CHAPTER 6

DIGESTIVE STABILITY AND BIOACCESSIBILITY OF β-CAROTENE AND LUTEIN OF CHLOROPLAST-RICH FRACTIONS (CRFs) FROM SWEET POTATO HAULM

6.1 Introduction

Agricultural biomass such as sweet potato haulm (SPH) potentially benefits human health as it contains abundant essential nutrients such as carotenoids. No present data have been reported on the bioavailability of carotenoids in SPH. Thus, this chapter aims to discuss the findings for research objective 3, which is *in-vitro* digestive stability and bioaccessibility of β-carotene and lutein of chloroplast-rich fractions (CRFs) from sweet potato haulm.

Bioaccessibility is a digestive process to release carotenoids from the food matrix. It presents the proportion of micellarised nutrients in the total digesta which are available for absorption in the gastrointestinal tract after digestion. The bioaccessibility can be influenced by two categories: carotenoid-related and unrelated groups. The carotenoid-related factors include dosage, chemical structure (isomeric forms), and interaction between different carotenoids, while the unrelated factors include thermal treatment, nutrient composition of co-consume foods, biometric of consumer, particle size of digested foods, micellarisation efficiency, transport from the enterocytes to the lymph system (Shilpa et al., 2020).

Carotenoids are released from the food matrix by heat, mechanical and physical food processing, the mastication process, and the action of salivary enzymes in the mouth (Shilpa et al., 2020). Carotenoids such as β-carotene and lutein are lipophilic in nature. Therefore, they must undergo solubilisation from the food matrix, followed by a micellarisation process.

In-vitro digestion is a prevalent method in determining bioaccessibility since it is inexpensive, rapid, versatile, and provides a preliminary investigation before executing *in-vivo* animal or human studies (Brodkorb et al., 2019; Courraud et al., 2013; Mackie et al., 2020; Minekus et al., 2014). The digestibility of nutrients in the CRF was determined using the static *in-vitro* digestion model by Minekus et al. (2014). It consists of a multi-compartment test, where oral, gastric, and small intestinal phases are simulated in sequence with gastrointestinal conditions representative of a healthy-fed state human adult. A time of two hours for gastric digestion is recommended in this static *in-vitro* digestion model, representing the half-emptying of a moderately nutritious and semi-solid meal (Minekus et al., 2014). Subsequently, the transit time of simulated intestinal digestion is suggested for two hours as well, to ensure the comparability of results between *in-vitro* digestion studies using the INFOGEST protocol by Minekus et al. (2014).

In this study, nutrient retention is defined as the percentage of nutrients present in the total digesta (end sample of the digestion process) compared with the initial nutrient concentration in the CRF. Meanwhile, nutrient bioaccessibility (BA) is defined as the percentage of nutrients in the total digesta that is micellarised and available for intestinal absorption. It is identified by comparing the nutrient in the micellar fraction to the nutrient in the total digesta. Determining nutrient stability during digestion and liberating the nutrient into the micellar phase is regarded as a nutrient accessible for uptake (NA). It is measured by the percentage of nutrients available for absorption, by comparing the nutrient concentration in the micellar fraction to the initial nutrient concentration in the CRF (pre-digested sample). The determination of nutrient retention after digestion, nutrient bioaccessibility (BA), and nutrient accessible for uptakes (NA) in the CRF from SPH are reported in this study.

In this chapter, the experiments include the inclusion of edible oil (2.5 % palm oil) to increase the BA and NA of β-carotene and lutein in the heat-treated CRFs involving heat treatments of conventional pasteurisation (CP), steam pasteurisation (SP), and water blanching (WB) as stated in the Section 3.1.3.2, Table 3.1. The optimisation of carotenoid accessibility of heat-treated CRFs in the presence of edible oil was determined by an *in-vitro* digestion model.

Results and Discussion

6.2 Pre-digested nutrient concentration

The carotenoid content in the starting material for the chloroplast-rich fractions (CRFs) ranged from 0.23 to 0.38 mg/g dw in β-carotene and from 0.25 to 0.41 mg/g dw in lutein (Table 6.1). Overall, a comparable range of β-carotene and lutein were found in the CRFs of sweet potato haulm (SPH).

