, fructose and glucose concentrations of fresh NFS samples may be due to the variation among fruit stalks and the origin of palm trees (Matsui et al., 2014).

Traditionally, palm sap syrup is produced by evaporating the palm sap in an open pan, and heated, using a wood fired stove, until it becomes concentrated. This heat causes the formation of dark colour, known as the browning development. Browning caused by Maillard reaction and caramelization may lead to unacceptable nutritional and sensory effects in sugar-

based food products and may be a limiting factor in the shelf life of products (Burdulu & Karadeniz, 2003). Dehydration technique suggested by Naknean et al. (2013) showed that evaporation heat treatment processing of palm sap at 70 to 80°C was better in retaining desired quality attributes in syrup than by heating with an open pan. According to a recent study by Yunos et al. (2018), evaporation heat treatment of palm sugar that maintained at low and controlled temperature exhibited the highest ability in ion chelating activity which related in antioxidant activity compared to freeze-drying and open pan methods. Besides, it could retain more sucrose content than further inverted compared to palm syrup heated by an open pan. This dehydration technique was also supported by Tamunaida and Saka (2011) showing that the saps treated at 70°C were higher in total sugars. In fact, in our study, the recorded temperature for the dehydration process was found to be 62°C which was lower than the recommended temperature so as to minimise the loss of bioactive compounds which might present in NFS samples. Non-enzymatic browning of palm sugar syrup during storage could also be reduced by storing at low temperature (-4°C). Chemical quality characteristics of concentrated NFS after processing at 62°C were shown in Table 3. Concentrated NFS obtained after processing was found to have a range of pH value of 5.24 to 5.34 from the four batches samples, higher than the pH of the fresh NFS. The increase of pH observed after processing of NFS might be

caused by the interaction between reducing sugars and amino compounds present in NFS samples resulted in the formation of Amadori rearrangement as an effect of elevated temperature. Besides, the Strecker degradation of amino acids might contribute to the increase in pH by losing CO₂ from the acid moiety (Akochi-K et al., 1997). This indicated that the NFS contains minerals and amino acids rather than simply a pure solution of sugar. From Table 3, moisture content of all four concentrated NFS samples ranged from only 15.76 to 15.82% and were not significantly different ($p > 0.05$) among the four batches. This result indicated that most of the moisture content in NFS had lost through the process of dehydration at 62°C. Brix values are commonly used for approximation of sugar content in sugar-rich samples and can be defined as the grams of sucrose per 100 g of sample material, thus, a refractometer can be used to determine the carbohydrate content of the sap and/or finished syrup (Willits & Hills, 1976). As the major chemical components of NFS are simple carbohydrate and water, the combination of these two values should be close to 100% (Stuckel & Low, 1996). Combining moisture with °Brix value of concentrated NFS in this study gave values in range of 99.58% to 101.19%. This again showed that low heat-dehydration technique at 62°C is an effective method in removing the water content without much sugar loss. According to reg. 132 of the Food Regulations 1985 (n. d.), molasses or syrup shall have TSS of not less than 85°Brix at 20°C. Under this definition, all four batches

of concentrated NFS, which have a range of TSS from 85.40 to 86.63°Brix, met this standard and could be directly incorporated into food. In the present study, concentrated NFS samples from batch J (July) were chosen to be further incorporated into the experimental cake formulations due to its highest value of TSS (86.73°Brix) and the lowest moisture content (15.76%) obtained from the analysis compared to other batches from different months (Table 3).

