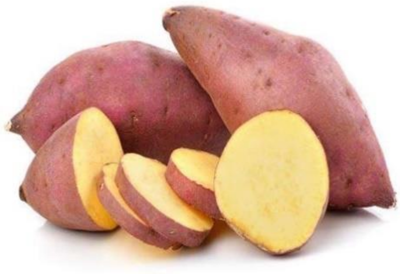
CHAPTER THREE

MATERIALS AND METHODS

3.1 Preparation of Sweet Potato Haulm Juice (SPHJ)

Sweet potato haulm (3 kg) was collected from Perlis Sweet Potato Farm in Kangar, Perlis in February 2021. The haulm was obtained from purple-skinned and yellow-fleshed sweet potato plants (Figure 3.1). Upon collection, the withered and dried leaves, stalks, and stems were removed before stored in a container in the chiller (4˚C). On the next day, the haulm was cleaned using running tap water to remove the remaining dirt and soil, and the remaining water was discarded using salad spinner. The haulm was cut for about 2 cm long each and juiced using a slow juicer (SAVTM JE-31).

From the haulm, 1.784 kilograms of juice and 0.955 kilograms of dregs were produced. The sweet potato haulm juice (SPHJ) was divided into two batches for i) unpasteurised SPHJ, and ii) pasteurised SPHJ. For the unpasteurised SPHJ, the sample was transferred into an aluminium tray and kept frozen (-20˚C, 48 hours) before freeze-drying. Meanwhile, the pasteurised SPHJ was pasteurised in a jacketed beaker (85˚C, 5 min) and immersed immediately in an ice-water bath to rapidly cool the juice down to room temperature (<20˚C, 30 min). The pasteurised sample was then transferred into an aluminium tray and kept frozen (-20˚C, 48 hours) before freeze-drying. All samples were performed in triplicate.



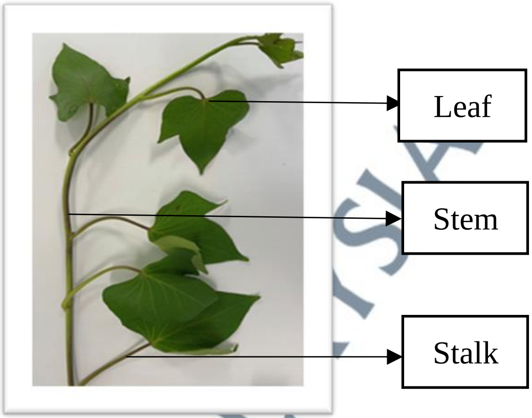


Figure 3.1: The haulm (stem, stalk, leaves) used in this study was obtained from the purple-skin yellow-fleshed sweet potato plant.

3.2 Analysis on Sweet Potato Haulm Juice (SPHJ)

3.2.1 Peroxidase Enzyme Test

A peroxidase enzyme test was done on the unpasteurised and pasteurised juice by measuring the colour change induced by the oxidation of guaiacol in the presence of hydrogen peroxide to assess the efficiency of pasteurisation according to (Bahçeci et al., 2005). One (1) mL of SPHJ was diluted with 9 mL deionised water to be added to an assay mix composed of 0.5 mL hydrogen peroxide and 0.5 mL guaiacol in a total volume of 100 mL deionised water. The rate of colour change induced by enzyme activity was measured using a spectrophotometer set at 420 nm. Readings were recorded every 2 seconds over 1.5 minutes period after 0.1 mL diluted juice was added to a 2.9 mL assay mix. The increase in absorbance in 1 minute was considered proportional to the peroxidase (POD) enzyme activity, and differences in the rate of pasteurised to non-pasteurised SPHJ were used to determine percentage enzyme activity reduction caused by the treatments.

3.2.2 Total Soluble Solid Content (TSS) of Sweet Potato Haulm Juice (SPHJ)

Total soluble solid of unpasteurised and pasteurised sweet potato haulm juice was calculated using a digital refractometer (Digital Pocket Refractometer PAL, ATAGO) and the values were expressed in ˚Brix.

3.2.3 Total Solid Content (TSC) and Moisture Content of Sweet Potato Haulm Juice (SPHJ)

The total solid content was calculated as the percentage of ratio from the weight of the dry sample to the weight of the wet sample. Percentage (g/100 g) of solid content was subtracted from 100 to obtain moisture content (g/100 g) in wet basis form (wb). The equations were as followed:

Total Solid Content (g/100 g wb) = (weight of freeze-dried juice/weight of SPHJ before freeze drying) × 100

Moisture Content (g/100 g wb) = 100 – Total Solid Content

3.3 Preparation of Sweet Potato Haulm Juice Powder (SPHJP)

The frozen unpasteurised and pasteurised SPHJ were freeze-dried (Freeze Dryer FD-550, Eyela) (-20˚C, 3 Pa, 48 hours) to form the sweet potato haulm juice powder (SPHJP). The unpasteurised and pasteurised SPHJP is then grounded using mortar and pestle to get homogenised powder. All SPHJP was sieved (75 μm), vacuum-sealed in aluminium pouches, and stored at -80˚C until further analysed.

