

CHAPTER 4

EFFECT OF DIFFERENT CROP BORDERS ON THE PHYSICOCHEMICAL VARIATION OF CHLOROPLAST-RICH FRACTIONS (CRFs) FROM SWEET POTATO HAULM

4.1 Introduction

The essential nutrients are certainly present in the green biomass's chloroplast-rich fraction (CRF); higher amounts were found in CRF compared to the juice of sweet potato haulm (Hanifah, 2022b). The CRF was recovered by centrifugation to obtain a layer of concentrated chloroplasts which presumably possess high nutritional compositions. The freeze-drying process removes any residual water from the CRF material. It was proven that sweet potato leaves' nutritional, antioxidant, and phenolic compositions were affected by harvesting periods (Suárez et al., 2020). As our green biomass was not collected in different harvesting periods, it is necessary to study whether there are variations present in essential nutrients and physical properties of the biomass collected from a single harvest period. Hence, the CRF of the sweet potato haulm (SPH) harvested from three different crop borders was studied.

Chapter 4 aims to discuss the findings of the first research objective, which is the effect of different crop borders on the physicochemical variation of chloroplast-rich fractions (CRFs) from sweet potato haulm. This study determined the nutritional, antinutritional, and physical properties of CRF from SPH harvested from different crop

borders (refer to Figure 1.3), and it is essential to improve the present understanding of food production patterns.

Results and Discussion

4.2 Yield of sweet potato haulm juice and CRF powder

Regardless of borders number or locations, all sweet potato haulm (SPH) juice recorded similar yields ranging between 0.78 – 0.84 ml per gram of wet biomass. The SPH juice yield was higher than the previous study on the juice of pea vine haulm, which recorded 0.6 ml per gram of wet biomass (Torcello-Gómez et al., 2019). The yield of chloroplast-rich fraction (CRF) powder from SPH was 4 times higher (mean value of 41.6 g/kg wet biomass) compared to the CRF from spinach (mean value of 10.1 g/kg fresh leaf) where both juices were prepared using a slow juicer (Syamila, 2019). The previous author stated that a viscous supernatant was observed after the centrifugation process, resulting in a relatively low yield of CRF.

A similar result was observed in our study, where the supernatant collected was very viscous and had a dark green-brownish colour even after the third time of centrifugation (Figure 4.1). Hence, continuous centrifugation (up to 6 times) is suggested to collect more CRF pellets. A dark green layer of CRF was obtained after juice centrifugation, separated from the supernatant. Comparable to research findings from Torcello-Gómez et al. (2019), our bottom solid phase also contains a small white layer (starch-rich) at the bottom of the tube.



Figure 4.1: The image of supernatant after three times of centrifugations

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

The total soluble solids (TSS) of sweet potato haulm (SPH) juice harvested from different crop borders (CB) was presented in Table 4.1. The TSS was analysed in the fresh SPH juice (before centrifugation and freeze-drying process).

Table 4.1: Total soluble solids of SPH juice, total solid content, and moisture content of CRF

	CB 1	CB 2	CB 3	Mean
Total soluble solids (°Brix)	5.40 ± 0.10 ^a	6.20 ± 0.20 ^a	5.70 ± 0.62 ^a	5.77
Total solid content (g/100 g fw)	16.63 ± 0.50 ^a	14.87 ± 0.36 ^b	14.74 ± 0.11 ^b	15.41
Moisture content (g/100 g fw)	83.37 ± 0.50 ^b	85.13 ± 0.89 ^a	85.26 ± 0.11 ^a	84.59

Different letters mean significant differences (Tukey's test, $p < 0.05$) between values. Data are means ± SD ($n = 3$). CB 1, CB 2, and CB 3 mean the samples were harvested from different crop borders: Crop Border 1, Crop Border 2, and Crop Border 3.

The Brix value exhibits a strong positive correlation with sugar mainly sucrose followed by other carbohydrates, protein, fats, minerals, vitamins, organic acids, and amino acids in the juice. No significant differences were recorded for TSS of the fresh SPH juice between the CB with values ranging from 5.4 to 6.2 °Brix ($p > 0.05$). Fresh juice of SPH prepared by Hanifah (2022b) had a higher value of TSS (8.93 °Brix) compared to our fresh SPH juice (on average of 5.77 °Brix). This might be due to the different harvest periods for the SPH. Moreover, our SPH juice recorded a lower TSS content than the spinach juice (7.1 °Brix), where both juices were prepared by a slow juicing method (Syamila, 2019).

Conversely, the total solid content (TSC) and moisture content were analysed on the chloroplast-rich fraction (CRF) of SPH, obtained after centrifugation and freeze-drying process. The TSC was obtained after decanting the water molecules originally present in the food without modification in the mass of the food matrix. Thus, the moisture content is based on the determination of the mass of water molecules in the food. Based on Table 4.1, CRF from CB 1 recorded a slightly higher amount of TSC than CB 2, and CB 3 with values of 16.63, 14.87, and 14.74 g/100 g fw, respectively ($p > 0.05$). This indicates that about 83 to 85 % of the water was removed after freeze-drying. The slight difference could be due to the different compositions of the SPH from each CB as the petiole (also known as stalk) holds greater water molecules compared to the leaf and stem (Ishida et al., 2000).

The moisture content of CRF from SPH had a lower value range (83.37 – 85.26 g/100 g fw) compared to the CRF from pea vine haulm (87.5 – 89.0 g/100 g fw) reported by Torcello-Gómez et al. (2019). On the contrary, Gedi et al. (2017) stated lower moisture content of CRF for all four selected green leaves, with values of 70, 72, 79,

and 82 g/100 g fw, respectively, for stinging nettles, grass, spinach, and kale. A previous study on sweet potato leaves harvested at three different periods reported a lower moisture content, with values ranging between 86.05 and 87.70 g/100 g fw (Suárez et al., 2020). This study found that the TSS of fresh juice and TSC of CRF in SPH between different CBs within the same harvest was not significant ($p > 0.05$).

4.4 Physical properties

4.4.1 Colours

The colour difference between the chloroplast-rich fraction (CRF) of sweet potato haulm (SPH) harvested from different crop borders was measured using a Hunter Lab colourimeter, and 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). Figure 4.2 shows the image of freeze-dried CRF powder from different crop borders (CBs). CB 1, CB 2, and CB 3 mean the samples were harvested from different crop borders: Crop Border 1, Crop Border 2, and Crop Border 3.

