CHAPTER 5

EFFECT OF HEAT TREATMENTS ON THE PHYSICOCHEMICAL VARIATION OF CHLOROPLAST-RICH FRACTIONS (CRFs) FROM SWEET POTATO HAULM

5.1 Introduction

In Chapter 4, it was evident that the chloroplast-rich fraction (CRF) of sweet potato haulm (SPH) harvested from different crop borders could substantially vary in specific chemical properties depending on the proportion and maturity of plant parts. However, CRF's physical properties and antinutritional factors (oxalic and phytic acids) did not significantly differ between crop borders. Some factors associated with these findings involve agricultural land area, bed size and spacing, selection of disease-resistant varieties, usage of fertilisers and pesticides, storage, and preservation methods.

Implementation of heat treatments is established to avoid physicochemical quality degradation with the action of native enzymatic activity and as well as to reduce the concentration of antinutrients naturally found in plants. Thermal treatment: blanching and pasteurisation are the most practical post-harvest treatments in inhibiting enzyme reactions, extending nutrient shelf life, stabilising texture, and extending the texture and flavour of plant materials (Wattanakul et al., 2019, 2021). Blanching before drying is a crucial step to remove pesticide residues and enhance the drying process, besides inactivating polyphenol oxidase and peroxidase activities which are responsible for the browning effect (Luo et al., 2020).

In addition, processing methods such as boiling, frying, and fermentation on sweet potato leaves reduced the antinutrients to different extents (Abong' et al., 2021). This chapter aims to discuss the findings on the second objective, which is the effect of heat treatments (refer to Table 3.1) on the physicochemical variation of chloroplast-rich fractions (CRFs) from sweet potato haulm. The nutritional, antinutritional, and physical properties of heat-treated CRFs were analysed. The CRF without any heat treatments (fresh) was used as a control and labelled as Fresh (F) in this chapter. The value for fresh CRF was retrieved from the mean values of the three crop borders studied in the previous chapter – Chapter 4.

Results and Discussion

5.2 Yield of sweet potato haulm juice and CRF powder

All heat-treated (treatments of conventional pasteurisation, steam pasteurisation, and water blanching) sweet potato haulm (SPH) juices recorded similar yields ranging between 0.63 – 0.68 ml per gram of wet biomass. In comparison to the fresh juice that was previously stated in Chapter 4 (Section 4.2), the mean value of the fresh juice (0.81 ml per gram of wet biomass) was higher. This was probably due to the loss of some wet biomass during the heat treatment process. Besides, based on observation by the naked eye, the heat-treated juices were more viscous compared to fresh juices, resulting in a lower yield of juice. The yield of chloroplast-rich fraction (CRF) powder from SPH decreased drastically after heat treatments where the yield of fresh CRF was 41.6 kg/g wet biomass (mentioned in Section 4.2), caused by the leaching of soluble solids.

Conventional pasteurised CRF had the lowest yield (mean value of 6.93 g/kg wet biomass) compared to steam pasteurised and water blanched CRFs (mean value of 15.54 and 12.24 kg/g wet biomass, respectively). The heating and juicing steps were varied between the heat treatments. In the conventional pasteurisation process, the haulm was juiced before being heated. In contrast, the haulm was heated first before being juiced in steam pasteurisation and water blanching treatment. Juicing of heat-treated haulm might further assist the release of chloroplasts from its cell wall, resulting in more amount of CRF collected.

5.3 Total soluble solids (TSS) of SPH juice, total solid content (TSC), and moisture content of CRF

The total soluble solids (TSS) of SPH juice, total solid content (TSC) and moisture content of CRF are shown in Table 5.1.

Table 5.1: Effect of heat treatments on total soluble solids of SPH juice, total solid content, and moisture content of CRF

	F	**CP**	**SP**	**WB**
Total soluble solids (°Brix)	5.77 ± 0.40^{a}	4.10 ± 0.10^{b}	2.60 ± 0.27^{c}	2.17 ± 0.06^{c}
Total solid content (g/100 g fw)	15.41 ± 1.06^{a}	10.48 ± 1.01^{b}	8.17 ± 0.97^{b}	8.47 ± 1.37^{b}
Moisture content (g/100 g fw)	84.59 ± 1.06^{b}	89.52 ± 1.01^{a}	91.83 ± 0.97^{a}	91.53 ± 1.37^{a}

Different letters mean significant differences (Tukey's test, $p < 0.05$) between values. Data are means ± SD (n = 3). F, CP, SP, and WB mean the CRF samples were treated with different heat treatments: F (fresh, no heat treatment), CP (conventional pasteurisation), SP (steam pasteurisation), and WB (water blanching).

The total soluble solids (TSS) were analysed on the fresh (F) and heat-treated juice of haulm (before the centrifugation and freeze-drying process). Meanwhile, the total solid content (TSC) and moisture content were measured on the fresh (F) and heat-treated chloroplast-rich fractions (CRFs), obtained after the centrifugation and freeze-drying process. From Table 5.1, all heat treatments significantly reduced the TSS (around 2° to 4 °Brix) in the haulm juice and TSC (around 5 to 7 g/100 g fw) in the CRF ($p < 0.05$). The losses were probably due to the breakdown of the food matrix and the release of low molecular weight compounds during the heat treatments (Arias-Rico et al., 2020). Hence, food matrix with low density might be separated or removed during the isolation of the CRF material.

The heat-treated CRFs had significantly higher moisture content compared to the fresh, but no significant difference was found between the heat treatments. The increase of moisture content in the heat-treated CRFs (around 5 to 7 %) could be owing to the water absorbed or trapped during the thermal processing. Overall, the heat treatments significantly affect the total soluble solids of the haulm juice, total solid content, and moisture content of the CRFs.

5.4 Physical properties

5.4.1 Colours

The colour difference between the chloroplast-rich fractions (CRFs) of sweet potato haulm (SPH) after heat treatments was measured using a Hunter Lab colourimeter, expressed by three components: *L** for the lightness of the colour (0 = black, 100 = white), *a** for greenness to the redness of the colour (+*a* = red, -*a* = green), and *b** for blueness to the yellowness of the colour (+*b* = blue, -*b* = yellow). Table 5.2 shows the effect of heat treatments on colour parameters and the total colour difference of CRF powder.