3.4 Physicochemical Analysis of Sweet Potato Haulm Juice Powder (SPHJP)

3.4.1 Water Activity, Colour Analysis and Physical Morphology

Water activity was calculated using a water activity meter (AquaLab 4TE, Meter Food). Colour analysis of unpasteurised and pasteurised SPHJP was analysed using a colorimeter (LabScan XE Spectrophotometer, Hong Kong) using CIE colour system according to the manufacturer's instruction. The physical morphology of SPHJP was analysed through Scanning Electron Microscopy (SEM) (Jeol, JSM-IT800, Japan).

3.4.2 Water Solubility Index

For warm WSI, 1 gram of SPHJP was mixed with 12.5 mL distilled water in 50 mL centrifuge tube. The mixture was incubated in water bath (37˚C) for 30 min before centrifuged at 10, 000 rpm (4˚C). For cold WSI, the samples were not undergone incubation, and proceed to centrifugation (30 min, 10, 000 rpm, 4˚C) (Hanil Combi, 514R, Korea). The supernatant was obtained and oven-dried (70˚C) to obtain the weight of dried solute in dry weight (dw) form (Syamila, 2019; Moreira et al., 2009).

WSI (g/100 g dw) = Weight of dried solute/ Weight of initial SPHJP × 100

3.4.3 Proximate Analysis and Crude Fiber Analysis

The macronutrient composition such as moisture, fat, ash, protein, fibre and carbohydrate were analysed following the AOAC method (AOAC, 2005). Moisture content was calculated based on moisture loss of SPHJP after oven drying, while ash content was based on the remaining incinerated SPHJP after ashing (4 hours, 550˚C) in muffle furnace (Carbolite, England). The ash content was calculated using formula:

Ash content (g/100 g dw) = Weight of SPHJP after ashing/Weight of SPHJP before ashing × 100

The determination of fiber was determined under the digestion of SPHJP with sulphuric acid and sodium hydroxide using the FibreTherm automated instrument (Gerhardt, Fibretherm, German). Crude protein content was determined using Kjeldahl method and the percentage of protein was calculated as followed:

Protein content (g/100 g dw) = value of nitrogen (N) × 6.25

Crude fat content was determined based on extraction of fat using petroleum ether according to Soxhlet method. Total carbohydrate content was obtained by difference using formula:

Carbohydrate content (g/100 g dw) = 100 – (moisture + ash + protein + fat)

All analyses were performed in triplicate.

3.4.4 Total Phenolic Content (TPC)

Total Phenolic Content (TPC) was conducted using the Folin-Ciocalteau method according to Hue et al. (2012) with modification. Sample extracts of SPHJP (0.5 mL), Folin-Ciocalteau reagent (0.25 mL) and 7.5% sodium carbonate solution (0.75 mL) was incubated under dark condition (2 hours, room temperature). The absorbance readings had been taken at 760 nm using a UV-Vis spectrophotometer (Cary 50 Bio, UV-Vis, Malaysia), and the quantification of total phenolic content was based on the standard gallic acid calibration curve. TPC was expressed gallic acid equivalent (GAE) on dry weight (dw). The linear regression equation was as followed:

$$y = 0.4985x + 1.0354$$

$$R^2 = 0.9514$$

3.4.5 DPPH Scavenging Activity

The quantification of 2, 2 diphenyl-1-picrylhydrazyl (DPPH) scavenging activity (%) was based on the standard ascorbic acid calibration curve according to Suárez et al. (2020) with modifications. The absorbance readings for SPHJP extracts and standard were taken at 517nm using UV-Vis spectrophotometer (Cary 50 Bio, UV-Vis, Malaysia). The linear regression equation obtained as followed:

$$y = 0.1073x + 0.0238$$

$$R^2 = 0.9433$$

The formula for calculating the DPPH Scavenging Activity (%) showed below:

$$\text{DPPH Scavenging Activity (\%)} = [(\text{absorbance of control} - \text{absorbance of sample})/\text{absorbance of control})] \times 100$$

3.4.6 Ferric Reducing Antioxidant Power (FRAP) Activity

Ferric Reducing Antioxidant Power (FRAP) Assay had been conducted according to Benzie & Strain (1996) using ferrous sulphate ($FeSO_4$) as standard to get calibration curve, with linear regression equation of $y = 0.0044x + 0.8283$, $R^2 = 0.9879$. FRAP reagent was prepared in the ratio of 10:1:1 (300 mM sodium acetate buffer: 10 mM 2,4,6-tri [2-pyridyl]-s-triazine (TPTZ) solution: 20 mM $FeCl_3$. The pH value of the buffer was checked and maintained at pH 3.6. SPHJP extract (0.2 mL) was added to FRAP reagent (3.8 mL), followed by incubation at 37˚C in the water bath for 30 min. The absorbance readings were taken at 593nm using UV-Vis spectrophotometer (Cary 50 Bio, UV-Vis, Malaysia).

