CHAPTER FOUR

RESULTS AND DISCUSSIONS

In this study, the changes of concentrations or activities between the unpasteurised (initial) sample and pasteurised sample indicates the effect of heat treatment upon the specific analysis provided.

4.1 Total Solid Content (TSC), Moisture Content, and Total Soluble Solid of Unpasteurised and Pasteurised Sweet Potato Haulm Juice (SPHJ)

The total soluble solid in the sweet potato haulm juice was analysed before freeze-drying, while total solid and moisture content in the juice were calculated after the freeze-drying process. Table 4.1 shows the total solid content, moisture content, and total soluble solid of unpasteurised and pasteurised sweet potato haulm juice.

Table 4.1: Total Solid Content (TSC), Moisture Content, and Total Soluble Solid of unpasteurised and pasteurised Sweet Potato Haulm Juice (SPHJ)

SPHJP	Total Solid Content (g/100 g wb)	Moisture Content (g/100 g wb)	Total Soluble Solid (°Brix)
Unpasteurised	12.48 ± 0.10^{a}	87.52 ± 0.10^{b}	8.93 ± 0.40^{b}
Pasteurised	11.68 ± 0.14^{b}	88.32 ± 0.14^{a}	11.5 ± 0.53^{a}

wb: wet basis

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Values with similar letters within columns are not significantly different (Tukey's test, $p<0.05$)
All the samples were analysed in triplicate

Total solid content (TSC), also known as dry matter content, is the amount of all suspended, colloidal, and dissolved solids in water and can be calculated through a drying process. There was a significant change in the TSC content of unpasteurised and pasteurised SPHJ ($p<0.05$). Total solid content in the unpasteurised sweet potato haulm juice was 12.48 g/100 g wb, indicating 87.52 g/100 g wb removal of water, while pasteurised juice had a lower TSC, which was 11.68 g/100 g wb, where 88.32g/100 g wb water was sublimated.

Table 4.1 shows more solids were lost after the pasteurisation process ($p<0.05$), however, the differences is low. Freeze drying had removed more than 85 g/100 g wb water in both samples of sweet potato haulm juice. According to Shonte et al. (2020), freeze-drying removed 92.47 g/100 g wb and 92.26 g/100 g wb water in nettle and spinach leaf.

Unpasteurised and pasteurised SPHJ had a significant difference in total soluble solids (TSS) content which was 8.93 and 11.5 ̊Brix ($p<0.05$). TSS measured the total amount of soluble constituents, majorly by sugars followed by vitamins, organic or amino acids present in solution. TSS quantifies water-soluble components in the food such as glucose, sucrose, pectin and others.

A higher value of TSS (11.5 ̊Brix) in pasteurised SPHJP compared to unpasteurised SPHJP (8.93 ̊Brix) could be due to the long carbohydrate chain such as starch hydrolyses into monosaccharides and disaccharides (sucrose, glucose, or fructose). These simple sugars dissolved in the juice when heating was applied, suggesting that increasing temperature caused a rise in soluble solids (Dewi et al., 2018). This explained that unpasteurised SPHJP might consist higher level of starch than pasteurised SPHJP.

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4.2 Physicochemical Properties of Unpasteurised and Pasteurised Sweet Potato Haulm Juice Powder (SPHJP)

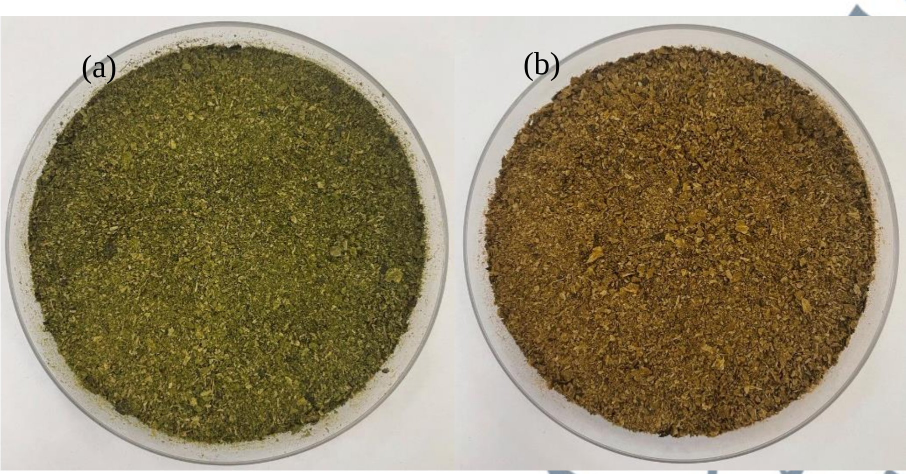

Figure 4.1: Unpasteurised (a) and pasteurised (b) Sweet Potato Haulm Juice Powder (SPHJP)

Figure 4.1 shows the unpasteurised and pasteurised SPHJP after freeze-drying. Analysis such as water activity and colour were important for dehydrated products as these can reflect the nutritional quality of the food products (Bozoglu & Erkmen, 2016; Syamila, 2019).

Water activity is essential when preserving low-moisture food and plays a role in inhibiting the growth rate of microorganisms, while colour gives an early impression to consumers and a significant sensory quality. In this study, plant juices must be immediately converted into stable powders with low water activity as one of the characteristics, aimed to achieve conveniency in storage. Colour is not only practically used to visualise physical quality, but also can reflect the nutritional content. Table 4.2 showed the physical analysis of unpasteurised and pasteurised SPHJP, which were water activity and colour analysis.

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Table 4.2: Water Activity and Colour Analysis of unpasteurised and pasteurised Sweet Potato Haulm Juice Powder (SPHJP)

SPHJP	**Water Activity (a_w)**	**Colour Analysis**			
		Lightness (L)	**Redness (a)**	**Yellowness (b)**	**Total Colour Difference (ΔE)**
Unpasteurised	0.40 ± 0.00[a]	32.32 ± 0.60[a]	1.40 ± 0.01[a]	22.70 ± 0.28[a]	5.98
Pasteurised	0.34 ± 0.01[b]	33.28 ± 0.24[a]	7.09 ± 0.09[b]	24.28 ± 0.38[b]	

SPHJP: Sweet potato haulm juice powder
Values with similar letters within columns are not significantly different (Tukey's test, $p<0.05$)
All the samples were analysed in triplicate

Table 4.2 showed the water activity and colour analysis of unpasteurised and pasteurised Sweet Potato Haulm Juice Powder (SPHJP). Pasteurised sample had a significantly reduced water activity in SPHJP ($p<0.05$) (Table 4.2). The unpasteurised sample had a value of 0.40, while pasteurised sample consisted of 0.34 of a_w. Water activity is crucial in preserving the dehydrated product. The combination of heating and freeze-drying had been used to reduce free water and this indirectly reduced the deterioration by microbes in food (Bozoglu & Erkmen, 2016).