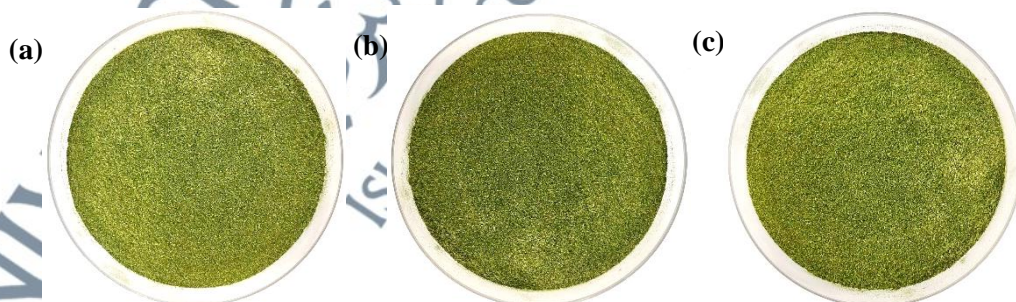


Figure 4.2: The image of CRF powder from a) CB 1, b) CB 2, and c) CB 3

When assessing the difference in colour (ΔE) between the CRF powders, the following ranges were applied: 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). Generally, CRF powder from SPH had a significant difference in colour parameters between each CB. From Table 4.2, CRF from CB 2 had the lowest value for lightness (L), and the highest value for greenness ($-a$) and yellowness ($-b$) compared to the other two CRF. This indicates that CRF from CB 2 was perceived as dark, probably reflecting from a higher number of concentrated chloroplasts collected as well as the lutein and β -carotene contents.

Table 4.2: Colour parameters and total colour difference of CRF

Colour properties	CRF from different crop borders		
	CB 1	CB 2	CB 3
L^*	38.41 ± 0.12^b	37.76 ± 0.37^c	39.04 ± 0.29^a
a^*	-2.67 ± 0.11^a	-4.73 ± 0.16^c	-4.18 ± 0.09^b
b^*	22.15 ± 0.60^c	26.37 ± 0.55^a	24.60 ± 0.25^b
Total colour difference (ΔE^*)			
CB 1 – CB 2	4.76 ± 1.14^a		
CB 1 – CB 3	3.11 ± 0.57^b		
CB 2 – CB 3	2.30 ± 0.78^b		

Different letters mean significant differences (Tukey's test, $p < 0.05$) between values. Data are means \pm SD ($n = 5$). CB 1, CB 2, and CB 3 mean the CRF samples were harvested from different crop borders: Crop Border 1, Crop Border 2, and Crop Border 3.

The high value for greenness ($-a$) in CRF might be attributed to the source of chloroplasts as the leaves part is usually greener in colour compared to the petioles and stems part of SPH (based on observation by the naked eye). The CRF powder had a noticeable to clear colour difference (ΔE : 2.30 – 4.76) between each CB. As there were no colour difference values of more than 5 ($\Delta E > 5$), it was estimated that the difference

in powder colour of CRF between every CB was hard to notice for inexperienced observers.

4.4.2 Water activity, bulk density, and dispersibility

Water activity (a_w) is a measure of the amount of available water for biological reactions. It has roles in moisture migration and chemical and physical reactions. Also, it acts as a support system for microorganisms to grow with values of at least 0.6 a_w for fungi and 0.91 a_w for bacteria. CB 1, CB 2, and CB 3 mean the samples were harvested from different crop borders: Crop Border 1, Crop Border 2, and Crop Border 3. From Figure 4.3 (a), CRF's water activity significantly differed between the crop borders (CBs) with values ranging from 0.24 to 0.45 a_w . Hence, the CRF might have good shelf-life stability due to the unfavourable conditions for microbial growth. Water activity was reported lower in the freeze-dried spinach juice with a value of 0.18 a_w (Syamila, 2019) compared to our study (mean value of 0.36 a_w).

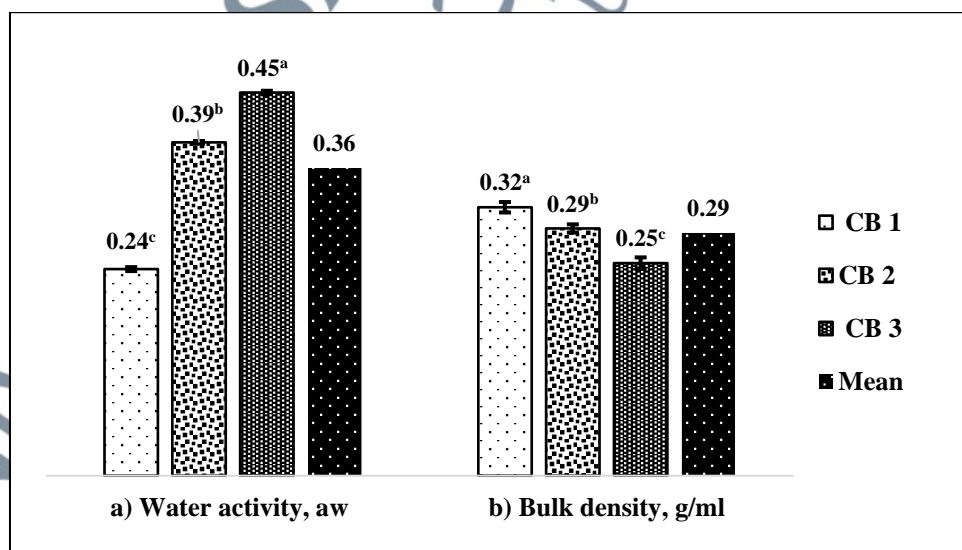


Figure 4.3: Water activity (a) and bulk density (b) of CRF. Results expressed with different letters mean significant difference (Tukey's test, $p < 0.05$)

Bulk density is defined as the weight of powder in a definite volume. Particles with a smaller size will accommodate more amount of powder in the same volume leading to a higher bulk density. Based on Figure 4.3 (b), the CRF powder between the CBs had significantly different values of bulk density ($p < 0.05$), ranging from 0.25 to 0.32 g/ml. High bulk density is desirable for reducing the cost of packaging and shipping whereas low bulk density influences the flowability and instant characteristics of powder properties (Kalyankar et al., 2016). A higher value of bulk density (0.53 g/ml) was reported by Raja et al. (2019) in freeze-dried *Carica papaya* L. leaf powder indicating that the leaf had lower porosity and higher moisture content compared to the CRF powder from sweet potato haulm.