Nutritional Composition of Carrot Cake

The nutritional composition of carrot cakes prepared from sugar replaced partially with concentrated NFS at different levels is shown in Table 4. The results revealed that the incorporation of concentrated NFS resulted in significantly different proportions of moisture, ash, carbohydrate and dietary fiber. Moisture content varied from 29.52 to 33.31% for carrot cakes prepared with the increasing amount of concentrated NFS to partially replace sugar. The result of this study indicated that all carrot cakes formulated with concentrated NFS recorded significantly higher ($p < 0.05$) moisture content ranging from 31.60 to 33.31% for 5, 10 and 20% NFS-incorporated cake compared to control cake (29.52%). Similar results were reported by Ahmadi et al. (2011) and Majzoobi et al. (2016) to replace sugar (sucrose) with date liquid syrup in cookies and cakes. This can be related to the different solubility rate of sucrose, fructose and glucose during mixing in cake preparation. Sucrose remains mostly in the crystalline form, having lesser interaction

Table 2
Chemical quality of fresh NFS samples collected on four different months in 2018

Sample batch	Quality characteristics						
	pH	Total soluble solids (°Brix)	Moisture (%)	Sucrose (g/L)	Fructose (g/L)	Glucose (g/L)	
M	4.82 ± 0.02 ^a	19.33 ± 0.06 ^a	80.25 ± 0.05 ^d	127.03 ± 0.14 ^a	21.92 ± 0.01 ^a	6.71 ± 0.03 ^a	
J	4.77 ± 0.02 ^b	18.23 ± 0.06 ^{ab}	81.78 ± 0.04 ^c	126.07 ± 0.23 ^b	19.40 ± 0.12 ^b	6.22 ± 0.04 ^b	
S	4.64 ± 0.02 ^c	18.13 ± 0.06 ^{ab}	82.12 ± 0.02 ^b	121.22 ± 0.41 ^c	17.38 ± 0.07 ^c	5.96 ± 0.05 ^c	
N	4.33 ± 0.01 ^d	17.70 ± 0.10 ^b	83.86 ± 0.07 ^a	115.45 ± 0.25 ^d	15.25 ± 0.06 ^d	5.86 ± 0.07 ^c	

Note. Four batches of NFS samples in this study were collected at different months (M=May, J=July, S=September, N=November) in the same year 2018. Each value is the mean of triplicate determinations ± standard deviation (n=3).

Mean values within the same column bearing the same subscript letter were not significantly different (p>0.05)

Table 3
Chemical quality of concentrated NFS samples dehydrated from fresh NFS collected on four different months in 2018

Sample batch	Quality characteristics						
	pH	Total soluble solids (°Brix)	Moisture (%)	Sucrose (g/100g)	Fructose (g/100g)	Glucose (g/100g)	
M	5.34 ± 0.02 ^a	86.63 ± 0.06 ^a	15.82 ± 0.03 ^a	74.04 ± 0.08 ^a	11.52 ± 0.01 ^a	6.61 ± 0.09 ^b	
J	5.31 ± 0.01 ^{ab}	86.73 ± 0.06 ^a	15.76 ± 0.06 ^a	72.01 ± 0.27 ^b	11.51 ± 0.01 ^a	6.46 ± 0.05 ^b	
S	5.27 ± 0.01 ^{bc}	85.43 ± 0.06 ^b	15.76 ± 0.07 ^a	70.76 ± 0.17 ^c	11.50 ± 0.25 ^a	6.84 ± 0.04 ^a	
N	5.24 ± 0.03 ^c	85.40 ± 0.00 ^b	15.79 ± 0.10 ^a	71.42 ± 0.11 ^{bc}	11.44 ± 0.06 ^a	6.49 ± 0.02 ^b	

Note. Four batches of NFS samples in this study were collected at different months (M=May, J=July, S=September, N=November) in the same year 2018. Each value is the mean of triplicate determinations ± standard deviation (n=3).