Table 6.1: Carotenoid content of CRF

CRF with different heat treatments	Carotenoid content (mg/g dw)	
	β-carotene	**Lutein**
a) Fresh (F)	0.38 ± 0.04^a	0.41 ± 0.05^a
b) Conventional pasteurisation (CP)	0.23 ± 0.01^b	0.25 ± 0.01^b
c) Steam pasteurisation (SP)	0.28 ± 0.02^b	0.30 ± 0.02^b
d) Water blanching (WB)	0.26 ± 0.05^b	0.31 ± 0.04^b

Different letters mean significant differences (Tukey's test, $p < 0.05$) between values. Data are means ± SD (n = 3).

The β-carotene and lutein content were highest in fresh CRF (F) with no significant difference found in the carotenoid concentration between the heat-treated CRFs ($p > 0.05$). Li et al. (2017) reported that the β-carotene content of leaves from fourteen varieties of sweet potato cultivars was slightly higher than CRF, with a mean value of 0.41 mg/g dw. However, a lower content of β-carotene was reported in the same study for stalks (mean value of 0.01 mg/g dw) compared to CRF. In another study reported by Abong' et al. (2020), the β-carotene content of nine varieties of Kenyan sweet potato leaves was lower than our finding, with a mean value of 0.19 mg/g dw.

The fresh CRF from SPH showed comparable lutein content with Kenyan sweet potato leaves (Abong' et al., 2020) and higher than South Korean sweet potato leaves (Li et al., 2017) with a mean value of 0.38 and 0.25 mg/g dw, respectively. The lutein content in the stalks from the aforementioned study was exceptionally lower compared to its leaves, with a mean value of 0.03 mg/g dw (Li et al., 2017). Surprisingly, the sweet potato leaves from India, reported by Krishna et al. (2018) had a greater concentration of lutein (14.7 mg/g dw) in comparison to our CRF which was probably due to the difference in sweet potato variety (Donado-Pestana et al., 2012). Variations in the lutein content of sweet potato cultivars might be influenced by the weather conditions, including sunlight exposure, rainfall level, temperature, or nutrient deficiencies in the seed roots as a result of repeated harvesting practices (Ishiguro, 2019).

A study reported that the β-carotene content of sweet potato leaves was about 30 times higher than the stalks while the lutein content of leaves was 10 times higher than the stalks (Li et al., 2017), suggesting that the leaf of sweet potato plants has an extremely greater amount of carotenoids compared to the stalks (petioles). As our study collected concentrated chloroplasts from SPH (which consist of leaves, petioles, and stems), a lower concentration of the micronutrients was expected owing to the combination of high nutrient part (leaves) and low nutrient part (petioles and stems) from the SPH.

The β-carotene and lutein content of fresh CRF from SPH was 0.38 and 0.41 mg/g dw, respectively. In a study on CRF from pea vine haulm (PVH), fresh juice of the haulm reported 0.99 mg/g dw for β-carotene and 4.81 mg/g dw for lutein (Wattanakul et al., 2022). Gedi et al. (2017) also reported a higher range of β-carotene (2.47 – 3.36 mg/g dw) and lutein (2.14 – 3.41 mg/g dw) content in CRFs from spinach, kale, nettles, and grass compared to our CRF. The fresh CRF from spinach was 4.35 mg/g dw for β-carotene and 4.70 mg/g dw for lutein (Syamila, 2019). Although the fresh CRF from SPH exhibited lower concentrations of carotenoids compared to the other CRFs reported previously, it certainly contains a beneficial amount and has potential use as health-promoting ingredients in food formulations for either animal or human consumption.

Apart from cultivar varieties, carotenoids in sweet potato leaves tend to decline gradually throughout the growing season (Ishiguro, 2019). The concentration of β-carotene also varies with harvesting, storage conditions farming sites, root age, and virus infection (Burri, 2011). Thus, cultivar selection and harvesting time, with optimal farming and climatic conditions are important considerations in achieving higher carotenoid content from SPH.