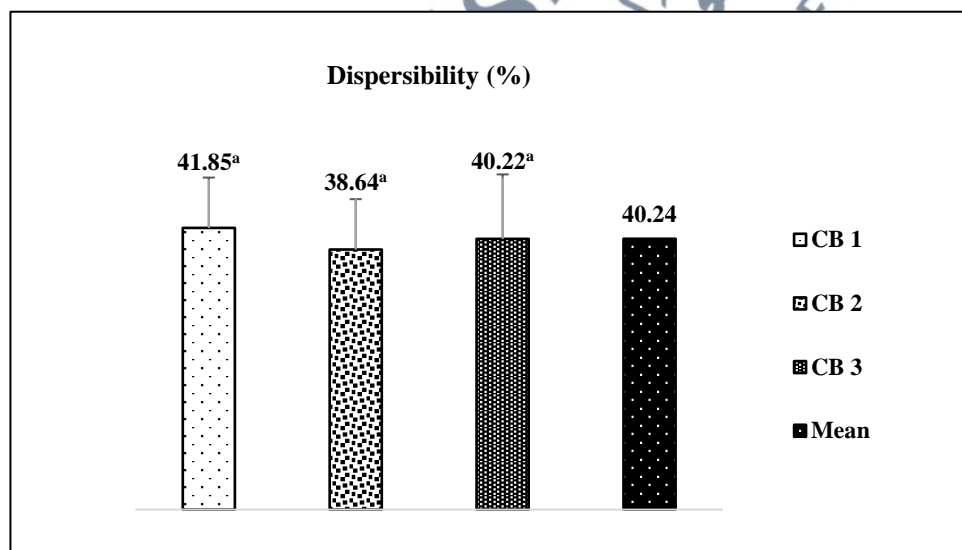


Figure 4.4: Dispersibility of CRF. Results expressed with different letters mean significant difference (Tukey's test, $p < 0.05$)

Dispersibility is one of the good functional properties of powder determined by measuring its ability to be dispersed throughout the water. From Figure 4.4, low dispersion of CRF powder was shown for the three CBs with a mean value of 40.24 %.

The intact green tea powder showed poor dispersing activity in water compared to the green tea powder coated with a 1 % solution containing a glidant agent (Sakurai et al., 2017). Lower dispersibility in water and poorer flow properties could happen when the size of the primary particles is small, leading to a larger formation of the adhesive force between them (Sakurai et al., 2017). However, previous studies reported that superfine particles of tea leaves improved the dispersibility properties in water due to the larger particle surface area and the breakdown of the leaf cell walls (Hu et al., 2012; Park et al., 2001). A wide range of dispersibility was found for the 10 commercial samples of cocoa powdered beverages with values ranging from 50 to 94.5 % (Shittu & Lawal, 2007). Thus, smaller particle sizes and lower surface tension are essential parameters to enhance the dispersibility of powder in water.

4.4.3 Water solubility index

The water solubility index (WSI) of chloroplast-rich fraction (CRF) powder was determined using the cold and warm WSI as demonstrated in Table 4.3.

Table 4.3: Water solubility index of CRF

CRF from different crop borders	Water solubility index (%)	
	Cold	Warm
a) Crop border 1 (CB 1)	26.36 ± 1.13 ^{a, A}	28.36 ± 2.65 ^{a, A}
b) Crop border 2 (CB 2)	18.09 ± 1.04 ^{b, A}	17.09 ± 1.04 ^{b, A}
c) Crop border 3 (CB 3)	17.38 ± 0.84 ^{b, A}	18.85 ± 1.96 ^{b, A}
Mean	20.61	21.43

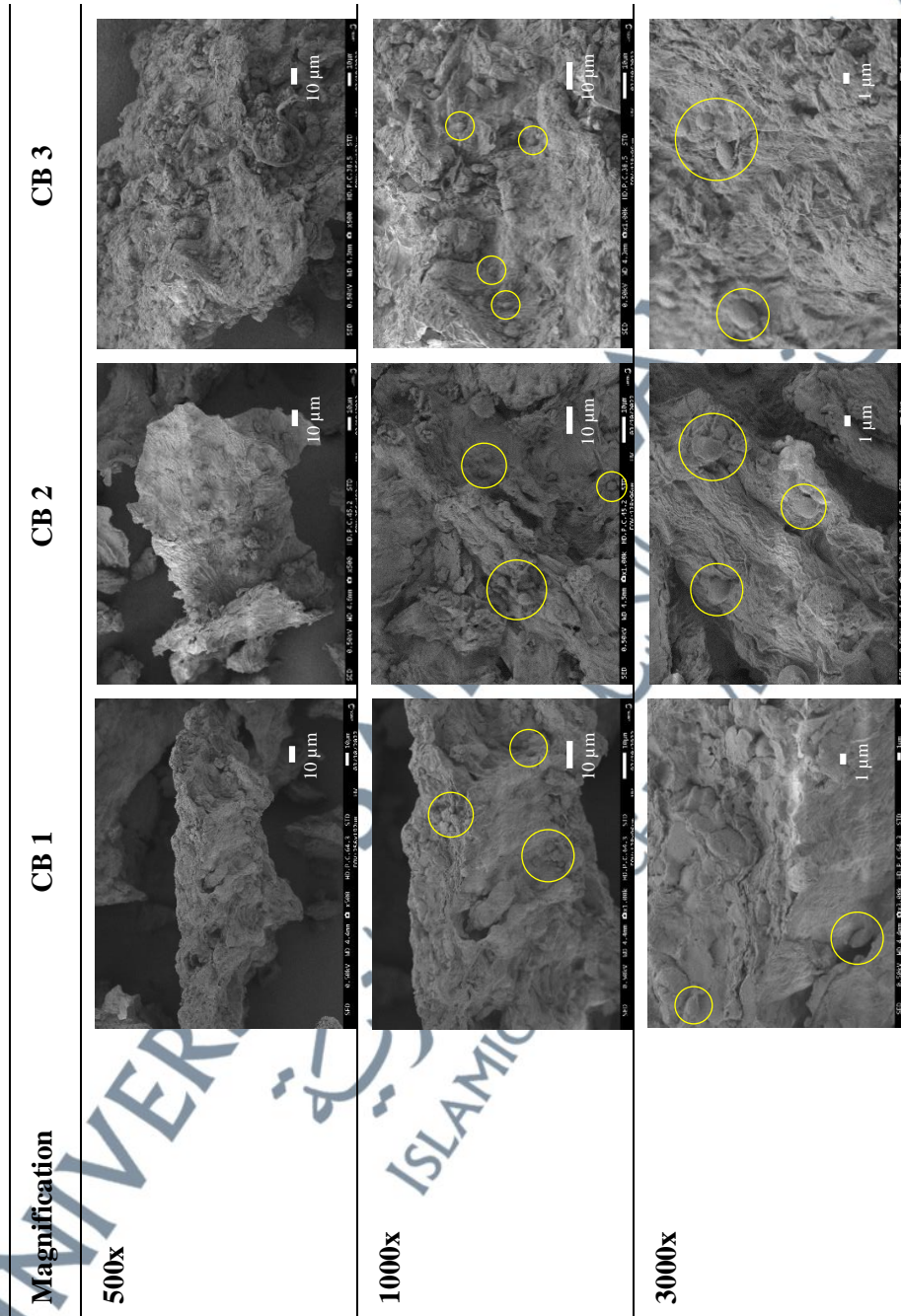
Cold water solubility (no incubation, centrifuged at 10 000 rpm at 4 °C for 15 min); warm water solubility (incubation at 38 °C for 30 min, centrifuged at 10 000 rpm at 4 °C for 15 min). Different lower letters within columns and different upper letters across the columns mean significant differences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

CB 1, CB 2, and CB 3 mean the samples were harvested from different crop borders: Crop Border 1, Crop Border 2, and Crop Border 3. Among the three crop borders (CBs), CRF powder from CB 1 had the highest WSI for both cold and warm methods. However, the difference was only between 8 to 11 %. The fresh sweet potato haulm (SPH) juice powder was more soluble in both cold and warm WSI with mean values of 28.70 and 23.52 %, respectively (Hanifah, 2022b) compared to our CRF powder from SPH. The higher amount of concentrated chloroplast present in the CRF could interfere with the powder properties, resulting in low solubility. Besides, crystalline solid formation and lack of porosity structures were seen from scanning electron microscopy (SEM) images – see Figure 4.5. However, the solubility of freeze-dried CRF powder (mean value of 20.61 %) was 4 times higher than the freeze-dried sweet potato leaves powder (5.71 %) studied by Sui et al. (2019). This showed that the slow juicing process involved in collecting the CRF layer might enhance the solubility of CRF powder.