From Figure 4.1, a noticeable colour change is observed between both samples. The Delta E value was 5.98 (Table 4.2), suggesting that the colour difference can be distinguished and is perceptible at human's normal vision (Obón et al., 2009). In our study for colour analysis using a colorimeter, only lightness (L*) exhibited non-significant values between unpasteurised and pasteurised SPHJP ($p>0.05$). Positive a* and b* values indicated redness and yellowness of the SPHJP. Pasteurised SPHJP had a higher value of redness which was 7.09, while unpasteurised SPHJP only had 1.40.

In food processing, enzymatic browning by polyphenoloxidase is prevented by dearaetion. Pasteurisation without deaeration to remove oxygen is known to caused losses of vitamin C and carotene (Fellows, 2017). Therefore, the changes of colour or browning of juice powder might be due to the absence of dearaetion prior to pasteurisation in this study. In this study, pasteurised samples can be seen in a darker colour, which might be contributed by the red or brown colour of the sample. In our study, the heat treatment applied on the SPHJP is higher than 80˚C and has shown deactivation of peroxidase enzyme activity.

However, the increasing temperature during pasteurisation without dearaetion probably is causing some extent of enzymatic browning to occur which encourage the oxidation of phenols in pasteurised SPHJP. A low value of a* value in the unpasteurised sample also reflected a negative a* values (-a*), indicated that the sample's original green colour is preserved. Pasteurisation also had a significantly increased of yellowness value in SPHJP ($p<0.05$).

Pasteurisation is a heat treatment that can inactivate the peroxidase enzyme, but still can cause discoloration in food and vegetable (Peng et al., 2017). It can be visually seen that pasteurised sample was brownish while the unpasteurised sample maintained a dark green colour (Figure 4.1). This may be due to common changes of chlorophyll to pheophytins when exposed to heat treatment and oxidation. This could be reflected by the lower total phenolic content of pasteurised SPHJP compared to the unpasteurised SPHJP (Table 4.6). However, research has also shown that the antioxidant activity by DPPH and FRAP between pasteurised and unpasteurised SPHJP shows no significant differences (Table 4.6).

Table 4.3: Water Solubility Index (WSI) of unpasteurised and pasteurised Sweet Potato Haulm Juice Powder (SPHJP)

SPHJP	Water Solubility Index (g/100 g dw)	
	Warm	**Cold**
Unpasteurised	23.52 + 1.23^{Aa}	28.70 + 0.92^{Ba}
Pasteurised	24.30 + 2.83^{Ab}	31.36 + 1.75^{Bb}

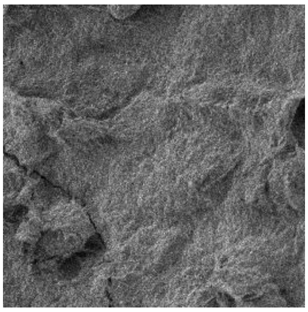
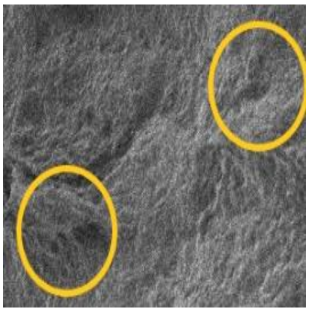
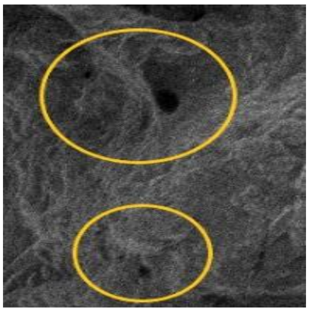
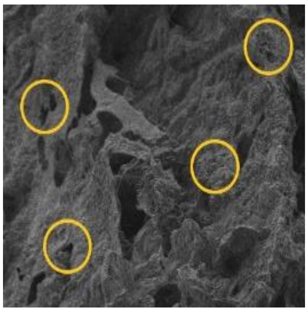
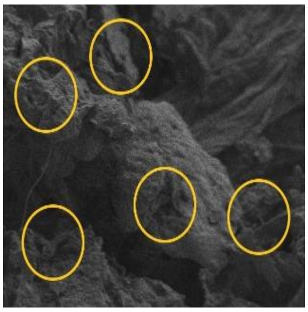
Cold water solubility (no incubation, centrifuged at 10,000 rpm at 4˚C); warm water solubility (incubation at 37˚C for 30 min, centrifuged at 10,000 rpm at 4˚C)
SPHJP: Sweet potato haulm juice powder, dw: dry weight
Values with similar small letters within columns are not significantly different (Tukey's test, $p<0.05$)
Values with similar capital letters across columns are not significantly different (Tukey's test, $p<0.05$)
All the samples were analysed in triplicate

Table 4.3 showed the warm and cold Water Solubility Index (WSI) of unpasteurised and pasteurised Sweet Potato Haulm Juice Powder (SPHJP). For both warm and cold WSI, pasteurised SPHJP was more soluble compared to unpasteurised SPHJP significantly ($p<0.05$).

This might be due to the presence of a higher amount of natural sugars (11.5 ˚Brix) in pasteurised samples that might help the solubility (Syamila, 2019). Carbohydrate is a hydrophilic substance consisting of simple sugars such as glucose and sucrose.

The solubility percentage for unpasteurised and pasteurised samples were higher than vacuum freeze-dried (5.71 g/100 g dw), hot air-dried (4.29 g/100 g dw), and microwave-vacuum dried (7.14 g/100 g dw) sweet potato leaves (Sui et al., 2019). SPHJP also had higher solubility index (g/100 g dw) in cold and warm water than purple potato flours which were 5.8 to 20.0 g/100 g dw (Qiu et al., 2019).