Table 5.2: Effect of heat treatments on colour parameters and total colour difference of CRF powder

Colour properties	**CRF with different heat treatments**			
	F	**CP**	**SP**	**WB**
L*	38.18 ± 0.55^{a}	32.42 ± 0.14^{c}	34.18 ± 0.50^{b}	29.69 ± 0.43^{d}
a*	-4.12 ± 0.88^{d}	7.09 ± 0.15^{a}	5.25 ± 0.02^{b}	1.67 ± 0.12^{c}
b*	25.12 ± 1.82^{a}	23.13 ± 0.54^{b}	22.11 ± 0.30^{b}	17.60 ± 0.12^{c}
ΔE*	-	13.38 ± 0.21^{a}	11.43 ± 0.43^{b}	13.55 ± 0.21^{a}
Sample colour				

Different letters mean significant differences (Tukey's test, $p < 0.05$) between values. Data are means ± SD (n = 5). F, CP, SP, and WB mean the CRF samples were treated with different heat treatments: F (fresh, no heat treatment), CP (conventional pasteurisation), SP (steam pasteurisation), and WB (water blanching). *ΔE** is determined by comparing the value from each heat-treated sample with fresh (F) sample.

The difference in colour (ΔE) between the CRF powders was applied by the following ranges: (ΔE): 0 – 1 - invisible difference, 1 – 2 - slight difference, 2 – 3.5 - noticeable difference, 3.5 – 5 - clear difference, > 5 - high difference (Mokrzycki & Tatol, 2011). $L*$ and $b*$ values significantly decreased in all heat-treated CRFs by around 2 to 8 degrees, which indicates that the heat treatments reduce the lightness of CRF powder. Conversely, all treatments showed a significant increase of $-a*$ value, causing the powder to become less green in colour. The degradation of the green colour was due to the conversion of chlorophyll structure to pheophytin, which results in green olive colour (Arias-Rico et al., 2020).

However, the water blanching treatment had the least colour changes for greenness ($-a*$) compared to the other two heat treatments. Previous studies reported that short-time blanching could give better colour retention (Raja et al., 2019) and could partially prevent chlorophyll degradation in comparison to steaming (Miglio et al., 2007). Therefore, heat treatment will affect the colour intensity of CRF powder caused by the high temperature applied during the process. Water blanching demonstrates the best heat treatment for preserving the natural green colour of CRF from SPH.

5.4.2 Water activity, bulk density and dispersibility

Water activity is an important food property in determining its safety and stability with regards to the microbial growth and rate of deteriorative reaction by determining the lower limit of available water for microorganisms to grow. F, CP, SP, and WB mean the CRF samples were treated with different heat treatments: F (fresh, no heat treatment), CP (conventional pasteurisation), SP (steam pasteurisation), and WB (water blanching).

The water activity and bulk density of chloroplast-rich fraction (CRF) from sweet potato haulm (SPH) are demonstrated in Figure 5.1 (a). No significant difference was found in the water activity of CRFs after heat treatment ($p < 0.05$). CRF from CP treatment had a slight decrease in water activity (0.33 a_w) while CRF from SP and WB had no changes (0.36 a_w) after heat treatment. A previous study reported a lower water activity in blanched (0.40 a_w) compared to fresh (0.49 a_w) *Carica papaya* L. leaf powder (Raja et al., 2019), resulting from the softened texture of blanched leaf which promotes water removal. Low water activity is favourable, as it indicates a decrease in water availability for microbial growth.

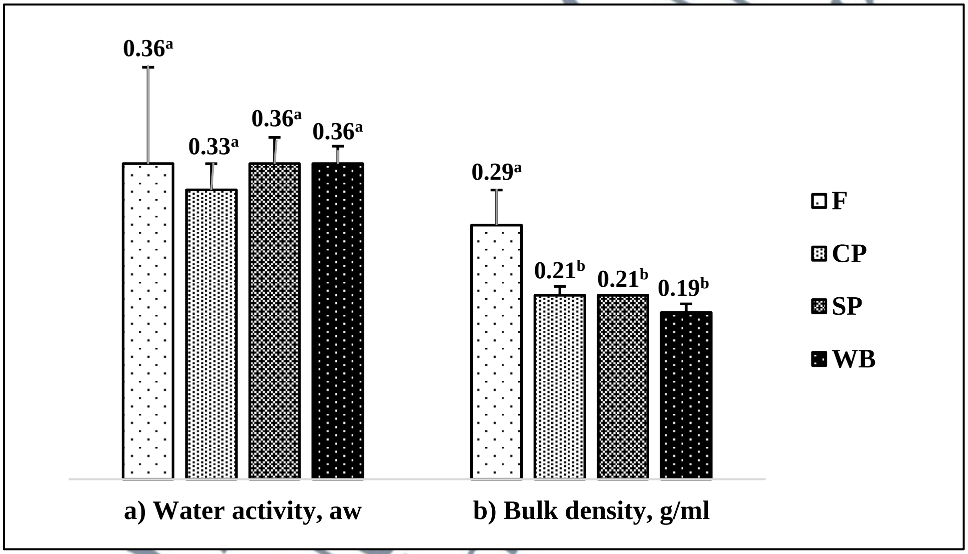


Figure 5.1: Water activity and bulk density of heat-treated CRFs. Results expressed with different letters mean significant difference (Tukey's test, $p < 0.05$)

Based on Figure 5.1 (b), a significant decrease was recorded for the bulk density of heat-treated CRFs (between 0.08 to 0.10 g/ml difference from fresh). Similarly, Raja et al. (2019) reported a lower bulk density in blanched (0.47 g/ml) compared to fresh (0.53 g/ml) *Carica papaya* L. leaf powder. The heat treatments resulted in a significant increase of bulk density for blanching (0.71 g/ml) while no significant difference was found for microwave heating (0.67 g/ml) in comparison to raw (0.69 g/ml) cabbage powder (Waseem et al., 2022). No significant difference in the bulk density of CRFs found between the heat treatments proposed in our study ($p > 0.05$), which explains that the treatments had a similar impact range on the CRF powder. The bulk density of powder can be influenced by the amount of air entrapped in the particles and particle internal porosity that can change during thermal processing. Lower porosity and moisture content will result in greater bulk density of powder (Raja et al., 2019).

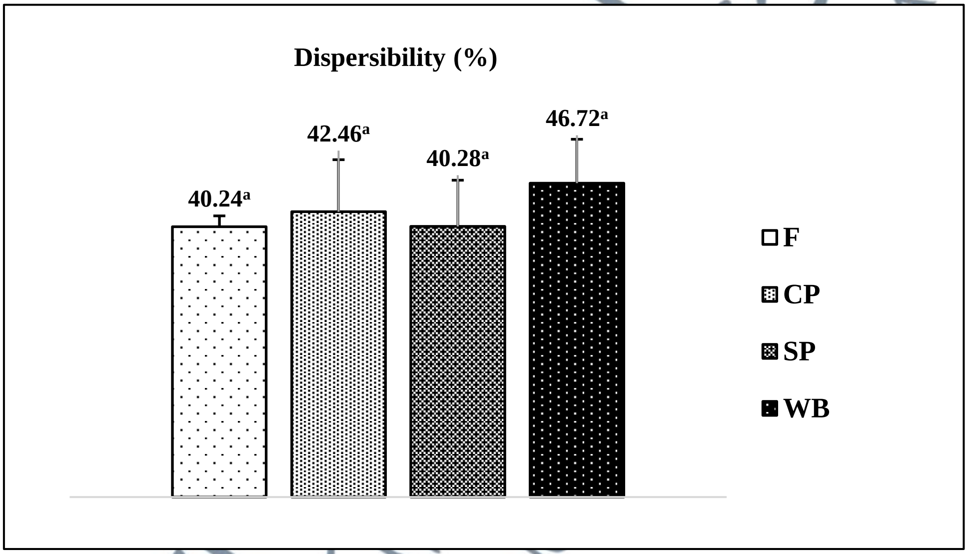


Figure 5.2: Dispersibility of heat-treated CRFs. Results expressed with different letters mean significant difference (Tukey's test, $p < 0.05$)