The freeze-dried CRF powder from spinach recorded a higher value for both cold and warm WSI with average values of 38 and 29.1 %, respectively (Syamila, 2019). The solubility may be related to the presence of natural sugars (Syamila, 2019). However, there was no correlation between the total soluble solids (TSS) content in the haulm juice (presented in Table 4.1) with the solubility of CRF powder. In comparison between the cold and warm WSI, both showed no significant difference between the CRF of all CBs ($p > 0.05$). Although smaller particle sizes may improve solubility, the previous study showed a negative impact on the CRF powder of spinach with particle sizes within the range of 75 to 250 μm (Syamila, 2019). It is recommended to determine the particle size of our CRF powder as well to facilitate findings from previous studies.

4.4.4 Morphology

The scanning electron microscopy (SEM) images of chloroplast-rich fraction (CRF) powder from sweet potato haulm (SPH) are shown in Figure 4.5.



Note: CB 1, CB 2, and CB 3 mean the CRF samples were harvested from different crop borders: Crop Border 1, Crop Border 2, and Crop Border 3. Yellow circles are intact chloroplasts.

Figure 4.5: The SEM images of CRF at different magnifications

The microstructure of powder is one of the crucial characteristics that determine its solubility. It was stated that amorphous solid products would have better solubility and oral bioavailability than crystalline products (Tomar et al., 2022; Vranić, 2004).

From Figure 4.5, the grainy-look or less smooth structure observed on the CRF powder was expected to be the effect of thylakoids stacking into the membrane in a half-protruding state. The images were similar to the fractured isolated thylakoids shown in the previous reports (Bogorad, 1981; Syamila, 2019). At 500x magnification, all CRF samples appeared like shard shapes with grittiness and lumpy surfaces. The porosity of CRF from SPH in every crop border was hardly noticeable, which may be related to their high viscosity and low free water content.

The low water availability disturbs the formation of ice crystals leading to a reduction in the porosity of the dried materials (Malik et al., 2017). Unlike the CRF from spinach, several intact chloroplasts (yellow circles) were spotted at higher magnifications (1000x and 3000x) in the CRF from SPH. Although our CRF showed crystalline solid formation and limited porosity structures, the intact chloroplast still can be seen, identified by the round or oval shape structure under the microscope. No intact chloroplasts from spinach were reported in the CRF due to its lack of porosity and high presence of crystalline form in the structure (Syamila, 2019).

4.5 Chemical properties

4.5.1 Proximate composition and chlorophyll content

The proximate composition and chlorophyll content of chloroplast-rich fraction (CRF) from sweet potato haulm (SPH) were presented in Table 4.4. CB 1, CB 2, and CB 3 mean the samples were harvested from different crop borders: Crop Border 1, Crop Border 2, and Crop Border 3.

Table 4.4: Proximate composition and chlorophyll content of CRF

Proximate composition (g/100 g dw)	CRF from different crop borders			
	CB 1	CB 2	CB 3	Mean
Crude protein	15.70 ± 0.03 ^c	19.44 ± 0.16 ^a	18.03 ± 0.03 ^b	17.72
Crude fibre	4.98 ± 0.70 ^b	4.11 ± 0.55 ^b	10.07 ± 1.21 ^a	6.39
Crude fat	2.88 ± 0.27 ^b	4.62 ± 0.09 ^a	5.32 ± 0.63 ^a	4.27
Ash	14.66 ± 0.16 ^a	11.54 ± 0.02 ^c	12.19 ± 0.01 ^b	12.80
Moisture	6.50 ± 0.27 ^c	7.51 ± 0.04 ^b	8.22 ± 0.08 ^a	7.41
Carbohydrate	55.25 ± 0.25 ^a	52.76 ± 0.61 ^a	46.14 ± 1.83 ^b	51.38
Pigment concentration (mg/g dw)				
Chlorophyll <i>a</i>	2.96 ± 0.02 ^c	5.02 ± 0.06 ^a	4.36 ± 0.02 ^b	4.11
Chlorophyll <i>b</i>	1.68 ± 0.06 ^b	2.63 ± 0.10 ^a	2.54 ± 0.10 ^a	2.28
Total chlorophylls	4.64 ± 0.08 ^c	7.65 ± 0.11 ^a	6.90 ± 0.10 ^b	6.40
Pigment ratios				
Chlorophyll <i>a/b</i>	1.76 ± 0.06 ^{ab}	1.91 ± 0.07 ^a	1.72 ± 0.07 ^b	1.80

Different letters mean significant differences (Tukey's test, $p < 0.05$) between values. Data are means ± SD ($n = 3$). CB 1, CB 2, and CB 3 mean the CRF samples were harvested from different crop borders: Crop Border 1, Crop Border 2, and Crop Border 3. Carbohydrate content was obtained by subtracting the sum of moisture, ash, crude protein, crude fat, and crude fibre contents from 100.

Based on the data, the largest composition in CRF of SPH for each crop border (CB) was contributed by carbohydrates. High carbohydrate content in the CRF of SPH

may originate from the sugar or starch content found in the green biomass (Torcello-Gómez et al., 2019). The carbohydrate content in CRF from CB 3 (46.14 g/100 g dw) was significantly lower than CB 1 (55.25 g/100 g dw) and 2 (52.76 g/100 g dw). The result was comparable to the CRF from pea vine haulm, in which the researcher stated 42 g/100 g dw of carbohydrates in the fresh biomass (Torcello-Gómez et al., 2019). Compared to the CRF from other green leaves, CRF from SPH had a higher carbohydrate content than CRF from spinach, kale, and grass but was similar to that of stinging nettles with values of 16.4, 17.4, 37.6, and 49.8 g/100 g dw, respectively (Gedi et al., 2017).

The moisture, ash, and crude protein content in the CRF of SPH were significantly different between the CB with values ranging from 6.50 to 8.22, 11.54 to 14.66, and 15.70 to 19.44 g/100 g dw, respectively ($p < 0.05$). These findings were in agreement with the previous report on the moisture, ash, and crude protein content of the dried sweet potato leaves from three different harvesting periods (Suárez et al., 2020). The CRF from CB 3 recorded the highest amount of crude fat (5.32 g/100 g dw) and crude fibre (10.07 g/100 g dw) compared to CB 1 (2.88 and 4.98 g/100 g dw, respectively) and CB 2 (4.62 and 4.11 g/100 g dw, respectively).