Table 4.4: Porosity of unpasteurised and pasteurised Sweet Potato Haulm Juice Powder (SPHJP)

SPHJP	**Magnification**		
	500x	**1000x**	**3000x**
Unpasteurised			
Pasteurised			

Yellow circle: porous structure of powder

Table 4.4 showed the porosity of unpasteurised and pasteurised Sweet Potato Haulm Juice Powder (SPHJP) obtained from Scanning Electron Microscopy (SEM) images. Based on Table 4.4, pasteurised SPHJP was more porous (marked as yellow circle) in physical morphology than unpasteurised SPHJP. At 500x magnification, no or too small porosity could be seen in unpasteurised SPHJP, and contrarily for pasteurised SPHJP. The scanned images are consistent with the relation to the WSI, where pasteurised SPHJP was more soluble in water than unpasteurised SPHJP (Table 4.3). The microstructure of the powder is important in determining the solubility of the substances, where a large surface area due to the porosity gave an advantage in solubility.

Proximate analysis is the quantitative analysis of macronutrients in food where it consists of the mass percentage of moisture, ash, fat, protein, and carbohydrate. In this study, proximate analysis was done on the unpasteurised, pasteurised sweet potato haulm juice powder (SPHJP). Table 4.5 shows the nutritional composition of unpasteurised and pasteurised sweet potato haulm juice powder.

Table 4.5: Nutritional Composition of unpasteurised and pasteurised Sweet Potato Haulm Juice Powder (SPHJP)

SPHJP	**Nutritional Composition (g/100 g dw)**					
	Carbohydrate	Protein	Ash	Moisture	Fibre	Fat
Unpasteurised	42.18 ± 0.70^{a}	35.23 ± 0.28^{a}	10.57 ± 0.02^{a}	9.59 ± 0.52^{a}	8.44 ± 0.41^{a}	2.42 ± 0.27^{a}
Pasteurised	39.33 ± 0.42^{b}	35.26 ± 0.47^{a}	13.24 ± 0.10^{b}	9.43 ± 0.18^{a}	7.72 ± 1.88^{a}	2.68 ± 0.06^{a}

SPHJP: Sweet potato haulm juice powder, dw: dry weight basis
Values with similar letters within columns are not significantly different (Tukey's test, $p<0.05$)
All the samples were analysed in triplicate

In this research, the carbohydrate content of the unpasteurised and pasteurised SPHJP samples is significantly different to each other ($p<0.05$) (Table 4.5). The reduction of the carbohydrate content in the pasteurised SPHJP results might be due to the thermal processes that degraded the starch of the haulm. The polysaccharides in pasteurised SPHJP had reduced due to the conversion of this polysaccharide (starch) into smaller constituents such as monosaccharides and disaccharides (sucrose, glucose, and fructose), which resulted in higher total soluble solid (TSS) (11.5 ˚Brix) (Table 4.1).

Meanwhile, unpasteurised SPHJP is expected to have larger number of carbohydrate content (polysaccharides), due to the unconverted starch into simple sugars yet as there is no thermal treatment applied, resulting in lower TSS value (Kong et al., 2020).

However, it should be noted that the total soluble solid (TSS) showed in Table 4.1, is different from the carbohydrate content in Table 4.5. This is because TSS only quantifies water-soluble components (a segment of carbohydrates) in the juice, meanwhile proximate analysis for carbohydrate content covers all types of carbohydrates including insoluble and soluble saccharides, fibre, or sugars as in this study, the carbohydrate is calculated only by difference of 100 with other macronutrients. In future, to improve the findings on SPHJP, the derivatisation of carbohydrate should be assessed using methods such as enzymatic methods, chromatographic and electrophoretic methods or physical methods such as refractive index (Cui & Brummer, 2005; Niaz et al., 2020).

The ash content in pasteurised SPHJ was significantly higher than unpasteurised SPHJP (Table 4.5) (p<0.05). It has been suggested that minerals such as zinc and iron were very stable under low heat conditions. However, low volatility minerals may contribute to high ash content upon exposure to heat during ashing or drying (Siti Mahirah et al., 2018; Morris et al., 2004). Higher concentrations of ash and mineral (iron, calcium, and potassium) in boiled cassava leaves than non-processed leaves were reported (Achidi et al., 2005). The loss of moisture and other nutrients such as fibre might also contribute to the higher ash concentration in pasteurised SPHJP than in unpasteurised SPHJP. Other than that, unpasteurised haulm samples in this study have a lower ash percentage than the unpasteurised sweet potato leaves (Ethiopia) (Awol, 2014), primarily due to the collective analysis on the stalk, stem, and leaves of the haulm.

An increase in certain nutrients after cooking could be demonstrated by water reduction, explaining the inverse relationship between moisture and other nutrients (Ersoy & Özeren, 2009). Thermal treatments such as boiling, steaming, dry roasting, and microwaving have increased cassava leaves' crude fat content (Ekpo & Baridia, 2020). In our study, the difference between the protein, fiber and fat content of unpasteurised and pasteurised SPHJP was insignificant (Table 4.5).

There was no significant difference in the protein percentage in the unpasteurised and pasteurised SPHJP, suggesting that heat treatment exposed to the pasteurised sample (85˚C, 5 min) did not change the protein content in SPHJP ($p>0.05$). Chirwa-moonga (2020) supported this finding, which reported that crude protein in purple sweet potato leaves was not affected by steaming (95˚C, 10-15 min). A high amount of protein was detected mainly in the sweet potato and cassava leaves (21.85 - 24.53 g/100 g dw) with essential amino acids such as glutamate, leucine, aspartate, and lysine (Iyaka et al., 2015), but the amino acids content of SPHJP has not yet been determined. It was suggested that thermal blanching might cause the leaching of water-soluble protein from the sample into the surrounding water (Xiou et al., 2017; Lee, 1958). In our study, the macronutrients of the juices were contained within the close-jacketed pasteuriser and dried using the freeze-drying method. Therefore, protein loss due to leaching has been successfully avoided.