Mean values within the same column bearing the same subscript letter were not significantly different (p>0.05)

with water (low solubility) compared to reducing sugar (glucose and fructose). During baking, more water can evaporate readily before sugar solubilise, resulting in a drier cake as observed in control (0%). In contrast, glucose and fructose in concentrated NFS, have more interactions with water (higher solubility) by hydrogen bonding and prevent it from evaporating during baking, causing higher moisture content of carrot cakes. Concentrated NFS used in this study are composed of three main sugars: sucrose, fructose and glucose as determined earlier (Table 3) compared to refined table sugar (>99.5% sucrose). Hence, the higher the substitution of concentrated NFS, the higher the moisture content of NFS. Another component of carrot cakes which increased significantly ($p < 0.05$) with every 5% substitution of concentrated NFS is ash content, which can be related to the inorganic minerals present in the palm saps used. According to Nguyen et al. (2016), palm sap contains high mineral contents such as potassium, chloride and sodium. Moreover, *N. fruticans* palm tree grows near the swampy seawater salt which directly contributes to the high content of sodium and chloride in NFS (Hamilton & Murphy, 1988).

Mineral analysis results from Table 4 showed that all formulations of carrot cake incorporated with concentrated NFS (5, 10, 15 and 20%) have a significantly higher sodium and potassium content as compared with the control carrot cake without addition of NFS. According to dietary guidelines issued by World Health Organization

[WHO] (2013), adults should consume at least 3510mg of potassium and less than 2000mg of sodium per day to reduce blood pressure and risk of cardiovascular disease, stroke and coronary heart disease. A study by Mierlo et al. (2010) which collected data across 21 countries reported that the mean potassium intakes ranged from 1700mg/day (China) to 3700mg/day (Finland, the Netherlands, and Poland). This suggested that an increase in potassium intake should be recommended, especially among Asian countries. In contrast to the low potassium intake, a recent Ministry of Health survey in Malaysia (2017) reported that the mean population salt intake had achieved 3419mg of sodium per day, which is 1.7 times than the limit set by WHO. Considering the high content of both sodium and potassium in cake formulations incorporated with NFS, a balance consumption of the cake should be considered in one's daily diet so that the intake of potassium is adequate and at the same time excessive intake of sodium could be avoided.

From the results shown in Table 2, all formulations of cake showed a high percentage of total dietary fiber content ranging from 9.20 to 10.75% due to the presence of grated carrot in the cake. Interestingly, total dietary fiber content of 20% concentrated NFS-substituted carrot cake (10.75%) was found to be significantly ($p < 0.05$) higher than the fiber content of control cake (9.75%). On the other hand, carrot cake containing 20% concentrated NFS, though not significant ($p > 0.05$), recorded the highest fiber content

(10.75%) compared with 5, 10 and 15% NFS-incorporated carrot cake. This is in accordance with the findings of Trinidad et al. (2010) which reported that minimal processed palm sugars contained significant amount of dietary fiber, especially inulin.

For other nutrients such as fat and protein, incorporation of concentrated NFS showed no statistically difference ($p > 0.05$) with the control cake, indicating that substituting sugar with concentrated NFS would not have a significant effect on the protein and fat contents.

Table 4
Proximate composition for different formulations of carrot cake

Component (%)	Concentrated NFS level (%)				
	0	5	10	15	20
Moisture	29.52 ± 0.23 ^c	31.60 ± 0.07 ^b	31.92 ± 0.42 ^{ab}	32.37 ± 0.74 ^{ab}	33.31 ± 0.77 ^a
Protein	7.02 ± 0.06 ^a	7.03 ± 0.11 ^a	7.09 ± 0.09 ^a	7.10 ± 0.27 ^a	7.15 ± 0.06 ^a
Fat	20.52 ± 1.38 ^a	19.66 ± 1.36 ^a	19.46 ± 0.06 ^a	19.40 ± 0.56 ^a	19.45 ± 0.20 ^a
Ash	1.18 ± 0.00 ^c	1.26 ± 0.02 ^d	1.31 ± 0.02 ^c	1.36 ± 0.01 ^b	1.47 ± 0.01 ^a
Sodium (mg/100g) ¹	6.90 ± 0.00 ^d	174.00 ± 4.24 ^c	176.50 ± 0.71 ^c	218.50 ± 4.95 ^b	253.50 ± 4.95 ^a
Potassium (mg/100g) ¹	4.05 ± 0.21 ^d	172.00 ± 4.24 ^b	152.50 ± 3.54 ^c	155.00 ± 4.24 ^c	222.50 ± 3.54 ^a
Carbohydrate	41.76 ± 1.67 ^a	40.45 ± 1.56 ^{ab}	40.22 ± 0.59 ^{ab}	39.8 ± 1.58 ^{ab}	38.62 ± 1.04 ^b
Dietary Fiber	9.20 ± 0.08 ^b	9.50 ± 0.25 ^{ab}	10.24 ± 0.60 ^{ab}	10.51 ± 0.29 ^{ab}	10.75 ± 0.18 ^a
Caloric Values ²	379.81 ± 7.87 ^a	366.82 ± 6.88 ^{ab}	364.42 ± 1.75 ^b	362.10 ± 3.67 ^b	358.13 ± 2.96 ^b