The significant difference in the nutritional content might be due to the different ratios of the haulm parts (leaf, petiole, and stem) in each CB. About 3 to 7 times higher protein content was reported by Ishida et al. (2000) in leaf (3.68 – 3.80 g/100 g dw) than in petiole (0.50 – 0.70 g/100 g dw) and stem (0.85 – 1.43 g/100 g dw) of two sweet potato varieties, cultivated in Japan. Similarly, the leaf presented the highest value for ash, followed by petiole and stem, with values ranging from 1.53 to 1.88, 0.94 to 1.65, and 0.84 to 1.30 g/100 g dw, respectively (Ishida et al., 2000). As for dietary fibre

content, Ishida et al. (2000) reported about 2 to 4 times higher value in stem (10.4 – 11.3 g/100 g dw) compared to petiole (2.42 – 4.61 g/100 g dw) and leaf (5.94 – 6.90 g/100 g dw).

The CRF from SPH recorded a greater percentage of carbohydrate, ash, and fat compared to the fresh SPH juice reported by Hanifah et al. (2022a) with values of 42.18, 10.57, and 2.42 g/100 g dw, respectively. However, the researcher stated a higher percentage of protein and fibre (35.23 and 8.44 g/100 g dw, respectively) than the CRF (Hanifah et al., 2022a). This might be due to the different harvest locations (crop borders) and different compositions of sweet potato haulm parts used throughout the study. To support our findings, we also collected protein and fibre data from our juice samples, and the results recorded were lower than our CRF samples (average values of 24.32 and 5.62 g/100 g dw, respectively for juice samples). In addition, a higher percentage of moisture in the freeze-dried SPH juice was observed by Hanifah et al. (2022a). This could be due to the centrifugation method that separates the CRF pellet and supernatant (containing more liquid) during the isolation of CRF.

The total chlorophyll content of CRF was significantly different between the CB, where CRF from CB 2 recorded the highest total chlorophyll content followed by CB 3 and CB 1 with mean values of 7.65, 6.90, and 4.64 mg/g dw, respectively (Table 4.4). This was due to the different proportions of haulm (leaf, petiole, and stem) in each CB, as leaves generally contain more chlorophyll pigments compared to petiole and stem. Li et al. (2017) reported that the chlorophyll in the sweet potato leaves had about 7.6 to 14.7 times higher content than the stalks. The mean values of chlorophyll *a* and *b* for leaves of fourteen sweet potato cultivars ranged from 3.29 to 5.19 mg/g dw and

from 1.12 to 1.94 mg/g dw, respectively while for stalks ranged from 0.27 to 0.57 mg/g dw and from 0.10 to 0.25 mg/g dw, respectively (Li et al., 2017).

A higher total chlorophyll content was exhibited by CRF from SPH (mean value of 6.40 mg/g dw) in comparison to the sweet potato leaves (5.68 mg/g dw) reported by the aforementioned study, indicating that the centrifugation method proposed in this study helps recover and concentrate the chloroplasts, regardless of the haulm proportion which consists of leaves, stems, and petioles (stalks). About nine genotypes of sweet potato leaves demonstrated remarkably high total chlorophyll content (ranging between 14.58 and 23.76 mg/100 g fw) after 30 days of planting, but the content gradually decreased with plant age (Hossain et al., 2019).

In a more recent study, drought and waterlogging stress significantly reduced the chlorophyll *a*, chlorophyll *b*, and total chlorophyll content of sweet potato leaves after 71 days of stress period (Orsák et al., 2023). Hence, plant parts, plant age and abiotic stress such as drought and waterlogging could have an impact on the chlorophyll content of sweet potato plants. Syamila (2019) recorded a greatly higher total chlorophyll content in freeze-dried spinach juice (11.66 mg/g dw), related to its greener colour (- 12.05) which was 3 times higher compared to the CRF from SPH (- 4.73).

The ratio of chlorophyll *a* to *b* is considered generally 3:1. This ratio is a key parameter to indicate the shade tolerance of plant species where shade-tolerant species display a lower ratio under shade in comparison to their counterparts grown under high light environments (Beneragama & Goto, 2010). Shade-tolerant species plants will produce a lower chlorophyll *a* to *b* ratio to absorb blue light in low-light environments efficiently (Yamazaki et al., 2005).

In our study, a low ratio of chlorophyll *a* to *b* was found in the CRF with a mean value of 1.80 mg/g dw (Table 4.4). The low chlorophyll *a* and high chlorophyll *b* content lead to a 2:1 ratio of chlorophyll *a* to *b*. A higher ratio of chlorophyll *a* to *b* was found in sweet potato leaves of five different strains with values ranging between 2.247 and 2.854 ratios (Katayama & Shida, 1970). Hence, the sweet potato plant (haulm) examined in our study can be considered a shade-tolerant species as a consequence of the lower proportion of chlorophyll *a* to *b* (2:1 ratio) present in the haulm probably due to the different planting areas.

In this study, the concentrated chloroplasts (CRF) of sweet potato haulm harvested from different crop borders had variations in their proximate compositions and chlorophyll content. This was attributable to the different ratios of haulm parts from each crop border which consists of leaves, stems, and petioles (stalks). Previous findings mentioned in this section clearly stated the difference in nutritional values present in the leaves and petioles (stalks) of sweet potato plants.

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

The total phenolic content (TPC) and antioxidant content (DPPH and FRAP assays) of chloroplast-rich fraction (CRF) from sweet potato haulm (SPH) were illustrated in Figure 4.6. CB 1, CB 2, and CB 3 mean the samples were harvested from different crop borders: Crop Border 1, Crop Border 2, and Crop Border 3.