Mechanical abruption and juicing are crucial pre-processing steps to release chloroplast organelles from their cell wall (Torcello-Gómez et al., 2019). There is a 0.72 g/100 g dw difference in the fibre content of the SPHJP whereby 8.44 g/100 g dw of unpasteurised SPHJP consisted of fibre as compared to pasteurised SPHJP, which had 7.72 g/100 g dw of fibre. It has been suggested that the modification of total dietary, soluble, and insoluble fibre are highly dependent on processing temperatures. Heat treatments such as boiling and pressure cooking also increased soluble fibre in barley (Bader Ul Ain et al., 2019).

In the research reported by Ishida et al. (2000), leaves from sweet potato mainly contributed to the soluble fiber (5.94 - 6.90 g/100 g dw) while its stem made up of insoluble fiber (10.40 – 11.30 g/100 g dw). The insoluble fibre in the haulm may have turned into soluble fibre in the presence of heat (85˚C), which is demonstrated by the pasteurisation of Aloe vera fillet at 85˚C for 15 minutes that resulted in a lower cell wall polymer (0.268 mg/g dw) than the fresh fillet (0.345 mg/g dw) (Rodríguez-González et al., 2011). As the temperature increases, the rate of breaking glycosidic bonds in polysaccharides is also increased, contributing to the release of oligosaccharides (Yi et al., 2014). Soluble dietary fibre could provide health benefits by forming a gel and increasing gut health by slowing down digestion, delaying gastric emptying, preventing constipation, and creating a fullness sensation (Axelrod & Saps, 2018; Li & Komarek, 2017). Hence, further study must be conducted on determining the concentration of soluble and insoluble fibre content of SPHJP.

Both freeze-dried SPHJP in this study had slightly higher moisture content (9.43-9.59 g/100 dw) compared to freeze-dried basil leaves (7.99 g/100 g dw) (Siti Mahirah et al., 2018). Moisture content higher than 7% may suggest that the freeze-drying process applied in this study could be enhanced to further reduce the moisture content of SPHJP. Moreover, a hygroscopicity test is suggested to confirm the physical characteristic of SPHJP at room temperature.

Preventive actions such as pasteurisation should be applied to minimise or avoid biological hazards in food (FSMA, 2011). Pasteurisation on *Justicia secunda* leaves has maintained its nutrient retention and quality (Neba et al., 2020). On top of that, heat treatment at 85˚C can deactivate the plant's enzyme and preserve the quality of nutrients like carotenoids and galactolipids in peavine haulm powder (Wattanakul et al., 2020).

In our study, the heat treatment applied on the SPHJP is higher than 80˚C and has shown deactivation of peroxidase enzyme activity, which may provide a stable shelf-life and safer food consumption. It was proven that pasteurisation could reduce the microbial load on food and juice samples such as *Listeria monocytogenes* and other vegetative pathogens (Peng et al., 2017). Further study to analyse the microbial safety of the SPHJ should be done to understand the impact of pasteurisation on the sample.

In response to the current trend to go green-based, wholesome, and absolute plant-based products, the high percentage of carbohydrate (39.33 g/100 g dw), protein (35.23 g/100 g dw), and fibre (7.72 g/100 g dw), ash (10.57 g/100 g dw) in both the unpasteurised and pasteurised SPHJP could be the key to sustainable food nutrients and production. These nutrients could be converted or further fermented into novel sugar and a new source of plant protein. Furthermore, carbohydrates helped to avoid the oxidation of polyphenol (Wang et al., 2016), which is linked to the ability of leaves as antioxidants.

Leaves were found to scavenge free radicals better than the skin and flesh of sweet potatoes (Makori et al., 2020). The leaf extracts can be utilised in food products such as juice, ice-cream or pasta and functional food as it is directly associated with human health as anti-diabetic, anti-cancer, and improves cardioprotective effect (Alam, 2021). This showed that data and information of the haulm are needed to ensure that haulm can be fully utilised and provide a sustainable nutrient.

However, further study must be conducted to determine its mineral compounds, soluble and insoluble dietary fibre, and amino acids concentration in the SPHJP samples. Investigation on the antioxidant and anti-nutrient properties of the haulm should also be set up.

Table 4.6: Total Phenolic Content (TPC) and antioxidant activity of unpasteurised and pasteurised Sweet Potato Haulm Juice Powder (SPHJP)

SPHJP	Total Phenolic Acid (g GAE/100 g dw)	DPPH Scavenging Activity (%)	Ferric Reducing Antioxidant Power (FRAP) (µmol Fe (II)/g dw)
Unpasteurised	2.09 ± 0.09[a]	67.17 ± 3.90[a]	424.13 ± 12.47[a]
Pasteurised	1.45 ± 0.34[b]	67.64 ± 3.27[a]	441.50 ± 22.60[a]

SPHJP: Sweet potato haulm juice powder, GAE: Gallic Acid Equivalent,
Values with similar letters within columns are not significantly different (Tukey's test, $p<0.05$)
All the samples were analysed in triplicate

Table 4.6 showed Total Phenolic Content (TPC) and antioxidant activity of unpasteurised and pasteurised Sweet Potato Haulm Juice Powder (SPHJP). The changes of antioxidant's concentration between the unpasteurised (initial) sample and pasteurised sample indicates the effect of heat treatment upon the antioxidant activities. In our study, it was found that there was a significant difference ($p<0.05$) in the total phenolic content (TPC) of unpasteurised (2.09 g GAE/100 g dw) and pasteurised (1.45 g GAE/100 g dw) SPHJP ($p<0.05$) (Table 4.6). Pasteurisation caused a significant reduction in the TPC of SPHJP, probably due to the presence of heat. Pasteurisation had been found to be the factor to the lower TPC amount in pasteurised than the unpasteurised SPHJP.

This was in agreement with previous studies that polyphenols were thermolabile compounds, and prolonged heat treatment caused chemicals and properties' changes to the compound (Kalt, 2005; Zhou et al. 2016). Honey beverages also exhibited a significant reduction ($p<0.05$) in TPC values after pasteurisation (Norhayati et al., 2019). García-Alamilla et al. (2017) agreed that the decrease of TPC is related to the thermal and oxidative of the compounds. In addition, some phenolic compounds are low in molecular weight; thus, it become easily volatile at high temperatures (Djikeng et al., 2018).