Note. Values are mean ± standard deviation

Mean values within the same row bearing the same subscript letter were not significantly different ($p > 0.05$)

¹Mineral analysis for sodium and potassium was done using ICP-OES performed by Biochem Laboratories Sdn. Bhd., Penang

²Caloric values (kcal/100 g) = (4×protein) + (9×fat) + (4×carbohydrate)

Texture Analysis

Effects of substitution of sugar with concentrated NFS on the textural profile of carrot cakes were shown in Table 5. The present result indicated that there was an inverse relationship between hardness and the level of concentrated NFS. Among all the formulations, control carrot cake showed the highest value for hardness. The hardness of carrot cakes varied from 5.23 to 14.59N. Apparently, carrot cake incorporated with 20% concentrated NFS had a hardness value of 5.23N, which was significantly ($p < 0.05$)

lower than the control cake without the addition of NFS (14.59N). Yet, there was no statistically difference of hardness between 5 and 10%, as well as 15 and 20% of NFS-incorporated cake. The large variation of hardness among the cakes might be due to the greater crystallisation rate of sucrose during cooling of cakes compared to fructose and glucose. Sugars do not just only play an important role in the overall sweetness of the cakes but also provide textural properties desirable in foods. According to Laos et al. (2007), crystallisation of pure

sucrose supersaturated solution occurred very quickly. In contrast, crystallisation of sucrose in presence of glucose or fructose led to slower crystallisation. This finding could be related back to the sugar content of the concentrated NFS being performed earlier in this study (Table 3). Hence, the purer the sucrose content added to the cake, the higher the hardness value of the carrot cakes.

Resilience and springiness of the carrot cakes also showed a decreasing trend when incorporated with concentrated NFS. There was a significant reduction ($p < 0.05$) of resilience in every 5% substitution of sugar with NFS. However, there was no statistically reduction in springiness by carrot cakes added with NFS up to 10%. At 15 and 20% level of NFS incorporation, springiness of cakes was 0.25 and 0.21, respectively which differ significantly ($p < 0.05$) from springiness of control cake which was 0.31. Manisha et al. (2012) showed that the sugar provided a considerable part of the bulking agent by competing with starch for available water, and this could delay the onset of gelatinisation, improving the size of air

bubbles due to carbon dioxide and water vapour before the cake sets. Moreover, sugar differs in their ability to delay gelatinisation, with the following sugars having the greatest impact (from least to greatest): fructose, glucose and sucrose (Brown, 2011). Refined sugar added in the control cake is more than the other cakes, therefore will have a greater effect in delaying gelatinisation, resulting in a cake with bigger air bubble size and higher volume. This could greatly affect the textural properties of carrot cakes in term of springiness and resilience. For other textural properties such as cohesiveness and chewiness, only slight differences were observed.