Dispersibility of powder was higher for all heat-treated CRFs compared to fresh with values increasing from around 2 to 6 % (Figure 5.2), but the difference was not significant ($p > 0.05$). Wattanakul et al. (2022) found that CRF from fresh juice was the least well-dispersed compared to the heat-treated pea vine haulm (HTPVH) and heat-treated juice (HJ) of pea vine haulm (based on qualitative observation). Hence, the authors assumed that heat treatment of the biomass or juice appears to reduce the robust physical nature of CRF material, which eventually results in well-dispersed particles of the CRF (Wattanakul et al., 2022). Although the heat treatment process for HTPVH and HJ was similar to our study (SP and CP, respectively), the type of biomass could also influence the dispersibility of CRF. This indicates that the thermal processing had little to no effect on the water activity, bulk density, and dispersibility of the CRF powder from SPH.

5.4.3 Water solubility index

Previously in Chapter 4 (Section 4.4.3), the solubility of chloroplast-rich fraction (CRF) powder was determined for both cold and warm water solubility index (WSI). No significant difference in solubility was found between the two different temperature methods. F, CP, SP, and WB mean the CRF samples were treated with different heat treatments: F (fresh, no heat treatment), CP (conventional pasteurisation), SP (steam pasteurisation), and WB (water blanching). Hence, the solubility of fresh (F) and heat-treated CRFs (CP, SP, and WB) was studied in this chapter (Table 5.3).

Table 5.3: Effect of heat treatments on water solubility index of CRF

CRF with different heat treatments	**Water solubility index (%)**	
	Cold	**Warm**
a) Fresh (F)	$20.61 \pm 4.99^{b, A}$	$21.43 \pm 6.06^{a, A}$
b) Conventional pasteurisation (CP)	$33.64 \pm 1.56^{a, A}$	$27.80 \pm 1.44^{a, B}$
c) Steam pasteurisation (SP)	$27.30 \pm 0.48^{ab, A}$	$24.65 \pm 0.53^{a, B}$
d) Water blanching (WB)	$24.80 \pm 0.36^{b, A}$	$21.60 \pm 1.96^{a, B}$

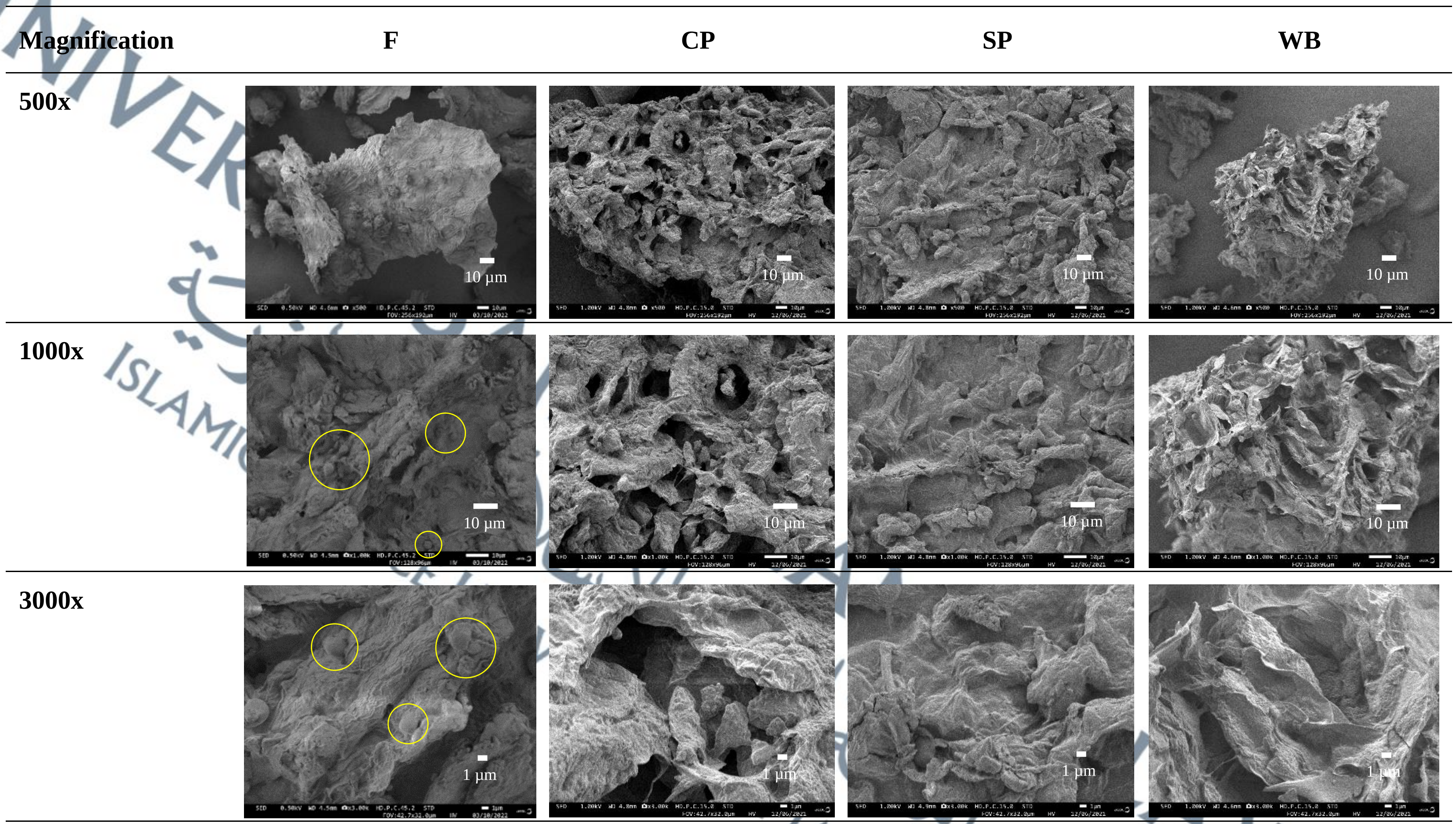
Cold water solubility (no incubation, centrifuged at 10 000 rpm at 4 °C for 15 min); warm water solubility (incubation at 38 °C for 30 min, centrifuged at 10 000 rpm at 4 °C for 15 min). Different lower letters within columns and different upper letters across the columns mean significant differences (Tukey's test, $p < 0.05$) between values. Data are means ± SD (n = 3).