3.4.7 Anti-nutrients Content

The anti-nutrients content that has been tested were phytic acid and oxalic acid. Megazyme Phytic Acid Kit and UV-VIS spectrophotometric method were used in the determination of phytic acid in SPHJP according to manufacturer's instruction.

Oxalic acid content was determined based on calcium oxalate precipitation. The method involves titration of acidic aqueous extracts of the sample with a standard solution of potassium permanganate (Ekanayake, 2011). SPHJP (0.5 g) was added with 25 mL distilled water and the mixture was homogenised (8000 rpm, 3 min, 30˚C). The mixture was then purified with 6N hydrochloric acid (2.75 mL) and caprylic alcohol (2 drops), and incubated in water bath (15 min, 95˚C).

After the mixture was left overnight (16 hours, 30˚C), it was filtered using filter paper in 50 mL falcon tube. The filtrate (25 mL) was added with tungstophosphoric acid reagent (5 mL) and left for 5 hours. The mixture was added with ammonium hydroxide (NH_4OH) until reach pH 4.5. The mixture was mixed with acetate buffer (pH 4.5, 5 mL) and left overnight (16 hours, 30˚C). The mixture was centrifuged (1700 rpm, 15 min, 30˚C) and the supernatant was discarded, leaving the precipitate.

The precipitate was washed using cold wash liquid and decant process was repeated for at least 3 times to obtain purified precipitate. It was then dissolved with 5 mL 10% sulphuric acid. Blank (5 mL 10% sulphuric acid) and sample were incubated in water bath (95˚C) and titrated against 0.01N potassium permanganate ($KMnO_4$). The endpoint reading was the first persistent pink colour more than 30 seconds. The calculation for oxalic acid content using following formula:

$$\text{Oxalic acid (mg/100 g dw)} = [\text{volume of } KMnO_4 \times 67.5 \times (\text{Net weight} + 100\text{ g})]/(\text{Net weight} \times \text{Wet slurry})$$

67.5 = 0.45 × (30/20) × (dilution factor) x 100 (convert to 100 mg)

Dilution factor = 25/25

Wet slurry = 15.0 g

0.45 = 0.45 mg anhydrous oxalic acid equivalent to 1.0 ml 0.01N $KMnO_4$

3.4.8 Statistical Analysis

The results were analysed statistically to one-way Analysis of Variance (ANOVA) using Minitab Software to analyse the significant difference between the nutritional concentration of unpasteurised and pasteurised SPHJP. The results were expressed in (M ± SD), and the means were compared using Tukey’s test at $p<0.05$.

3.5 Storage Stability of Sweet Potato Haulm Juice Powder (SPHJP)

Due to known sensitivity of antioxidant like ß-carotene to light; sample storage without and with light were executed. The unpasteurised and pasteurised freeze-dried samples were sealed in aluminium pouches for dark conditions (without light) and transparent packaging (with light). All pouches were stored in transparent container at 20±2˚C, and the temperature was monitored using digital thermometer. Stability analysis for Ferric Reducing Antioxidant Power (FRAP) (and Total Phenolic Content (TPC) in unpasteurised and pasteurised SPHJP were evaluated for Day 0, Day 14, Day 60, Day 120, and Day 180.

3.5.1 Total Phenolic Content (TPC)

TPC of unpasteurised and pasteurised SPHJP stored at day 0, 14, 60, 120 and 180 had been determined using method described in 3.4.4.

3.5.2 Ferric Reducing Antioxidant Power (FRAP) Activity

FRAP activity of unpasteurised and pasteurised SPHJP stored at day 0, 14, 60, 120 and 180 had been determined using method described in 3.4.6.

3.5.3 Statistical Analysis

The results were analysed statistically to one-way Analysis of Variance (ANOVA) using Minitab Software to analyse the significant difference between the nutritional concentration of unpasteurised and pasteurised SPHJP. The results were expressed in (M ± SD), and the means were compared using Tukey's test at $p<0.05$. All experimental data for storage stability of FRAP activity and TPC were evaluated for the order of degradation kinetic (zero, first, or second-order). The determination of kinetic model and half-life ($t^{1/2}$) of the FRAP activity and TPC in the SPHJP to degrade to 50% of their initial concentrations had been calculated using following equations (Equation 1-6).

Zero order reaction:

Kinetic reaction: $C_t = -kt + C_0$ (Equation 1)

Half-life: $t_{1/2} = C_0/2k$ (Equation 2)

First order reaction:

Kinetic reaction: $\ln C_t = -kt + \ln C_0$ (Equation 3)

Half-life: $t_{1/2} = 0.693/k$ (Equation 4)

Second order reaction:

Kinetic reaction: $1/C_t = -kt + 1/C_0$ (Equation 5)

Half-life: $t_{1/2} = 1/k(C_0)$ (Equation 6)

k = rate constant,

t = storage time,

C_0 = Nutrient's initial concentration,

C_t = Nutrient's concentration at time t