Antioxidant Capacity of Carrot Cake Extract

Characterization of a new food product often involves the usage of antioxidative capacity. There is a variety of *in vitro* test procedures to carry out for evaluating antioxidant activities with the samples of interest. Hence, antioxidant activity should not be concluded based on a single antioxidant test model. In our present study, three methods

Table 5
Textural properties for different formulations of carrot cake

Properties	Concentrated NFS Level (%)				
	0	5	10	15	20
Hardness (N)	14.59 ± 0.78 ^a	9.95 ± 0.58 ^b	8.63 ± 0.72 ^b	5.95 ± 0.16 ^c	5.23 ± 0.13 ^c
Springiness	0.31 ± 0.04 ^a	0.29 ± 0.31 ^a	0.28 ± 0.12 ^{ab}	0.25 ± 0.13 ^{bc}	0.21 ± 0.08 ^c
Cohesiveness	0.35 ± 0.02 ^a	0.27 ± 0.28 ^b	0.33 ± 0.13 ^a	0.26 ± 0.01 ^b	0.13 ± 0.00 ^c
Chewiness (N)	0.56 ± 0.11 ^b	0.56 ± 0.04 ^b	0.62 ± 0.14 ^{ab}	0.65 ± 0.08 ^{ab}	0.72 ± 0.04 ^a
Resilience	0.60 ± 0.21 ^a	0.49 ± 0.32 ^b	0.42 ± 0.07 ^c	0.30 ± 0.23 ^d	0.21 ± 0.30 ^e

Note. Each value is the mean of triplicate determinations ± standard deviation (n=3)
Mean values within the same row bearing the same subscript letter were not significantly different ($p > 0.05$)

were chosen for evaluating antioxidant activities, which are Folin-Ciocalteu (FC) method to evaluate TPC, free radical scavenging capacity of DPPH and RP assay to determine the reducing power of bioactive compounds. These assays were selected to confirm the presence of phenolic content and antioxidant in food samples as they are simple, inexpensive and rapid (Badarinath et al., 2010). In fact, antioxidant test models vary in different respects. Hence, it is difficult to compare fully one method to another (Alam et al., 2013).

Table 6 shows the antioxidant capacity for different formulations of carrot cake incorporated with concentrated NFS. The TPC of carrot cakes prepared from sugar and concentrated NFS ranged from 0.11 to 0.34. The result obtained in this study indicated that TPC in carrot cake extract increased proportionally with the level of NFS added in the formulations in the following order: $0\% < 5\% < 10\% < 15\% < 20\%$. Carrot cake extract substituted with 20% concentrated NFS was found to have the highest TPC content (0.14 mg GAE) compared to the control carrot and other cake extracts of different formulations. However, carrot cake incorporated with 20% concentrated NFS was not significantly differed ($p > 0.05$) with 15% concentrated NFS-incorporated cake. The high TPC in carrot cake formulated with concentrated NFS was mainly due to the presence of a large amount of polyphenol compound in concentrated NFS that is being substituted for sugar in the carrot cake. It is possible to hypothesise that a high content of total phenolic compounds is likely present in NFS as a result of there being no purification

process applied during its manufacture, whereas refined sugar is produced by employing additional steps which remove colour and any other non-sugar components. According to Sukairi et al. (2018), NFS sample reported to highly inhibit α -amylase enzyme due to the presence of phenolic compound, for example phenolic acids and flavonoids. The phenolic compounds work by inhibiting α -amylase activity, causing a retardation in the intake of monosaccharide in the body.