Total phenolic content (TPC) of SPHJP were slightly lower than the total polyphenol content of unblanched (6.42 g CAE/100 g), uncut-blanched sweet potato leaf powder (4.91 g CAE/100 g) and cut-blanched sweet potato leaf powder (2.90 g CAE/100 g) (Luo et al., 2020). It was expected that blanching caused the leaching of micronutrients into the water (Wattanakul et al., 2020), contributing to the reduction of total polyphenol in the sweet potato leaf powder. It was confirmed that the cut leaf produced the least polyphenol content due to the more surface area exposed to the water. However, the losses due to the leaching of nutrients did not occur as we utilised jacketed pasteuriser for the pasteurisation process, explaining the sensitivity of antioxidants towards heat. In addition, Sun et al. (2014) stated that carbohydrates may provide a protective mechanism against polyphenols. This is because, in nature, polyphenols are in the glycosides form and bound to the sugar, discovering the interaction between carbohydrate and phenols (Mrduljaš et al., 2017; Zhang et al., 2014). Unpasteurised SPHJP had more carbohydrate content, which might be the factor to the higher TPC amount than the pasteurised SPHJP.

The sweet potato tops consisted of stem and leaf, contained greater TPC than the root and tuber itself (Islam et al., 2002). Studies on the total polyphenol content of orange-fleshed tubers of sweet potato resulted in a range of 0.946-0.1361 g GAE/100 g (Alam et al., 2016), was relatively low compared to the unpasteurised and pasteurised SPHJP.

A DPPH (1,1-diphenyl-2-picryl hydrazyl) is a compound composed of free-radical molecules. In the presence of antioxidants, the dark coloured of DPPH turned yellow due to the reduction of the stable DPPH radicals to diphenyl-pricrylhydrazine depending on the antioxidant capacity. The scavenging activity of unpasteurised and

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pasteurised SPHJP against DPPH did not significantly differ (p>0.05) (Table 4.6). Ali Ghasemzadeh (2012) studied that the DPPH scavenging activity of leaf from six varieties of sweet potatoes ranged from 32.8 to 62.12%, slightly lower than scavenging activity of SPHJP (67.17 – 67.64%) (Table 4.6)., and this might be attributed to the changes in cultivars, or different medium of growing sweet potato plants.

A FRAP assay determines the antioxidant's ability in a sample to reduce ferric ions (Xu et al., 2010). In this study, the FRAP value of unpasteurised (424.13 µmol Fe (II)/g dw) and pasteurised (441.50 µmol Fe (II)/g dw) SPHJP did not show any significant difference (p<0.05) (Table 4.6). However, both of these values were in line with freeze-dried sweet potato leaf extracts collected from Universiti Putra Malaysia, which were between 320.5 to 746.2 µmol Fe (II)/g DW (Ali Ghasemzadeh, 2012).

High correlation between TPC and FRAP activity of unpasteurised SPHJP (r= 0.91) and pasteurised SPHJP (r= 0.94). High correlation indicated that phenolic compounds in terms of its antioxidant activities, contributed most to the ion reducing ability of both SPHJP in this study (Wern et al., 2016). However, the scavenging activity and ferum reducing the capacity of pasteurised and unpasteurised SPHJP was not significantly different, possibly due to only mild heat treatment applied to the pasteurised SPHJP (85˚C). This suggested that pasteurisation (85˚C, 5 min) had significantly affected the phenolic content but insignificantly affected antioxidant activities.

The ability of SPHJP to scavenge DPPH as it contained many phenolic compounds such as 3,5-di-O-caffeoylquinic acid mainly, followed by 3-O-caffeoylquinic acid, 3,4-di-O-caffeoylquinic acid and Isoquercetin (Suárez et al., 2020). Moreover, the difference in the scavenging activity of sweet potato leaves from previous studies might be attributed to the changes in the cultivars (Luo et al., 2020). A FRAP activity in nectarine juice after pasteurisation 15 at 20 minutes at 80˚C also did not show any significant difference, showing the stability of antioxidants activity following a mild heat treatment (Laslo et al., 2017).

Each of the antioxidant analysis, like TPC, DPPH scavenging activity and FRAP activity has advantages and disadvantages due to its characteristic and mechanism of actions. This explains why different tests produce different data but reliable enough to give information on the products.

Moreover, these methods of determination actually depend on the condition of the sample, its particular characteristics and specific requirement. Total phenolic content (TPC) has many advantages such as produce highly reliable data, simple and robust method (Munteanu & Apetrei, 2021). In case of overestimation with Folin-Ciocalteau method, samples with low TPC, such as pasteurised SPHJP, might provide larger antioxidant activities, in such case due to the other methanol-soluble compounds (Belščak et al. 2009). Therefore, characterization of each phenolic compounds in SPHJP is encouraged to be studied, to rule out any non-phenolic reducing agents (organic acids and sugars).

DPPH scavenging activity and FRAP activity accounts antioxidant activities based on the electron transfer (Wern et al., 2016). For DPPH, which is a nitrogen radical, has a longer half-life compared to the peroxyl radicals. Antioxidants react very reactive towards peroxyl radicals, resulting in slow action on DPPH radical. Therefore, the determination of the scavenging activity might be inaccurate as the antioxidants are reacting slowly or inert to DPPH (Pokorná et al., 2015). Furthermore, DPPH is highly sensitive to light, therefore control of light exposure should be imposed strictly. Although DPPH activity is rapid and simple method, DPPH is only soluble in organic medium, thus it limits the activities of the hydrophilic antioxidants (Hidalgo & Almajano, 2017; Ulewicz-Magulska & Wesolowski, 2019).

Meanwhile, the sensitivity of FRAP activity can be seen on the acidity requirement where it needed to be conducted at pH<3.6, to keep the solubility of the iron, avoid it from precipitating, and form solids (Carlsen et al. 2010). Moreover, the advantage of low level pH is allowing a decrease in ionisation force and increase in redox reaction and drive electron transfer (Hegerman et al., 1998). Nevertheless, in this study, it is found best to be test the stability of the antioxidants in unpasteurised and pasteurised SPHJP in terms of TPC and FRAP activity.