Heat treatments improved the solubility of CRFs in both cold and warm methods with CRF from CP showing the highest increment among the heat treatments. It is assumed that the heat treatments modify the chloroplast structure by inducing porosity (based on SEM images, see Section 5.4.4 – Figure 5.3) which subsequently improved the solubility. In comparison between WSI methods, all heat-treated CRFs reported significantly higher cold WSI (ranging from 24 to 33 %) compared to warm WSI (ranging from 21 to 27 %) indicating that higher temperature condition did not influence the solubility of CRF powder. Even so, no significant difference was reported for the solubility of fresh CRF between the WSI methods ($p > 0.05$).

The solubility of CRF from CP was slightly higher than the pasteurised sweet potato haulm juice powder reported by Hanifah (2022b) for both cold (33. 64 and 31.36 %, respectively) and warm WSI (27.80 and 24.30 %, respectively). Previous studies reported that the presence of higher amounts of natural sugars might help solubility (Hanifah, 2022b; Syamila, 2019). Still, there was no direct correlation between total soluble solids – see Section 5.3, Table 5.1, and the solubility of CRF. As particle size may influence solubility, fractionation of CRF powder using different particle size ranges is suggested for future works.

5.4.4 Morphology

Thermal treatment of raw food during food processing could affect the cell structure as well as its nutritional composition. Figure 5.3 demonstrates the scanning electron microscopy (SEM) images of chloroplast-rich fraction (CRF) from sweet potato haulm (SPH) with different heat treatments: CP (conventional pasteurisation), SP (steam pasteurisation), and WB (water blanching).



Note: F, CP, SP, and WB mean the CRF samples were treated with different heat treatments: F (fresh, no heat treatment), CP (conventional pasteurisation), SP (steam pasteurisation), and WB (water blanching). Yellow circle: intact chloroplast.

Figure 5.3: The SEM images at different magnifications on a CRF from SPH with different heat treatments

The fresh CRF with no heat treatment (F) had a relatively smooth and homogenous surface while heat-treated CRFs (CP, SP and WB treatments) had a rough and rugged surface. Heat treatment causes damage to the organised structure of the cell wall, resulting in more tightly packed and more amorphous extracellular material being formed (Arias-Rico et al., 2020; Jiang et al., 2022). In addition, some glycosidic linkages could be broken, and the dietary fibre polysaccharides of the plant cell wall may be polymerised forming protein fibre complexes (Arias-Rico et al., 2020).

No intact chloroplast (yellow circle) was shown in all heat-treated CRFs, suggesting that the chloroplast had a cell wall breakage, releasing the thylakoids from the membrane. At 500 and 1000x magnifications, a porous structure was observed in the CRF treated with CP and WB. In addition, a larger pore size with the formation of cavity structure was noticeable in the CRF from CP (3000x magnification). The structure of CRF from SP was likely stuck together, forming a dense scale-like structure.

It is assumed that porosity is related to the solubility of powder. Therefore, the solubility of CRF treated with CP (33 %) was significantly higher than fresh (20 %), SP (27 %) and WB (24 %) – see Section 5.4.3, Table 5.3. A previous study exhibited a significant alteration in the microstructure of brown seaweed (*Undaria pinnatifida*) after blanching, steaming, boiling, and baking processes (Jiang et al., 2022). Heat treatments could modify the microstructure of CRF, and CP treatment is capable of promoting porosity and amorphous structure in the CRF from SPH.

5.5 Chemical properties

5.5.1 Proximate composition and chlorophyll content

The proximate composition of fresh and heat-treated chloroplast-rich fractions (CRFs) from sweet potato haulm (SPH) was demonstrated in Table 5.4. The crude protein and crude fat contents of CRF significantly reduced after steam pasteurisation (SP) treatment ($p < 0.05$). The decrease in protein content was comparable to the study conducted by Chirwa-Moonga et al. (2020) where the crude protein content of green sweet potato leaves was reduced significantly after steaming for 10 and 15 min.

Table 5.4: Effect of heat treatments on proximate composition and chlorophyll content of CRF

Proximate composition (g/100 g dw)	**CRF with different heat treatments**			
	F	**CP**	**SP**	**WB**
Crude protein	35.47 ± 0.88^{a}	35.00 ± 0.49^{a}	32.08 ± 0.03^{b}	35.29 ± 0.29^{a}
Crude fibre	5.68 ± 0.73^{a}	6.31 ± 1.24^{a}	7.09 ± 0.60^{a}	7.71 ± 0.94^{a}
Crude fat	2.68 ± 0.25^{b}	2.68 ± 0.26^{b}	1.99 ± 0.17^{c}	3.85 ± 0.04^{a}
Ash	13.27 ± 1.05^{a}	13.67 ± 0.16^{a}	13.41 ± 0.07^{a}	12.82 ± 0.11^{a}
Moisture	10.45 ± 1.45^{a}	9.61 ± 0.27^{ab}	9.36 ± 0.77^{ab}	8.10 ± 0.24^{b}
Carbohydrate	38.13 ± 1.37^{b}	39.04 ± 0.51^{b}	43.16 ± 0.54^{a}	39.93 ± 0.53^{b}
Pigment concentration (mg/g dw)				
Chlorophyll *a*	4.11 ± 1.05^{a}	1.45 ± 0.01^{b}	1.50 ± 0.01^{b}	2.68 ± 0.05^{b}
Chlorophyll *b*	2.28 ± 0.52^{a}	0.45 ± 0.03^{c}	0.58 ± 0.07^{c}	1.36 ± 0.03^{b}
Total chlorophylls	6.40 ± 1.57^{a}	1.90 ± 0.03^{c}	2.08 ± 0.07^{bc}	4.04 ± 0.06^{b}
Pigment ratios				
Chlorophyll *a/b*	1.80 ± 0.10^{c}	3.26 ± 0.23^{a}	2.59 ± 0.31^{b}	1.97 ± 0.06^{c}

Different letters mean significant differences (Tukey's test, $p < 0.05$) between values. Data are means ± SD (n = 3). F, CP, SP, and WB mean the CRF samples were treated with different heat treatments: F (fresh, no heat treatment), CP (conventional pasteurisation), SP (steam pasteurisation), and WB (water blanching).

Conversely, crude protein content in sweet potato leaves was increased significantly after boiling, steaming, microwaving, and baking processes whereas crude fat content cooked by the aforementioned process was reduced significantly (Sun et al., 2014a). Comparable protein content was found in our conventional pasteurised (35.0 g/100 g dw) and water blanched (35.29 g/100 g dw) CRF to both unpasteurised (35.23 g/100 g dw) and pasteurised (35.26 g/100 g dw) sweet potato haulm juice reported by Hanifah et al. (2022a).