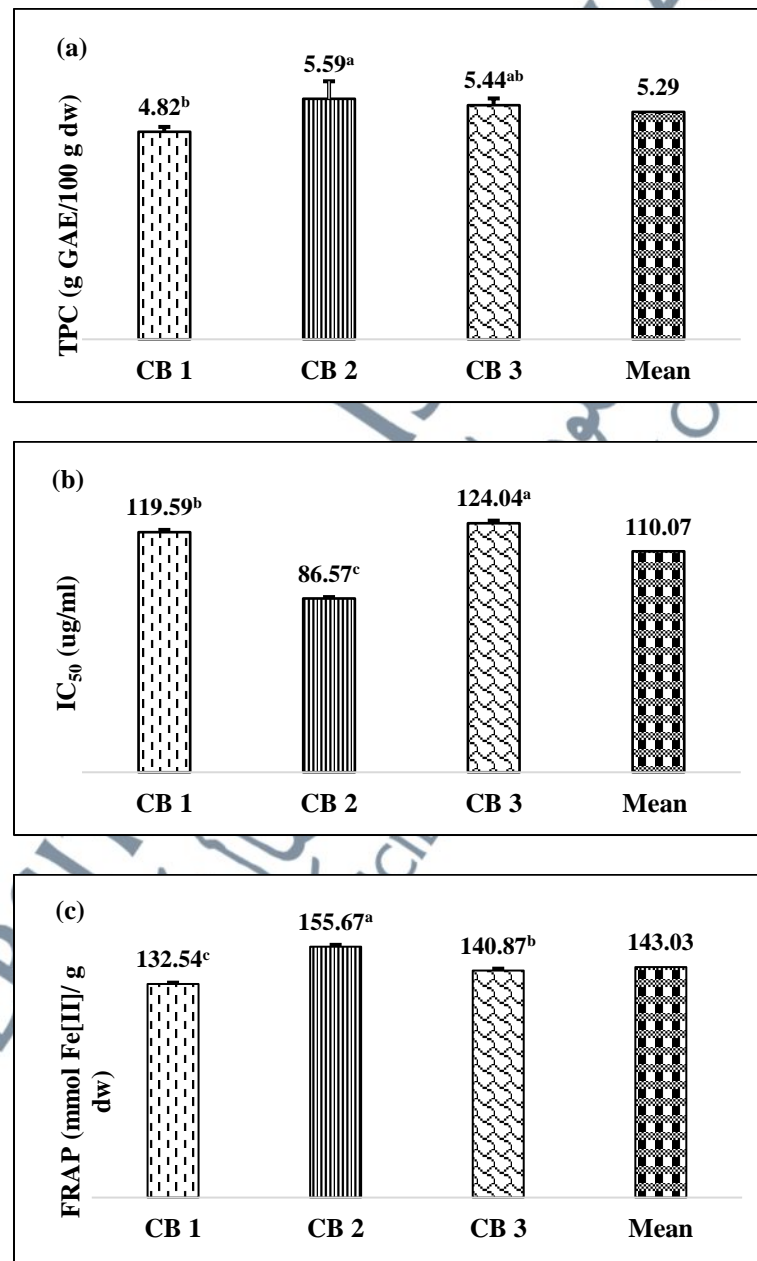


Figure 4.6: TPC (a), DPPH (b), and FRAP (c) of CRF. Results expressed with different letters mean significant difference (Tukey's test, $p < 0.05$)

The TPC of the CRF harvested from the three crop borders (CBs) was between 4.82 and 5.59 g GAE/100 g dw (Figure 4.6 [a]). These findings were congruent with the previous literature reports. Hue et al. (2012) reported that the TPC of six different sweet potato leaf cultivars ranged from 2.78 to 5.35 g GAE/100 g dw. Conversely, the TPC of 40 varieties of sweet potato leaves were found to be broader in range, with values between 2.73 and 12.46 g/100 g dw (Sun et al., 2014a). Islam et al. (2002) reported comparable TPC values with the earlier study, which varied from 1.42 to 17.1 g/100 g dw in 1389 sweet potato genotypes collected from all over the world.

The CRF from CB 1 (4.82 g GAE/100 g dw) showed a significantly lower value of TPC than CB 2 (5.59 g GAE/100 g dw) while no significant difference was found for CB 3 (5.44 g GAE/100 g dw). The difference observed might be due to the disproportionate parts of haulm (leaf, petiole, and stem) processed at each CB. The concentration of phenolics is possibly dependent on the maturity stage of different plant parts. Padda and Picha (2007) reported that the young leaf of sweet potato had a significantly higher amount of TPC compared to the mature leaf, old leaf, and petiole with values of 87.29, 27.77, 21.56, and 7.62 mg/g dw, respectively. The authors stated that the higher TPC may be related to the higher auxin production in the young leaf tissue. Moreover, sweet potato leaves contained about 10 to 30 folds higher phenolic content than petioles (Jang & Koh, 2019). Generally, the leaf part was found to have a greater TPC than the petiole and stem.

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay is a free radical scavenging method that reduces the violet DPPH solution into a colourless solution in the presence of antioxidant compounds in the plant extract. IC₅₀ is the concentration required to scavenge 50 % of the free radicles and the amount is inversely proportional to the

scavenging activity of the CRF. Hence, a lower value of the IC_{50} indicates a higher activity of antioxidants. The radical scavenging activity of the CRF extracts is presented in Figure 4.6 (b). All the extracts showed significant differences in IC_{50} values among the CBs. The CRF from CB 2 had the highest scavenging activity ($IC_{50} = 86.57 \mu\text{g/ml}$) while the CRF from CB 3 had the lowest scavenging activity ($IC_{50} = 124.04 \mu\text{g/ml}$).

Suárez et al. (2020) recorded a significant difference in the value of DPPH radical scavenging activity in sweet potato leaves between two different harvesting periods which might be affected by the disproportionate parts of haulm as stated before, and the harvesting practices. Interestingly, our findings reported higher antioxidant activity compared to the previous study on six varieties of *Ipomoea batatas* leaves with IC_{50} values ranging between 372.40 and 597.61 $\mu\text{g/ml}$ (Hue et al., 2012). This demonstrated that the sweet potato haulm's CRF had more antioxidants than the sweet potato leaves. Moreover, higher scavenging activity (IC_{50}) was reported for our CRF in comparison to both *Ipomoea aquatica* varieties; water spinach (15.83 mg/ml) and land spinach (50.26 mg/ml) reported by Mariani et al. (2019).

The ferric reducing antioxidant potential (FRAP) is an assay that reduces the colourless ferric tripyridyltriazine (Fe^{3+} -TPTZ) complex into an intense blue ferrous tripyridyltriazine (Fe^{2+} -TPTZ) complex solution at low pH in the presence of antioxidant. The ferrous complex was maximally absorbed at 593 nm. The lower value of absorbance indicates a higher content of antioxidants. Similar to the DPPH radical scavenging assay, the antioxidant activity in CRF extracts of SPH harvested from CB 1, CB 2, and CB 3 were significantly different. The reducing activity of ferric ions in the three CRF extracts was presented in Figure 4.6 (c). A comparable trend was observed in our findings where CB 2 (155.67 mmol Fe [II]/g dw) had recorded the

highest FRAP activity followed by CB 3 and CB 1 (140.87 and 132.54 mmol Fe [II]/g dw, respectively).

However, CB 1 recorded the lowest FRAP activity, which was in contrast with its DPPH radical scavenging activity where the extract had a higher antioxidant capacity than CB 3. This may be the result when iron compounds are under the environment's influence, leading to different oxidation-reduction potentials (Ivanova et al., 2015). Therefore, the results attained by the FRAP assay will likely differ as not all antioxidants react with the selected oxidiser. The reducing power in CRF from SPH was higher compared to the previous study that reported on the six varieties of *Ipomoea batatas* leaf with values ranging from 320.5 to 565.3 $\mu\text{mol Fe (II)}/\text{g dw}$ (Ghasemzadeh et al., 2012). It was proven that our concentrated chloroplast extract possessed a much greater reduction of iron complexes in the presence of greater antioxidant compounds.