From Table 6, control carrot cake extract showed a significant ($p < 0.05$) lower percentage of scavenging or inhibition of DPPH free radical activity compared to 10, 15 and 20% of NFS-incorporated carrot cake extract. However, substitution of 5% concentrated NFS carrot cake extract did not differ significantly ($p > 0.05$) from the control cake extract in term of DPPH radical scavenging activity. The 20% level of concentrated NFS-incorporated cake extract showed the highest scavenging activity towards DPPH (3.89% of inhibition). Both TPC and DPPH demonstrated identical trends in the comparison of the antioxidant activities in which the presence of polyphenols in concentrated NFS play a significant role in contributing to the overall phenolic content of the cake extract. The antioxidants present in the phenolic fractions were able to reduce the violet colour, stable 1,1-diphenyl-2-picrylhydrazyl radical to the yellow colour 1,1-diphenyl-2-picrylhydrazine. Previous studies have also presented positive correlations between the quantity of phenolic compounds and the DPPH free radical scavenging effect

(Kedare & Singh, 2011; Liu et al., 2009). This finding is also in accordance with the result from Sukairi et al. (2018) which reported that higher scavenging activity was observed as the concentration of NFS sample was increased, showing once again that this natural sweetener has a positive effect on the enhancement of antioxidant activity in carrot cake samples.

Another effective method to determine an extract's antioxidant properties is the reducing power (RP) assay. As shown in Table 6, the reducing power assay showed a pattern of oxidant strength identical to the TPC and DPPH assays. The reducing power of carrot cake extract increased as the substitution of sugar with concentrated NFS becomes higher, though the increment was not significant ($p > 0.05$) as could be seen between 0 and 5%, as well as 15 and 20% level of concentrated NFS incorporation. The reducing power was the highest when sugar was replaced with 20% concentrated NFS in carrot cakes while the least reducing power was shown to be the control cake without any incorporation of concentrated

NFS. The presence of reducers (antioxidant) in the concentrated NFS used in the cake formulation caused the reduction of the Fe^{3+} /ferricyanide complex to the ferrous form. Therefore, measuring the formation of Perl's Prussian blue at 700 nm can monitor the Fe^{2+} concentration. Again, this confirmed the presence of antioxidative compounds in the concentrated NFS sample used which directly led to an increase in the antioxidative activities of carrot cake extract when NFS was added into the carrot cakes.

Sensory Acceptability

Table 7 shows the sensory evaluation scores for carrot cakes incorporated with concentrated NFS. The result showed that the scores of all sensory attributes were in a range of 4.27 to 5.27. The present sensory data showed that all carrot cakes formulated with concentrated NFS (5, 10, 15 and 20%) were not significantly different ($p > 0.05$) compared to the control cake for all attributes. Among all the carrot cake formulations, 20% concentrated NFS-incorporated cake received the highest scores for all sensory

Table 6
Antioxidant capacity of carrot cake samples determined against TPC, DPPH and RP assays

% of concentrated NFS incorporated carrot cake	Antioxidant Capacity		
	TPC (mg GAE/ g)	DPPH (% of inhibition)	Reducing power (mg ascorbic acid/g)
0	0.11 ± 0.01 ^d	2.90 ± 0.19 ^b	1.18 ± 0.01 ^b
5	0.12 ± 0.01 ^c	2.92 ± 0.19 ^b	1.18 ± 0.01 ^b
10	0.13 ± 0.01 ^{bc}	3.52 ± 0.19 ^a	1.27 ± 0.06 ^{ab}
15	0.13 ± 0.02 ^{ab}	3.56 ± 0.12 ^a	1.32 ± 0.03 ^a
20	0.14 ± 0.00 ^a	3.89 ± 0.07 ^a	1.34 ± 0.07 ^a

Note. Each value is the mean of triplicate determinations ± standard deviation (n=3)
Mean values within the same column bearing the same subscript letter were not significantly different ($p > 0.05$)

attributes (colour, texture, appearance and overall acceptance) except for taste and flavour. On the other hand, carrot cake incorporated with 15% concentrated recorded 5.18 and 5.10 score values for taste and flavour, respectively. These values are the highest among treatments but did not show significant ($p>0.05$) differences when compared to other cake formulations (0, 5, 10 and 15%). The result also showed that carrot cakes containing 20% concentrated NFS received the highest score for overall

acceptance (5.10) followed by carrot cakes containing 15% NFS (5.08). The result obtained indicated that consumers prefer 20% concentrated NFS to be incorporated in carrot cakes. Besides, consumers also preferred the 15% concentrated NFS-incorporated carrot cake in terms of taste and flavour. This data indicated that consumers generally accept the carrot cakes prepared with concentrated NFS up to 20% of sugar replacement.