Table 4.7: Anti-nutritional content of unpasteurised and pasteurised Sweet Potato Haulm Juice Powder (SPHJP)

SPHJP	Antinutrient Content	
	Oxalic Acid (mg/100 g dw)	Phytic Acid (g/100 g dw)
Unpasteurised	1038.66 ± 65.18[a]	0.04 ± 0.00[a]
Pasteurised	767.89 ± 63.45[a]	0.01 ± 0.00[b]

SPHJP: Sweet potato haulm juice powder, dw: dry weight basis
Values with similar letters within columns are not significantly different (Tukey's test, p<0.05)
All the samples were analysed in triplicate

Table 4.7 showed the anti-nutritional content of unpasteurised and pasteurised Sweet Potato Haulm Juice Powder (SPHJP). This study determined the oxalic acid composition in unpasteurised and pasteurised SPHJP.

Oxalic acid and oxalates exist in different amounts and levels in various plants such as leafy vegetables. In plants, these compounds act as a mechanism for self-defence against insects, pests, and other animals, also play a role in photoremediation of toxic soils caused by heavy metals (Prasad & Shivay, 2017). However, for humans, oxalic acid is one of the anti-nutrients that can distort with mineral's availability in our bodies. A high concentration of oxalates may cause the development of kidney stones. Therefore, any vegetables which are high in this compound should not be consumed in large amounts (Savage & Vanhanen, 2019).

This study found that unpasteurised SPHJP contained 1038.66 mg/100 g dw while pasteurised SPHJP contained 767.89 mg/100 g dw of oxalic acid (Table 4.7). Compared to the findings by Ooko Abong' et al. (2020), leaves from two varieties of Kenyan's orange-fleshed sweet potato which are *Kabode* and *Yellow sp* varieties consisted of 1369.09 and 1618.71 mg/100 g dw, showed a higher amount of oxalates than both SPHJP. In other studies, boiled fat hen leaves had a lower total oxalate (682.79 mg/100 g dw) than raw leaves (1112.40 mg/100 g dw). The amount of calcium bound to the insoluble oxalate is also higher in raw leaves than boiled leaves, suggesting that heat treatment might reduce the formation of calcium oxalate (Savage & Vanhanen, 2019).

Phytic acid is another anti-nutritional factor that may chelate with minerals and essential nutrients. Based on Table 4.7, a significant reduction was detected in phytic acid levels of unpasteurised SPHJP (0.04 mg/g dw) and pasteurised SPHJP (0.01 mg/g dw) (p<0.05). This showed that the pasteurisation process could enhance the reduction in phytic acid in the sweet potato leaves. Inyang Udousoro et al. (2013) stated that heat treatment at 90˚C decreased the phytate level (74.6%) in the curry leaf (*Ocimum canum sims* L.) while heating at 90˚C for 15 minutes significantly caused a loss in all anti-nutrients such as oxalate, phytate, tannin, and cyanides. In addition, obtained phytic acid values in both SPHJP in this study were lower than leaves of Kenyan's orange-fleshed sweet potato, which ranged from 1.14-5.33 g/100 g dw (Ooko Abong' et al., 2020).

In general, pasteurised SPHJP had higher mineral content and better water solubility index and consisted lower water activity, carbohydrate content, TPC and phytic acid amount (p<0.05) than unpasteurised SPHJP. However, pasteurisation did not significantly affect protein and fat content, antioxidant activities and oxalic acid (p>0.05).

It is suggested that sweet potato haulm should be pasteurised upon consumption due to the higher nutrients' availability as revealed.

Further study should be conducted on the effect of pasteurisation on the protein fraction, profiles of vitamins and mineral available in the SPHJP. In addition, the data on other physical characteristics of powder such as hygroscopicity and dispersibility. As for the antioxidant compounds, analysis on the carotenoids or flavonoids content, can be additional information regarding the benefits of SPHJP. However, anti-nutrients could become another threat to the existing beneficial nutrients, hence the content of other anti-nutrients such as tannins and cyanide, should also be determined.

4.3 Storage Stability of Unpasteurised and Pasteurised Sweet Potato Haulm Juice Powder (SPHJP) using Ferric Reducing Antioxidant Power (FRAP) Assay and Total Phenolic Content (TPC)

During storage, the deterioration of nutrient's quality may be contributed by certain factors such as temperature, light, oxygen, or water activity. Therefore, it is crucial to evaluate the effect of pasteurisation and light exposure during SPHJP's storage (180 days, 20˚C) on Ferric Reducing Antioxidant Power (FRAP) activity and Total Phenolic Content (TPC). Table 4.8 showed the FRAP Activity of unpasteurised and pasteurised SPHJP during storage under light and dark conditions.

Table 4.8 FRAP Activity of unpasteurised and pasteurised SPHJP during storage under light and dark conditions

SPHJP	Day	FRAP Activity (μmol Fe (II)/g dw)	
		Light	Dark
Unpasteurised	**0**	424.13 ± 12.47^{Aa}	
	14	407.72 ± 8.05^{Aa}	359.54 ± 16.70^{Bb}
	60	411.14 ± 17.03^{Aa}	360.96 ± 15.78^{Bb}
	120	364.99 ± 13.01^{Ab}	348.96 ± 15.99^{Ab}
	180	330.01 ± 14.10^{Ac}	349.00 ± 41.70^{Ab}
Pasteurised	**0**	441.50 ± 22.60^{Aa}	
	14	457.93 ± 14.13^{Aa}	392.40 ± 22.30^{Ba}
	60	356.13 ± 12.34^{Ab}	377.40 ± 22.20^{Aa}
	120	322.90 ± 20.10^{Ab}	400.59 ± 16.85^{Ba}
	180	314.00 ± 26.50^{Ab}	375.30 ± 32.10^{Aa}

Values with similar capital letters across columns are not significantly different (Tukey's test, $p<0.05$)
Values with similar small letters within columns (for each treatment's storage) are not significantly different (Tukey's test, $p<0.05$)
All the samples were analysed in triplicate

The FRAP activity of unpasteurised SPHJP under both conditions was significantly reduced after 6 months storage ($p<0.05$) (Table 4.8). In unpasteurised SPHJP there was 22.19% loss in FRAP activity under light conditions while 17.71% of loss under dark conditions ($p<0.05$).

It was also revealed that light affected the FRAP activity at the early storage period, which were day 14 (407.72 μmol Fe (II)/g dw) and day 60 (411.14 μmol Fe (II)/g dw) ($p<0.05$). However, on days 120 and 180, there was no significant difference in the FRAP activity regardless of light or dark conditions ($p>0.05$).