Polyphenols have been extensively reported to be significantly associated with the antioxidant activities in extracts of various plant species (Ghasemzadeh et al., 2012; Hue et al., 2010; Padda & Picha, 2007; Suárez et al., 2020; Zhang et al., 2019). Therefore, the antioxidant capacity in the CRF extract, as determined by DPPH and FRAP assays should be correlated with TPC. In our study, a negative correlation between TPC and the DPPH assay ($r = -0.5634$) was observed on the CRF. A negative correlation means the higher value of total phenolic content will give a lower IC_{50} DPPH scavenging activity value. The moderate correlation of TPC with their IC_{50} DPPH value can be explained as not all phenolic compounds give a strong antioxidant property. Xu et al. (2010) and Ghasemzadeh et al. (2012) observed a higher correlation between radical scavenging activity and TPC ($r = 0.7704$ and 0.8270 , respectively) of sweet potato leaves.

A strong positive correlation between TPC and the FRAP assay ($r = 0.8776$) has been reported for the CRF from SPH. This was in agreement with the findings by Xu et al. (2010), which reported a strong relationship between TPC and FRAP assay ($r = 0.9654$) on the sweet potato leaves. Moreover, the value of TPC of sweet potato leaf extracts was highly correlated with ferric-reducing activity ($r = 0.997$) in a previous study reported by Zhang et al. (2019). Hence, our findings have proven that the polyphenols content present in the CRF predominantly contributes to the antioxidant activity specifically observed by FRAP activity.

In a comparison of antioxidant assays used in our study, a strong negative correlation ($r = -0.8905$) was found between DPPH and FRAP assays for the CRF from SPH, indicating that the antioxidant compounds exhibit proton-donating ability. A negative correlation means the higher value of ferric-reducing activity (FRAP assay) will give a lower IC_{50} value (DPPH assay). A previous study by Xu et al. (2010) on sweet potato leaves also reported a strong correlation between both assays ($r = 0.8425$).

4.5.3 Antinutrients content (Oxalic and phytic acids)

Antinutrients can be indigenously present in plants acting as a defence mechanism against herbivore attacks. Nevertheless, the antinutrients are responsible for detrimental effects related to the digestion, absorption, and utilisation of nutrients and micronutrients. Based on Table 4.5, two antinutritional factors (oxalic and phytic acids) were determined in the chloroplast-rich fraction (CRF) of sweet potato haulm (SPH) harvested from different crop borders (CBs).

Table 4.5: The antinutrient content of CRF

Antinutrient content	CRF from different crop borders			
	CB 1	CB 2	CB 3	Mean
Oxalic acid (g/100 g dw)	1.89 ± 0.16 ^a	2.40 ± 0.14 ^a	2.20 ± 0.73 ^a	2.16
Phytic acid (mg/100 g dw)	71.70 ± 6.32 ^a	62.23 ± 13.16 ^a	55.62 ± 3.67 ^a	63.18

Different letters mean significant differences (Tukey's test, $p < 0.05$) between values. Data are means ± SD ($n = 3$). CB 1, CB 2, and CB 3 mean the CRF samples were harvested from different crop borders: Crop Border 1, Crop Border 2, and Crop Border 3.

Generally, no significant difference between the CBs for both antinutrients (oxalic and phytic acids) was noticed in the CRF ($p > 0.05$). The propagation technique, chemical usage, storage, and preservation methods used in growing the crop can be highly influential on the amount of antinutrients present in plants (Thakur et al., 2019). As the haulms were grown in one farming area and constantly implemented the same agriculture practices, the selection of disease-resistant varieties and the selection of biological agents to control pests and diseases were controlled for every CB.

The oxalates content in the CRF (mean value of 2.16 g/100 g dw) was slightly higher than the leaves of three sweet potato varieties in Kenya with values ranging from 1.35 to 1.82 g/100 g dw (Abong' et al., 2021). A previous finding on leaves of nine Kenyan sweet potato varieties reported a broader range of soluble oxalate content varying from 511.62 to 1618.71 mg/100 g dw (Abong' et al., 2020). The concentrated chloroplasts (CRF) from SPH were assumed to possess a greater concentration of oxalic acid compared to the dried sweet potato leaves. Hence, further treatment or processing mechanisms are strongly recommended for proper utilisation of the haulm.

The CRF from the three CBs was found to have a phytic acid content with a mean value of 63.718 mg/100 g dw (Table 4.5). This was extremely lower than the phytic acid content of sweet potato leaves reported by both findings with values ranging from 1.14 to 5.33 g/100 g dw (Abong' et al., 2020) and 0.55 to 1.07 g/100 g dw (Abong' et al., 2021) in nine and three sweet potato varieties, respectively. In contrast, Antia et al. (2006) reported an exceptionally lower value of phytic acid in *Ipomoea batatas* leaves collected from Nigeria with an average value of 1.44 mg/100 g dw. The disparity of plant parts, genetic diversity, and physiological makeup are some of the factors that contribute to the presence of phytates in plants (Abong' et al., 2020).

4.5.4 Minerals content

The chloroplast-rich fraction (CRF) from sweet potato haulm (SPH) harvested from different crop borders was overall rich in minerals as stated in Table 4.6.

Table 4.6: Mineral compositions (mg/100 g dw) of CRF

Elements	CB 1	CB 2	CB 3	Mean
K	9453.00 ± 467 ^a	9347.00 ± 175.0 ^a	9253.00 ± 218.0 ^a	9351.00
Ca	4887.00 ± 205 ^a	5183.30 ± 104.1 ^a	5293.33 ± 196.0 ^a	5121.21
Cl	1630.00 ± 151 ^a	1540.00 ± 45.8 ^a	1570.00 ± 34.6 ^a	1580.00
S	698.30 ± 64.2 ^a	728.30 ± 23.9 ^a	705.00 ± 21.9 ^a	710.53
P	599.70 ± 54.5 ^a	563.00 ± 15.13 ^a	581.70 ± 19.4 ^a	581.47
Si	380.70 ± 33.6 ^a	414.30 ± 21.9 ^a	381.30 ± 20.8 ^a	392.10
Mg	281.70 ± 23.7 ^a	282.00 ± 13.08 ^a	279.33 ± 13.32 ^a	281.01
Rb	121.33 ± 8.02 ^a	120.67 ± 0.58 ^a	116.67 ± 4.51 ^a	119.56
Fe	102.40 ± 8.96 ^b	130.33 ± 2.52 ^a	117.00 ± 4.0 ^{ab}	116.58
Sr	31.97 ± 3.41 ^a	36.63 ± 1.29 ^a	37.13 ± 1.70 ^a	35.24
Mo	29.90 ± 1.22 ^a	28.87 ± 0.49 ^a	29.63 ± 0.85 ^a	29.47
Zn	26.13 ± 1.33 ^a	24.07 ± 1.43 ^a	25.60 ± 1.32 ^a	25.27
Al	22.40 ± 0.96 ^a	24.90 ± 2.50 ^a	26.30 ± 3.06 ^a	24.53
Cu	19.17 ± 1.50 ^a	19.57 ± 1.42 ^a	19.33 ± 1.88 ^a	19.36
Br	14.33 ± 0.87 ^a	13.13 ± 0.70 ^a	15.13 ± 1.47 ^a	14.20
Mn	12.70 ± 0.35 ^a	11.30 ± 0.20 ^b	10.05 ± 0.33 ^c	11.35
Ni	4.40 ± 0.25 ^a	4.79 ± 0.93 ^a	5.51 ± 1.28 ^a	4.90

Different letters mean significant differences (Tukey's test, $p < 0.05$) between values. Data are means ± SD (n = 3). CB 1, CB 2, and CB 3 mean the CRF samples were harvested from different crop borders: Crop Border 1, Crop Border 2, and Crop Border 3.