Both blanching and microwave heating processing showed a considerable loss of crude protein, crude fat, and crude dietary fibre contents in cabbage powder (Waseem et al., 2022). Volatile and water-soluble fatty acids could be partially lost or destroyed during the steaming, boiling, and microwaving treatments, causing a decline in the fat amount of sweet potato leaves (Sun et al., 2014a). The crude fat content was significantly increased ($p < 0.05$) in water blanched CRF, comparable with the steam blanched *Moringa* leaves reported by Wickramasinghe et al. (2020). A significant reduction in moisture content was observed in water blanched CRF, which could be due to the leaching of soluble constituents into the water during the blanching process.

However, the contents of crude fibre and ash in CRF were not affected significantly ($p > 0.05$) by the heat treatments. Although steaming and microwaving treatments could induce a large amount of cytochylema to flow out, creating a loss of water-soluble fibre in sweet potato leaves (Sun et al., 2014a), no extensive effect on crude fibre content was observed in our study. A substantial increase in carbohydrate content was recorded in steam pasteurised CRF. The possible reason was the loss of crude protein and crude fat content, resulting in the relative increase of starch in CRF. Compared to the heat treatments conducted in this study, steam pasteurisation (SP) appeared to exhibit an impact on the crude protein, crude fat, and carbohydrate contents of CRF. Conversely, conventional pasteurisation (CP) and water blanching (WB) treatments succeeded in retaining almost every proximate composition in the CRF.

From Table 5.4, the results show that all heat treatments caused a significant reduction in the pigment concentration of CRF ($p < 0.05$). The CRFs had lost about 65, 64, and 35 % of chlorophyll *a* content after exposure to the CP, SP, and WB treatments, respectively. The highest value of chlorophyll *a* recorded for water blanched CRF was in agreement with the greenness value reported previously in Section 5.4.1, Table 5.2, compared to the conventional pasteurised and steam pasteurised CRF. A study on brown seaweed (*Undaria pinnatifida)* reported a lower degree of chlorophyll *a* loss in blanching (24.5 %), boiling (38.45 %), steaming (49.32 %), and baking (67.59 %) compared to the raw sample with 83.43 % loss (Jiang et al., 2022).

The chlorophyll is susceptible to heat loss (Chen & Chen, 1993) which is caused by cell rupture and degradation of chlorophyll to pheophytins during the thermal treatment (Jiang et al., 2022). Our study reported a significant reduction in the total chlorophyll concentration of all heat-treated CRFs with the highest loss in CP (70 %), followed by SP (68 %), and WB (37 %). In a study by Pellegrini et al. (2010), the total chlorophylls were decreased in all cooked fresh broccoli with different levels of chlorophyll loss in cooking treatments (microwaving > boiling > basket steaming > oven steaming). A significant increase in the ratio of chlorophyll *a* to *b* was found in the CRF from CP and SP, while little to no changes in the CRF from WB. The longer heating time in CP and SP treatments (5 min) probably caused a 2 folds degradation of chlorophyll *a* than WB treatment (3 min) in the heat-treated CRFs. WB preserved the highest value in total chlorophyll concentration of CRFs in comparison between heat treatments. This indicates that WB treatment is effective in minimising the degradation of chlorophyll in the CRF from SPH.

5.5.2 Total phenolic and antioxidant content (DPPH and FRAP assays)

The free-radical scavenging activity was determined using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay while the reducing activity of ferric ions was determined using a ferric reducing antioxidant potential (FRAP) assay. F, CP, SP, and WB mean the CRF samples were treated with different heat treatments: F (fresh, no heat treatment), CP (conventional pasteurisation), SP (steam pasteurisation), and WB (water blanching). Figure 5.4 shows the effect of heat treatments on total phenolic content (TPC) and antioxidant content (DPPH and FRAP assays) of chloroplast-rich fraction (CRF) from sweet potato haulm (SPH).

The TPC of all heat-treated CRFs decreased significantly ($p < 0.05$, Figure 5.4 [a]) with about 50 to 76 % of phenolic compounds lost through the heat treatment. A significant reduction of TPC (63.8 % loss) in blanched sweet potato leaves may be attributed to their leaching loss into the cooking water, as a small amount of phenolic acids was found in the cooking water (Jang & Koh, 2019). A different trend of retention in TPC was found in the boiled leaves of three sweet potato varieties with values of 27, 75, and 94 % for *Kabode*, *White* sp, and *Yellow* sp varieties, respectively (Abong' et al., 2021). Hence, the genotype of plants could influence the rate of nutrient loss during the cooking process. The depletion of TPC in sweet potato leaves after boiling, microwaving, and frying could be due to the breakdown of phenolic compounds during cooking (Sun et al., 2014a).