The highest reduction in FRAP value was noted (28.88%) ($p<0.05$) under light condition, while lowest reduction was under dark conditions (15.01%) ($p>0.05$) in pasteurised SPHJP. After 180 days (6 months) of storage, pasteurised SPHJP under dark conditions did not contain any significant reduction in the FRAP value, suggesting that

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the pasteurisation process and the absence of light when storing SPHJP could retain the FRAP mechanism. Heat treatment has been proved to improve physicochemical properties and enhance the activities of antioxidants due to the breakdown of antioxidant compounds in honey (Sulaiman & Sarbon, 2020).

Light exposure caused a loss in FRAP activity in both pasteurised and unpasteurised SPHJP. Similarly, FRAP value in chia microgreens also had a notable decrease of light-exposed microgreen than dark-grown microgreen due to the presence of light (Mlinarić et al., 2020). Ioannou et al. (2020) studied that the degradation of flavonoid compound, naringin, respectively reduced the antioxidant activity under light conditions after 11 days. Exposure to light had a negative effect on the antioxidant activity of flavonoids (Ioannou et al., 2020). Naringin had been found to be one of the phenolic compounds that present in the *Ipomoea batatas* L. leaf extracts (Zengin et al., 2017).

In general, the loss of FRAP activity in all storage conditions in 180 days demonstrated that the antioxidant compounds undergo an oxidation process. Specifically, major degradation of FRAP activity under light conditions for both SPHJP, probably due to the photo-oxidation of bioactive compounds such as phytochemicals (Lu & Zhao, 2017). The formation of products from the oxidation process could decrease the concentration and action phenols, consequently reducing antioxidant capacity. The highest depletion of FRAP activity in pasteurised SPHJP was expected to be encouraged by thermal oxidation, which occurs at high temperatures and produces free fatty acid (Perkins, 1992). Therefore, in this study, the pasteurised SPHJP under light conditions was more prone to photo-oxidation than unpasteurised SPHJP.

High standard deviation obtained in the FRAP values in Table 4.8, might be due to the human error happened when conducting laboratory work. However, all results obtained have reliable coefficient of variation (COV) which were less than 15%. Some of the improvement that can be done to reduce human errors when conducting FRAP assay is the ferrous sulphate standard solution should be stored in amber volumetric flask to ensure complete blockage of light as iron is easily oxidised and the solution could change from colourless to pale yellow. For the pH meter, it should be calibrated before each use to ensure accurate pH value obtained. This is because buffer should be maintained at pH 3.6 to ensure iron solubility and avoid its precipitation due to the low acidic environment.

Kinetic degradation can be used to predict the quality degradation and shelf life of a product during storage. Table 4.9 showed the kinetic degradation for FRAP activity of unpasteurised and pasteurised SPHJP during storage under light and dark condition.

Table 4.9: Kinetic degradation for FRAP Activity of unpasteurised and pasteurised SPHJP during storage under light and dark conditions

SPHJP		Zero Order			First-Order			Second-Order		
		k (day^{-1})	R^2	Half-life (day)	k (day^{-1})	R^2	Half-life (day)	k (day^{-1})	R^2	Half-life (day)
Unpasteurised	Light	0.5032	0.9360	107	0.0013	0.9336	533	4.0×10^{-6}	0.9294	10×10^{7}
	Dark	0.2847	0.4590	60	0.0007	0.4729	990	2.0×10^{-6}	0.4875	21×10^{7}
Pasteurised	Light	0.8182	0.8399	181	0.0022	0.8634	315	6.0×10^{-6}	0.886	73×10^{6}
	Dark	0.2159	0.3667	48	0.0005	0.3691	1386	1.0×10^{-6}	0.3713	44×10^{7}

The FRAP activity of unpasteurised SPHJP under light conditions followed a zero and first-order kinetic degradation with a R^2 value of 0.9360 and 0.9336, respectively (Table 4.9). Meanwhile, pasteurised SPHJP followed first and second-order reaction with a R^2 value of 0.8634 and 0.886, respectively. However, the determination of reaction order for the SPHJP stored under dark conditions cannot be concluded due to low R^2 values, which were less than 0.4875.

Low rate of constant in kinetic degradation explains that the product can retain FRAP activity during storage (Tavares et al., 2020). A lower reaction rate (first-order reaction) was found in unpasteurised SPHJP than pasteurised SPHJP, which was 0.0013 and 0.0022 day^{-1}, indicating that pasteurised SPHJP had a higher rate of FRAP activity's degradation ($p<0.05$) (Table 4.8). A high temperature might degrade bioactive compounds and lessen the antioxidant capacity of the substance (Zhou et al., 2016). This was in line with Table 4.8, where unpasteurised SPHJP had lower degradation (22.19%) in FRAP value compared to pasteurised SPHJP (28.88%) significantly under light condition ($p<0.05$).

Antioxidant compounds such as anthocyanin were thermosensitive compounds, and high-temperature process might increase the rate of degradation reaction (Su et al., 2019; Tavares et al., 2020). In addition, the half-life for pasteurised SPHJP in reducing 50% of the FRAP activity was 315 days, lower than unpasteurised SPHJP, which was 533 days. The degradation of the FRAP activity in the unpasteurised and pasteurised SPHJP followed a first-order reaction, based on the good R^2 values and relevant half-life discovered.

Phenolic compounds include simple phenols, phenolic acids and its derivatives, flavonoids, coumarins, tannins, lignin, and others, have the ability as antioxidants. (Blainski et al., 2013). Table 4.10 showed the Total Phenolic Content (TPC) of unpasteurised and pasteurised SPHJP during storage under light and dark conditions.