Macroelements abundantly found in the CRF were magnesium (Mg), phosphorus (P), sulphur (S), chlorine (Cl), calcium (Ca), and potassium (K). Conversely, microelements detected were zinc (Zn), iron (Fe), copper (Cu), manganese (Mn), silicon (Si), bromine (Br), molybdenum (Mo), rubidium (Rb), strontium (Sr), and

nickel (Ni). Every crop border (CB) showed comparable concentrations for all seventeen elements (except Mn and Fe). The sweet potato crops were cultivated in one agricultural land area, along with the controlled application of fertilisers and pesticides.

Mineral K was reported as the most abundant macroelement found which was in agreement with previous studies (Suárez et al., 2020; Sun et al., 2014a; Chuang et al., 2011). Our K values (9253 – 9453 mg/100 g dw) were at least 5 folds of values recorded by Sun et al. (2014a) on the leaves of forty sweet potato cultivars (average content of 1625.1 mg/100 g dw) and 3 folds of values recorded by Suárez et al. (2020) on the sweet potato leaves harvested from three different periods (1963.8 – 3341.7 mg/100 g dw). Similarly, the Ca content in our CRF (4887.0 – 5293.33 mg/100 g dw) was significantly higher than those reported in previous studies with values ranging only between 25.21 and 1958.1 mg/100 g dw (Chuang et al., 2011; Iyaka et al., 2015; Suárez et al., 2020; Sun et al., 2014a).

The third highest macroelement found in the CRF was Cl, followed by S with mean values of 1580.0 and 710.53 mg/100 g dw, respectively. The average value of mineral P was 581.47 mg/100 g dw, which was lower than the findings from Sun et al. (2014a) with an average content of 1248.2 mg/100 g dw. However, Suárez et al. (2020) stated lower P values compared to our study, ranging from 137.7 to 153.3 mg/100 g dw. The value of the mineral Mg ranged from 279.33 to 282.0 mg/100 g dw, comparable to the previous findings (Chuang et al., 2011; Tang et al., 2021).

The CRF from all CBs were overall rich in various trace elements (Si, Rb, Fe, Sr, Mo, Zn, Al, Cu, Br, Mn, and Ni) as stated in Table 4.6. The Fe, Zn, Cu, and Mn content (average content of 116.58, 25.27, 19.36, and 11.35 mg/100 g dw, respectively)

was considerably higher than values reported by previous studies on the sweet potato leaves (Nguyen et al., 2021; Sun et al., 2014a; Tang et al., 2021). For element Mo, the values stated by Chuang et al. (2011) were significantly lower than our study with average values of 0.06 and 29.47 mg/100 g dw, accordingly. Other trace elements found in the CRF (Si, Rb, Sr, Al, Br, and Ni) were greatly high in content with average values of 392.1, 119.56, 35.24, 24.53, 14.20, and 4.9 mg/100 g dw, respectively.

No element Na was found in our CRF compared to the findings by Sun et al. (2014a) with an average content of 159.98 mg/100 g dw. This could be due to the extremely high value of K⁺ present, which is considered the dominant monovalent cation in chloroplasts, compared to Na⁺ (Robinson & Downton, 1984). Moreover, the accumulation of Na⁺ generally occurs in the cytoplasm which is prone to be excluded during the isolation of the CRF process (Nieves-Cordones et al., 2016).

There was no traceable number of toxic metals such as arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) in the CRF from every CB. A higher concentration of essential elements discovered in our study suggested that the CRF had a greater amount of minerals than the sweet potato leaves.

4.6 Conclusions

This study indicates the variation in nutritional, antinutritional and physical properties of chloroplast-rich fraction (CRF) of sweet potato haulm (SPH) harvested from three different crop borders (CBs).

It is concluded that;

1. The CRF from SPH had desirable properties of powder due to the low water activity (0.24 – 0.45 a_w) and low dispersibility (38 – 41 %) which indicates good shelf life and great flowability.
2. Low water solubility in the CRF was observed in both cold and warm conditions, with values ranging between 17 and 28 %. This is possibly affected by the crystalline solid formation and limited porosity structures seen from scanning electron microscopy (SEM) images.
3. It can be observed that moisture, ash, and crude protein were significantly different among the CBs ($p < 0.05$) which might be due to the various ratios of the haulm parts (leaf, petiole, and stem) in each CB.
4. Total chlorophyll content significantly differed among the CBs ($p < 0.05$), with CRF from Crop Border 2 (CB 2) showing the highest value (7.65 mg/g dw). The chlorophyll content of CRF was higher than in stalks (0.10 – 0.57 mg/g dw) and leaves (1.12 – 5.19 mg/g dw) of fourteen sweet potato cultivars in South Korea.
5. Notable differences in the total polyphenol contents and antioxidant activity were found with CRF from CB 2 having the most potent antioxidant compounds among the CBs. The CRF showed remarkably greater antioxidant activity (86.57

$\mu\text{g/ml}$ (IC_{50}) and 155.67 mmol Fe (II)/g dw) than both varieties of *Ipomoea aquatica* (water spinach and land spinach) and sweet potato leaves.

6. A moderate and strong correlation of total phenolic contents against the antioxidant activity was shown in the IC_{50} DPPH ($r = -0.5634$) and FRAP ($r = 0.8776$) assays, demonstrating that the polyphenols in the CRF were accountable for the antioxidant activity.
7. Both antinutrient factors of CRF were low in concentrations (mean values of 2.16 g/100 g dw and 63.18 mg/100 g dw) for oxalic and phytic acids, respectively. This reveals that the haulm is a safe ingredient to be incorporated in health-promoting products for human or animal consumption.
8. The composition of almost all elements in CRF between the CBs was comparable. The CRF contains essential minerals including potassium, calcium, phosphorus, iron, magnesium, zinc, copper, and manganese.
9. The isolation of chloroplast by centrifugation method to obtain CRF considerably contributed to the greater level of nutritional properties beneficial to human and animal consumption. In conclusion, the sweet potato haulm's CRF could be a natural source of essential nutrients in developing new ingredient innovations.