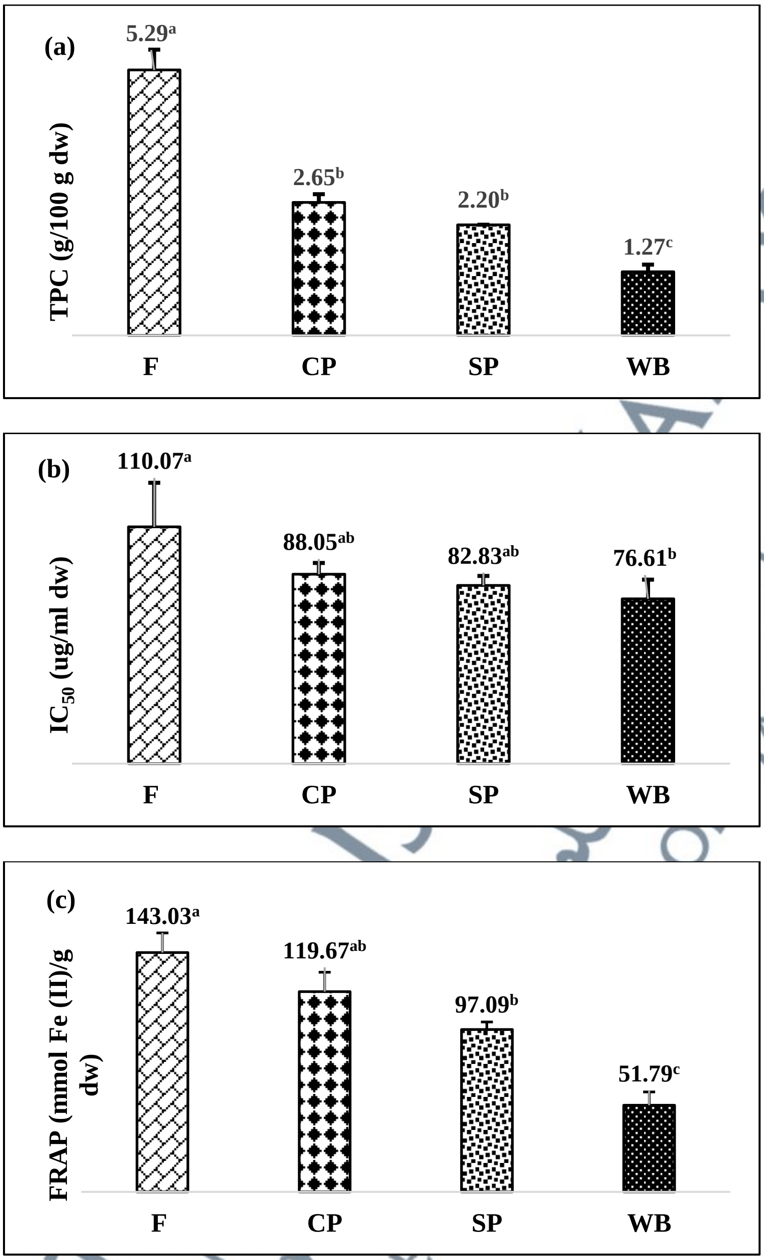


Figure 5.4: TPC (a), DPPH (b), and FRAP (c) of heat-treated CRFs. Results expressed with different letters mean significant difference (Tukey's test, $p < 0.05$)

In comparison to the findings by Hanifah (2022b), about 70 % of TPC was retained in SPH juice after the pasteurisation process. This suggests that the CRF material collected from SPH carries more thermolabile compounds of polyphenols that undergo chemical changes after prolonged heat treatment. The steamed brown seaweed

(*Undaria pinnatifida*) lost the most in TPC (32 %), followed by blanching (23 %) and boiling (21 %) samples (Jiang et al., 2022). Similarly, the CRF reported the greatest loss of TPC in WB, followed by SP and CP treatment.

The DPPH radical scavenging activity of CRFs increased after heat treatments with up to 20 to 30 % increment (Figure 5.4 [b]). A lower IC_{50} value indicates a stronger antioxidant activity of a sample. This was in agreement with a significant increase in the antioxidant activity of broccoli and spinach during boiling, steaming, and microwaving (Turkmen et al., 2005). Sun et al. (2014a) found that the antioxidant activity of sweet potato leaves cooked by steaming, baking, and frying was increased significantly but sweet potato leaves cooked by boiling and microwaving decreased significantly. The *Moringa oleifera* leaves had a 40 % loss of antioxidant activity after steam blanching for 3 min (Wickramasinghe et al., 2020). Three types of cabbage (Red cabbage, Chinese cabbage, and Mustard cabbage) showed a reduction in radical scavenging activity from 15 to 35 % after blanching for 5 min (Amin & Lee, 2005). Moreover, a greater loss of antioxidant activity in sweet potato leaves was found in higher heating temperatures (80 and 100 °C) for the same treatment time (Sun et al., 2017). Heat processing could cause little changes or even enhance the antioxidant potential of vegetables due to the formation of novel compounds such as Maillard reaction products that exhibit antioxidant activity (Turkmen et al., 2005).

The effect of heat treatments on the FRAP activity of CRF is shown in Figure 5.4 (c). The FRAP activity diminished in all heat-treated CRFs ranging from 51.79 to 119.67 mmol Fe (II)/g dw. The CRF had the highest loss of ferric-reducing activity in WB (64 %), followed by SP (32 %) and CP (16 %) treatment. The steamed pumpkin leaves reduced FRAP activity loss compared to the boiling cooking technique with 19 and 73 % loss, respectively (Mashiane et al., 2021). In other findings reported by Mashitoa et al. (2021), steaming retained antioxidant capacity (FRAP assay) of pumpkin leaves higher than water bath blanching treatment (89 and 26 % retention, respectively). Similarly, steaming for 5 min demonstrated higher retention of ferric reduction activity compared to hot water bath blanching with the same heating time in nightshade leafy vegetables (Managa et al., 2020).

The results demonstrated that heat treatments could cause fluctuations in TPC and antioxidant activity (FRAP assay) of CRF from SPH. Among the heat treatments, water blanching (WB) presented the lowest TPC and antioxidant activity (FRAP assay) of CRFs. Blanching of SPH in hot water could release some phenolic compounds into the hot water causing the most loss of polyphenols and antioxidant activity. Moreover, blanching may result in a loss of antioxidants due to the surface area of vegetables in contact with the hot water and the high solubility of antioxidant compounds in boiling water (Amin & Lee, 2005). Steaming has been reported to have better retention of phenolic compounds and antioxidants compared to boiling (Mashiane et al., 2021; Sun et al., 2014a) and water blanching (Mashitoa et al., 2021; Wickramasinghe et al., 2020) as the treatment enhances the extractability by matrix softening.

No significant difference in TPC and antioxidant content (DPPH and FRAP assays) between CRF treated by conventional pasteurisation (CP) and steam pasteurisation (SP), indicates that both treatments had a similar impact on the essential nutrients present in CRF ($p > 0.05$). Although previous studies reported a positive impact of cooking treatments on antioxidant activity, the results may vary depending on the structural property of the cell wall to withstand the heat treatment in different types of vegetables (Burns et al., 2003).

It has been hypothesised that polyphenols possess antioxidant activity in plants. A strong positive correlation was shown between TPC and IC_{50} DPPH (r = 0.9987) of CRF, suggesting that phenolic compounds were not correlated with the antioxidant properties of heat-treated CRFs. A positive correlation means the higher value of TPC will give a higher IC_{50} value. However, TPC presented a strong correlation with FRAP activity (r = 0.9339) of CRF in our study. Sun et al. (2014a) implied that the correlation between phenolic compounds and the antioxidant activity of sweet potato leaves could be mainly attributed to individual phenolic compounds specifically 4,5-di-O-caffeoylquinic acid, 3,4-di-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, and 3,4,5-tri-O-caffeoylquinic acid. The antioxidant activity of phenolic compounds is essentially due to their redox properties, allowing them to act as hydrogen donors, reducing agents, heavy metal chelators, singlet oxygen quenchers, and hydroxyl radical quenchers (Kaur & Kapoor, 2002). The IC_{50} DPPH was not correlated with FRAP (r = 0.9268), indicating that heat treatments could influence the antioxidant properties of CRF in different approaches. A positive correlation means the higher value of IC_{50} will give a higher ferric-reducing antioxidant activity.

The above results suggested that optimisation of heat treatments is recommended concerning lower heating temperatures and heating times that result in a minimal loss of antioxidants compared with the fresh CRF. Blanching treatment could be improvised by packing the haulm into a vacuum-sealed bag first before the heating process to prevent the leaching of water-soluble compounds. Determination of individual phenolic compounds in the CRF is recommended for future work to identify specific phenolic compounds that are responsible for the TPC and antioxidant activity.