Heat treatment is a well-known process to inactivate endogenous enzymes after harvesting, to preserve the nutritional value of plant material and extend the shelf life of nutrients during storage. In our study, a significant reduction of β-carotene and lutein was observed in all heat-treated CRFs ($p < 0.05$). The reduction of carotenoid content was in agreement with the antioxidant activity (FRAP assay) recorded in heat-treated CRFs – see Section 5.5.2, Figure 5.4 (c). However, no significant impacts on both micronutrients were found between the heat treatments ($p > 0.05$). CRF treated by conventional pasteurisation (CP) had the highest loss in β-carotene, followed by CRF from water blanching (WB) and steam pasteurisation (SP) for about 40, 32, and 26 % losses, respectively. The loss of lutein was highest in CRF from CP, followed by SP and WB with a loss of 39, 27, and 24 %, respectively.

A comparable reduction of nutrients was found in a recent study by Wattanakul et al. (2022) which the CRF of heated pea vine haulm (HPVH) had about 34 % loss in β-carotene and about 40 % loss in lutein. The heat treatment for HPVH is similar to our heating protocols for SP, where the biomass is heated first before the juicing process. Regardless of the nutrient loss in HPVH, the β-carotene and lutein content were consistent between the CRF of fresh juice PVH and heated juice (HJ) PVH (Wattanakul et al., 2022). The heat treatment for HJ in the aforementioned study is according to our heating protocols for CP treatment, where the biomass is juiced first before the heating process. Surprisingly, our CRF from CP had the highest loss of β-carotene and lutein while CRF from HJ was consistent after heat treatment (Wattanakul et al., 2022).

Hence, heating the SPH after the juicing process (CP treatment) appears to cause a greater loss of carotenoids in comparison to heating the haulm before the juicing process (SP and WB treatments). The loss of nutrients in the CRF could be affected by the enzyme activity or thermal decomposition. Thus, optimising commercial heat treatment conditions is recommended to efficiently inhibit enzyme activity and retain carotenoids (Wattanakul et al., 2022).

6.3 Effect of heat treatments on carotenoid retention and bioaccessibility of CRF

The nutrient retention (%), nutrient bioaccessibility (BA %), and nutrient accessible for uptake (NA in % and mg/g dw) of fresh and heat-treated (conventional pasteurisation, steam pasteurisation, and water blanching treatments) chloroplast-rich fractions (CRFs) from sweet potato haulm (SPH) are tabulated in Table 6.2.

6.3.1 Nutrient retention

The stability of lipophilic nutrients in the chloroplast-rich fraction (CRF) of sweet potato haulm (SPH) was determined by the percentage of carotenoid retention after the digestion process. The effect of conventional pasteurisation (CP), steam pasteurisation (SP), and water blanching (WB) treatments on the nutrient retention of CRFs is demonstrated in Table 6.2. Generally, the CRFs seem to have good retention of β-carotene and lutein with percentages ranging from 41 to 88 % and from 59 to 94 %, respectively.

The results demonstrated that lutein was more stable in the digested CRFs compared to β-carotene. The highest stability of β-carotene was recorded in the CRF from CP, followed by WB, fresh, and SP treatment, with retention ranging from 41 to 88 %. The highest stability of lutein was found in the CRF from WB, followed by fresh, CP, and SP treatment, with retention ranging from 59 to 94 %.

Table 6.2: The concentration of β-carotene and lutein accessible for uptake after *in-vitro* digestion in heat-treated chloroplast-rich fractions (CRFs) from sweet potato haulm

Nutrient concentrations	**F**	**CP**	**SP**	**WB**
β-carotene				
Initial concentration (mg/g dw)	0.38 ± 0.04^{a}	0.23 ± 0.01^{b}	0.28 ± 0.02^{b}	0.26 ± 0.05^{b}
Nutrient retention (%)	68.78 ± 11.32^{ab}	88.25 ± 5.08^{a}	41.15 ± 3.86^{b}	79.90 ± 20.50^{a}
Nutrient bioaccessibility (BA%)	11.12 ± 2.21^{c}	11.90 ± 0.29^{bc}	17.30 ± 2.03^{a}	15.75 ± 1.11^{ab}
Nutrient accessible for uptake (NA%)	7.50 ± 0.86^{b}	10.49 ± 0.37^{ab}	7.07 ± 0.38^{b}	12.47 ± 2.75^{a}
Nutrient accessible for uptake (NA mg/g dw)	0.028 ± 0.00^{a}	0.024 ± 0.00^{b}	0.020 ± 0.00^{c}	0.032 ± 0.00^{a}
Lutein				
Initial concentration (mg/g dw)	0.41 ± 0.05^{a}	0.25 ± 0.01^{b}	0.30 ± 0.02^{b}	0.31 ± 0.04^{b}
Nutrient retention (%)	88.72 ± 16.62^{ab}	76.34 ± 7.30^{ab}	59.33 ± 8.13^{b}	94.21 ± 10.21^{a}
Nutrient bioaccessibility (BA%)	13.93 ± 2.28^{b}	8.45 ± 2.85^{b}	29.23 ± 2.16^{a}	25.35 ± 2.12^{a}
Nutrient accessible for uptake (NA%)	11.47 ± 1.68^{c}	6.34 ± 1.68^{d}	17.29 ± 2.28^{b}	23.74 ± 0.72^{a}
Nutrient accessible for uptake (NA mg/g dw)	0.047 ± 0.01^{b}	0.016 ± 0.00^{c}	0.051 ± 0.00^{b}	0.073 ± 0.01^{a}