Table 7
Sensory acceptability of different formulations of carrot cake

Properties	Concentrated NFS level (%)				
	0 %	5 %	10 %	15 %	20 %
Colour	5.27 ± 1.25 ^a	5.07 ± 1.34 ^a	4.87 ± 1.50 ^a	5.13 ± 1.41 ^a	5.27 ± 1.39 ^a
Appearance	4.82 ± 1.10 ^a	4.72 ± 1.14 ^a	4.72 ± 1.25 ^a	4.83 ± 1.26 ^a	5.00 ± 1.30 ^a
Texture	4.65 ± 1.29 ^a	4.28 ± 1.41 ^a	4.28 ± 1.50 ^a	4.55 ± 1.51 ^a	4.72 ± 1.40 ^a
Taste	4.70 ± 1.28 ^a	4.82 ± 1.37 ^a	4.52 ± 1.55 ^a	5.18 ± 1.10 ^a	5.03 ± 1.39 ^a
Flavour	4.80 ± 1.31 ^a	4.60 ± 1.30 ^a	4.27 ± 1.57 ^a	5.10 ± 1.02 ^a	5.00 ± 1.35 ^a
Overall Acceptance	4.87 ± 1.13 ^a	4.87 ± 1.27 ^a	4.62 ± 1.34 ^a	5.08 ± 1.01 ^a	5.10 ± 1.13 ^a

Note. Each value is the mean of determinations ± standard deviation (n=60)

Mean values within the same row bearing the same subscript letter were not significantly different ($p>0.05$) (Score 1 = dislike extremely, score 7 = like extremely)

CONCLUSION

The addition of concentrated NFS up to 20% to partially replace sugar in carrot cake formulation resulted in a significant increment of ash content compared to other cake formulations. Carrot cake containing 20% concentrated NFS also recorded significantly higher ($p<0.05$) total dietary fiber content at 10.75g/100g as compared with control carrot cake with 0% NFS at 9.20g/100g. Texture profile analysis revealed that there was a decreasing trend of hardness,

springiness and resilience in carrot cakes with the increasing levels of concentrated NFS substituted for sugar. TPC of carrot cake showed the highest value when 20% concentrated NFS was formulated in the cake but did not significantly ($p>0.05$) differ with 15% concentrated NFS-incorporated cake. The presence of antioxidant as a result of enhancement by concentrated NFS was further proved by the radical scavenging effect of DPPH and RP assay, in which a significantly ($p<0.05$) higher percentage

of inhibition and reducing power was observed in carrot cake incorporated with 20% concentrated NFS, comparing with the control cake. Sensory evaluation indicated that carrot cakes incorporated with 20% concentrated NFS, though not significant ($p>0.05$) differ among all formulations, received the highest scores for all sensory attributes except for taste and flavour. Meanwhile, carrot cake containing 15% concentrated NFS had 5.18 and 5.10 score values of taste and flavour, respectively. Using carrot cake as a bakery product model, this study showed that novel food ingredients like NFS could be used as a natural alternative sweetener up to 20% to replace table sugar to enhance nutritional compositions and antioxidative properties while maintaining the sensory quality of carrot cakes. Even though an improvement was observed in cakes formulated with NFS up to 20%, low values in antioxidant capacity were detected in the present study. Other processing methods such as freeze-drying or higher incorporation of concentrated NFS to replace sugar in cake formulations could be suggested in further research to improve and compare the antioxidant properties of the formulated cake. Besides, further study can be done to investigate the glycaemic response of processed foods incorporated with NFS.

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