5.5.3 Antinutrients content (Oxalic and phytic acids)

The contribution of vitamins and minerals from edible plants to human nutrition is however limited due to the presence of antinutritional factors. Oxalic and phytic acids will bind with minerals in their salt form, limiting the bioavailability of minerals and assimilation of proteins.

Heat treatment in the course of cooking has been a reliable method to reduce the concentration of antinutritional factors in edible plants (Abong' et al., 2021; Arias-Rico et al., 2020; Issa et al., 2020; Waseem et al., 2022). Variations in oxalic and phytic acids were affected by heat treatments in chloroplast-rich fraction (CRF) from sweet potato haulm (SPH), as shown in Table 5.5.

Table 5.5: Effect of heat treatments on antinutritional content of CRF

Antinutrient content	CRF with different heat treatments			
	F	**CP**	**SP**	**WB**
Oxalic acid (g/100 g dw)	2.16 ± 0.26^{a}	0.84 ± 0.37^{b}	0.93 ± 0.43^{b}	1.09 ± 0.33^{b}
Phytic acid (g/100 g dw)	0.06 ± 0.00^{c}	0.06 ± 0.00^{c}	1.16 ± 0.00^{a}	0.73 ± 0.00^{b}

Different letters mean significant differences (Tukey's test, $p < 0.05$) between values. Data are means ± SD (n = 3). F, CP, SP, and WB mean the CRF samples were treated with different heat treatments: F (fresh, no heat treatment), CP (conventional pasteurisation), SP (steam pasteurisation), and WB (water blanching).

Oxalic acid content was reduced significantly after heat treatments ($p < 0.05$), comparable to the previous findings on cooked sweet potato leaves (Abong' et al., 2021; Issa et al., 2020; Mwanri et al., 2011). At least half of the antinutrient was diminished (from 50 to 61 % reduction), indicating that oxalic acid present in SPH is a heat-sensitive compound that degrades during thermal processing. Correspondingly, a study on pasteurised SPH juice recorded a lower concentration of oxalic acid compared to the unpasteurised SPH juice with values of 767.89 and 1038.66 mg/100 g dw, respectively (Hanifah, 2022b).

Previous studies reported that boiling of sweet potato leaves diminished the oxalates by 0.5 to 23 % by interrupting the cell walls of plants, resulting in the degradation, and leaching of antinutrients into the cooking water (Abong' et al., 2021). The oxalic acid content in sweet potato leaves studied by Mosha et al. (1995) decreased by 9 % with conventional blanching (for 10 min) and 4 % with microwave blanching (for 60 sec). No significant difference was shown in the depletion of oxalic acid content between all heat-treated CRFs with loss of 50, 57, and 61 % for CRF treated by water blanching (WB), steam pasteurisation (SP), and conventional pasteurisation (CP), respectively. Hence, the heat treatments proposed in our study induced a similar impact on the oxalic acid concentration of CRF from SPH.

In contrast, CRF from CP had no changes in phytic acid concentration while CRF from SP and WB had an extensive increase up to 12 times its value. This was contradictory to the reduction of phytic acid content in cooked sweet potato leaves reported by previous findings (Abong' et al., 2021; Mosha et al., 1995; Mwanri et al., 2011). Hanifah (2022b) reported a significant reduction in pasteurised (0.01 g/100 g dw) SPH juice compared to the unpasteurised (0.04 g/100 g dw) sample. A study reported about 16 to 30 % loss of phytic acid concentration in boiled sweet potato leaves from three different varieties (Abong' et al., 2021). Our study found a significant increase in the phytic acid content of CRF treated with SP and WB ($p < 0.05$).

Conventional blanching of sweet potato leaves for 10 min at 98 °C reduced phytic acid by 67 % while microwave blanching for 60 sec reduced phytic acid concentration by 58 % (Mosha et al., 1995). Although a similar treatment was conducted in our study, blanching of SPH was done at 85 °C, for 5 min which the temperature and heating time were lower than in the previous study. The temperature and time during heat treatment probably influence the rate of phytic acid degradation in green biomass. Furthermore, lactic acid fermentation prior dehydration process reported the highest reduction of phytic acid (50 – 70 %) compared to boiling (16 – 30 %) and dehydration (36 – 56 %) processes of sweet potato leaves (Abong' et al., 2021).

CP, SP, and WB treatments had successfully diminished at least half of the oxalic acid concentration in the CRF from SPH. However, the heat treatments had a negative to no effect on the phytic acid concentration. Even though various cooking processes could remove antinutrients in vegetables, the effectiveness is highly dependent on temperature, process type, vegetable type and its characteristics (Dagostin, 2017). The step of heating before juicing in both SP and WB treatments might contribute to some physicochemical alterations specifically the heat-labile property of the phytic acid compound present in the haulm. Hence, a further approach involving variation in temperature, time and method of treatments is proposed to level up the percentage loss of phytic acid in the CRF from SPH.

5.5.4 Minerals content

Previously in Chapter 4 (Section 4.5.4, Table 4.6), the chloroplast-rich fraction (CRF) from sweet potato haulm (SPH) was reported to have various elements consisting of magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), potassium (K), calcium (Ca), chlorine (Cl), sulphur (S), phosphorus (P), silicon (Si), rubidium (Rb), strontium (Sr), molybdenum (Mo), bromine (Br), and nickel (Ni). The comparative analysis of the mineral composition of fresh (F, no heat treatment) and heat-treated CRFs: CP (conventional pasteurisation), SP (steam pasteurisation), and WB (water blanching) from SPH was determined.

As shown in Table 5.6, a significant increase of minerals K, Br, and Mn was reported in all heat-treated CRFs ($p < 0.05$). Conventional pasteurised CRF had the highest concentration of mineral K, followed by steam pasteurised, and water blanched CRFs (CP > SP > WB). The higher concentration of mineral K found in heat-treated CRFs in comparison to fresh CRF was contradictory to the studies reported by Chirwa-Moonga et al. (2020) and Luo et al. (2019), who found a significant reduction of mineral K in steamed and blanched sweet potato leaves, respectively. Interestingly, mineral Br increased drastically to about 9 to 11 times higher while mineral Mn increased about 8 to 10 times greater than fresh CRF, indicating that the heat treatments proposed in this study had an enormous effect on these two elements. CP and WB treatments increased S content in CRF while no significant change was observed for CRF treated with SP. Conversely, P content was reduced in CRF after SP and WB treatments but had no effect after CP treatment, comparable to the finding by Chirwa-Moonga et al. (2020) where steaming for 10 minutes did not affect the P content in green sweet potato leaves.