Table 4.10: Total Phenolic Content (TPC) of unpasteurised and pasteurised SPHJP during storage under light and dark conditions

SPHJP	Day	TPC (g GAE/100 g dw)	
		Light	Dark
Unpasteurised	**0**	2.09 ± 0.09^{Aa}	
	14	1.35 ± 0.42^{Aab}	1.69 ± 0.22^{Aa}
	60	1.19 ± 0.07^{Ab}	1.03 ± 0.11^{Ab}
	120	0.77 ± 0.43^{Ac}	0.61 ± 0.43^{Ab}
	180	0.48 ± 0.16^{Ac}	0.56 ± 0.09^{Ab}
Pasteurised	**0**	1.45 ± 0.34^{Aa}	
	14	1.27 ± 0.21^{Aab}	1.16 ± 0.09^{Aa}
	60	0.74 ± 0.34^{Aab}	0.92 ± 0.40^{Aa}
	120	0.57 ± 0.58^{Aab}	0.75 ± 0.54^{Aa}
	180	0.41 ± 0.15^{Ab}	0.53 ± 0.31^{Aa}

SPHJP: Sweet potato haulm juice powder
Values with similar capital letters across columns are not significantly different (Tukey's test, $p<0.05$)
Values with similar small letters within columns (for each treatment's storage) are not significantly different (Tukey's test, $p<0.05$)
All the samples were analysed in triplicate

Based on Table 4.10, there was a significant loss in TPC value of unpasteurised SPHJP under light and dark condition and pasteurised under light condition ($p<0.05$), but no significant change in TPC value for pasteurised SPHJP under the dark condition from day 0 until day 180 ($p>0.05$). This was in line with the findings from Table 4.8, where pasteurised SPHJP under dark condition did not exhibit any significant loss in FRAP value in 6 months of storage.

The highest losses of TPC were noted in unpasteurised SPHJP under light condition (76.87%), followed by unpasteurised SPHJP under dark conditions (73.13%), pasteurised SPHJP under light conditions (71.38%) and the lowest loss found in pasteurised SPHJP under dark conditions (63.72%).

TPC started to degrade on day 14 for unpasteurised and pasteurised SPHJP under light conditions ($p<0.05$). This explained that exposure to the light decreased the amount of TPC in the first two weeks. Similarly, TPC values in unpasteurised and pasteurised honey beverages degraded significantly ($p<0.05$) after 2 weeks (Norhayati et al., 2019). Further degradation occurred on day 120, where the TPC for unpasteurised SPHJP was 0.77 g GAE/100 g dw ($p<0.05$).

Another possible factor contributing to this might be the presence of peroxidase enzyme in unpasteurised SPHJP as significant loss ($p<0.05$) of TPC stopped at day 14 for pasteurised SPHJP under light condition. Enzymes such as peroxidase and polyphenol oxidase negatively affected TPC by oxidizing polyphenols into quinones, forming brown products (Hutabarat & Halbwirth, 2019; Walker & Ferrar, 1998). These enzymes would contribute to the phenolic degradation and further quality loss in products.

All samples except for pasteurised SPHJP under dark conditions possessed significant TPC losses ($p<0.05$) after 180 days. In fact, the pasteurised SPHJP under light conditions showed a similar observation with Noriham et al. (2012), where the pasteurisation (25˚C, 94 seconds) had significantly degraded TPC in winter melon puree after 180 days of storage.

Kinetic degradation of TPC also should be assessed to study the rate of reaction and the half-life of the polpyhenols compound in unpasteurised and pasteurised SPHJP during storage under light and dark conditions.

Table 4.11: Kinetic degradation for Total Phenolic Content (TPC) of unpasteurised and pasteurised SPHJP during storage under light and dark conditions

SPHJP		**Zero Order**			**First-Order**		
		k (day^{-1})	**R^2**	**Half-life (day)**	**k (day^{-1})**	**R^2**	**Half-life (day)**
Unpasteurised	**Light**	0.0075	0.8489	0.0078	0.0073	0.9528	95
	Dark	0.0083	0.8560	0.0087	0.0075	0.9269	92
Pasteurised	**Light**	0.0060	0.8872	0.0041	0.0069	0.9611	100
	Dark	0.0046	0.9185	0.0033	0.0051	0.9737	136

Table 4.11 showed the kinetic degradation for TPC of unpasteurised and pasteurised SPHJP during storage under light and dark condition. TPC degradation for SPHJP under both conditions had demonstrated to follow first-order reaction (Table 4.11). Similar results were obtained in *Bixa orellana* L. and *Criollo cocoa* beans, where the degradation rate of phenolic compounds followed first-order kinetic (Fernández-Romero et al. 2020; Zapata et al. 2021). The TPC of hardy kiwi puree at 25˚C acted on first-order kinetic degradation in 72 hours of storage (Kim et al., 2018).

Pasteurised SPHJP under dark conditions seemed to be fit the first-order kinetic degradation with the best R^2 = 0.9737, with the lowest rate of reaction, k of 0.0051 day^{-1} and most extended half-life of 136 days. This showed that TPC degraded slowest under dark conditions with the help of pasteurisation. Pasteurised SPHJP under light conditions had a higher reaction rate, 0.0069 day^{-1} and shorter half-life (100 days) than pasteurised SPHJP under dark conditions.

Unpasteurised SPHJP under dark conditions has the shortest half-life of 92 days and the highest rate of degradation, 0.0075 day^{-1}. Meanwhile, unpasteurised SPHJP under light conditions had k value of 0.0073 day^{-1} , and half-life of 95 days, not much different with the light-exposed unpasteurised SPHJP. This indicated that pasteurisation helped slow down the rate of phenolics content degradation and retain the highest shelf-life (136 days). The inactivation of peroxidase (through pasteurisation) that degraded phenolic compounds and its antioxidant activities, retain the ability of phenols to maintain shelf-life (Rabie et al., 2015; Odriozola-Serrano et al., 2008).

Another antioxidant compound existed in sweet potato leaf tips, carotenoids, in which its degradation also followed a first-order reaction model (Tang et al., 2021; Šeregelj et al., 2020). In the same study, the freeze-dried carotenoid's level from sweet potato peel extract was significantly degraded under light conditions. This might indicate that antioxidant compounds such as polyphenols and carotenoids were susceptible to degradation, especially at high temperatures, under light exposure, and prone to oxidation. However, the half-life of the carotenoid was shorter, which was 55 days under dark conditions and 15 days under light conditions (Šeregelj et al., 2020), compared to SPHJP.

In fact, it could be suggested that light exposure can fasten the rate of degradation of TPC in the SPHJP and other agriculture products. Nevertheless, pasteurisation and dark condition also may help the longer half-life of reducing 50% of TPC and low loss of nutritional content during storage. Further study should be conducted on the storage at chilled or lower temperature as results from the previous study showed that kinetic parameters of phenolic compounds, antioxidant activities and stability of nutrients are strongly dependant on storage temperature (Wattanakul et al., 2020; Jerry & Bright, 2019; Ali et al., 2018; Kim et al., 2018).