Table 5.6: Mineral compositions (mg/100 g dw) of heat-treated CRFs

Elements	F	CP	SP	WB
K	9351.0 ± 100.1^{d}	14133.3 ± 57.7^{a}	13533.0 ± 289.0^{b}	12900.0 ± 300.0^{c}
Ca	5121.2 ± 210.0^{a}	2503.3 ± 70.9^{c}	2586.7 ± 50.3^{c}	3450.0 ± 87.2^{b}
Cl	1580.0 ± 45.8^{b}	1876.7 ± 20.8^{a}	1633.3 ± 45.1^{b}	1550.0 ± 45.8^{b}
S	710.5 ± 15.75^{b}	869.7 ± 5.69^{a}	718.0 ± 27.8^{b}	818.3 ± 36.9^{a}
P	581.5 ± 18.4^{a}	559.0 ± 10.0^{a}	507.3 ± 10.0^{b}	504.0 ± 27.5^{b}
Si	392.1 ± 19.2^{a}	138.7 ± 1.5^{bc}	110.3 ± 1.5^{c}	155.3 ± 11.6^{b}
Mg	281.0 ± 1.5^{b}	321.7 ± 5.5^{a}	289.3 ± 17.2^{ab}	315.0 ± 19.2^{ab}
Rb	119.6 ± 2.5^{a}	29.6 ± 1.4^{b}	2.9 ± 0.4^{c}	25.9 ± 1.5^{b}
Fe	116.6 ± 14.0^{b}	176.7 ± 2.1^{a}	164.7 ± 25.3^{ab}	193.7 ± 23.7^{a}
Sr	35.2 ± 2.9^{a}	8.5 ± 1.2^{b}	9.6 ± 0.8^{b}	10.3 ± 1.0^{b}
Mo	29.5 ± 0.5^{a}	ND	ND	27.5 ± 3.0^{a}
Zn	25.3 ± 1.1^{a}	19.5 ± 0.5^{b}	19.7 ± 1.4^{b}	20.6 ± 0.5^{b}
Al	24.5 ± 2.0^{a}	ND	22.1 ± 5.6^{a}	ND
Cu	19.4 ± 0.2^{ab}	20.0 ± 1.5^{ab}	17.5 ± 1.2^{b}	21.8 ± 1.3^{a}
Br	14.2 ± 1.0^{c}	151.7 ± 3.2^{a}	130.3 ± 5.5^{b}	128.0 ± 7.8^{b}
Mn	11.4 ± 1.3^{c}	115.3 ± 5.0^{a}	95.73 ± 2.0^{b}	113.0 ± 1.0^{a}
Ni	4.9 ± 0.6^{a}	6.1 ± 1.0^{a}	5.9 ± 1.5^{a}	5.7 ± 1.0^{a}

Different letters mean significant differences (Tukey's test, $p < 0.05$) between values. Data are means ± SD (n = 3). F, CP, SP, and WB mean the CRF samples were treated with different heat treatments: F (fresh, no heat treatment), CP (conventional pasteurisation), SP (steam pasteurisation), and WB (water blanching). 'ND' stated in the table means 'not detected'.

In addition, Fe content in CRF was increased after heat treatments, similar to a study on steam blanched *Moringa oleifera* leaves (Wickramasinghe et al., 2020). Exposure to heat could accelerate the oxidative cleavage of the prophyrin ring in the non-heme Fe (a form of Fe found in vegetables) resulting in Fe liberation which elevates its content (Chirwa-Moonga et al., 2020). However, Chirwa-Moonga et al. (2020) reported a significant reduction in Fe content found in green sweet potato leaves after steaming for 10 min. Element Mg was slightly higher in every heat-treated CRF compared to the fresh CRF. The result was not complimentary with a recent study, which reported a significant depletion of Mg content in sweet potato leaves due to the leaching and diffusion of nutrients during water blanching (Luo et al., 2019). CP treatment significantly increased mineral Cl in CRF while no significant effect was shown for CRF treated with SP and WB.

Heat treatments significantly reduced the concentration of Ca, Si, Rb, Sr, and Zn in CRF ($p < 0.05$). The significant loss in Ca content was similar to the previous finding reported on blanching and microwave heating of white cabbage (Waseem et al., 2022). A similar result was obtained on *Moringa oleifera* leaves where element Ca was lost after steam blanching for 3 min influenced by the elevation of temperature during the cooking process that disrupts cells and subsequently leaches out minerals into the water (Wickramasinghe et al., 2020).

In agreement with our finding, the Zn element was diminished significantly in sweet potato leaves after blanching for 1 min (Luo et al., 2019) and after steaming for 15 min (Chirwa-Moonga et al., 2020). A low concentration of Mo and Al minerals was present in fresh CRF, and heat treatments reduced the amount to an extent where the elements were undetectable in CRF. The minerals Cu and Ni showed no significant differences ($p > 0.05$) between the heat treatments. To conclude, heat treatments: CP, SP, and WB had diverse impacts on the mineral composition of CRF from SPH.

5.6 Conclusions

This study provides information on the physicochemical variations of chloroplast-rich fraction (CRFs) from sweet potato haulm (SPH) after heat treatments. Heat treatments proposed in this study were conventional pasteurisation (CP), steam pasteurisation (SP), and water blanching (WB).

It is concluded that;

1. The colour intensity changed with heat exposure. WB is the best heat treatment for retaining the green colour of CRF powder.
2. The solubility of CRFs powder improved in cold (24 – 33 %) and warm (21 – 27 %) methods, but no significant difference was shown between the methods. Heat-treated CRFs had lower bulk density than fresh CRF while no impact on the water activity and dispersibility was observed after thermal treatments.
3. Heat treatments could alter the microstructure of CRFs powder. CP gives the greatest condition to induce the formation of porosity and amorphous structure in CRF (seen at 1000x and 3000x magnifications of scanning electron microscopy images).
4. SP treatment exhibits an impact on the proximate composition; crude protein, crude fat, and carbohydrate contents with values of 32.08, 1.99, and 43.16 g/100 g dw, respectively. CP treatment succeeded in retaining entirely proximate compositions present in CRF. The loss of total chlorophyll was effectively minimised in WB treatment (35 %), compared to CP (65 %) and SP (64%).
5. The retention of TPC ranged from 24 to 50 % and higher in antioxidant activity (FRAP assay) with retention ranging from 36 to 84 % in the heat-treated CRFs.

Antioxidant activity (DPPH assay) increased in heat-treated CRFs up to 20 – 30 % increment. CP treatment preserves TPC and antioxidant activity (FRAP assay) greatest, followed by SP, and WB.

6. Heat treatments have successfully diminished about 50 to 61 % of the oxalic acid concentration in CRF. Even so, a negative to no effect was observed on the phytic acid content of all heat-treated CRFs.
7. There were variations in the mineral compositions of CRFs with some elements increased (K, Cl, S, Mg, Fe, Br, and Mn) and decreased (Ca, P, Si, Rb, Sr, Zn, Mo, and Al), while the other elements (Cu and Ni) had little to no effect after heat treatments.