Different letters mean significant differences (Tukey’ s test, $p < 0.05$) between values. Fresh, CP, SP, and WB mean the CRF samples were treated with different heat treatments: Fresh (no heat treatment), CP (conventional pasteurisation), SP (steam pasteurisation), and WB (water blanching).

Heat treatment of biomass or juice tends to reduce the robust physical nature of CRF material, resulting in a more efficient release of carotenoids during the extraction of digesta (Wattanakul et al., 2022). However, the degradation of nutrients could occur due to pH changes and a slight increase in temperature through the digestion process. Carotenoids are more sensitive to acidic than alkaline conditions, proven by the significant loss of β-carotene (52 % loss) in carrot juice during the gastric phase (Courraud et al., 2013).

Heat treatments (CP and WB) improve the retention of lipophilic nutrients in the CRFs. This was in agreement with a study on heat-treated pea vine haulm and juice CRFs, owing to the breakdown of enzymes that are responsible for carotenoid degradation (Wattanakul et al., 2022). Nonetheless, the treatment SP exhibited the utmost impact on the loss of β-carotene (59 %) and lutein (41 %) in the CRF, probably influenced by the higher cooking temperature (100 °C) compared to other treatments (85 °C), and subsequently demolished heat-sensitive micronutrients.

Although the trend for nutrient stability (CP > WB > SP) in heat-treated CRFs was not compatible with the retention of antioxidant activity (CP > SP > WB) – see Section 5.5.2, Figure 5.4. Perhaps, the lipophilic nutrients present in the CRF from SP were more sensitive towards *in-vitro* digestion conditions compared to those from CP and WB treatments. Thus, it may be proposed that heat treatment aids in improving the retention of carotenoids. Among the treatments, CP and WB efficiently retained both β-carotene and lutein in the CRF from SPH.

6.3.2 Nutrient bioaccessibility (BA) and nutrient accessible for uptakes (NA)

Carotenoid bioaccessibility is limited and influenced by a variety of factors, crucially the food matrix composition and the degree of food processing (Castenmiller et al., 1999; Deming & Erdan, 1999). As nutrient bioaccessibility (BA) compares the value of micellarised nutrients in micelle with the digesta, the nutrient accessible for uptake (NA) instead gives the value of the final micellar phase compared to the nutrient present in the initial CRF. This is to give a better understanding of how much nutrient is available for absorption by the body, relative to the initial concentration of the nutrient in the chloroplast-rich fractions (CRFs) of sweet potato haulm (SPH).

The accessibility of nutrients in the CRFs treated with conventional pasteurisation (CP), steam pasteurisation (SP), and water blanching (WB) was determined (Table 6.2). The β-carotene bioaccessibility (BA %) and nutrient accessible for uptake (NA %) in all digested CRFs ranged from 11.12 to 17.30 % and from 7.07 to 12.47 %, respectively. The β-carotene bioaccessibility was highest in CRF of SP, followed by WB, CP and fresh. Even so, the highest nutrient accessible for uptake (NA mg/g dw) of β-carotene was in CRF of WB (0.032 mg/g dw), followed by CP (0.024 mg/g dw), fresh (0.028 mg/g dw) and SP (0.02 mg/g dw).

The BA % and NA % for lutein in all digested CRFs ranged from 8.45 to 29.23 % and from 6.34 to 23.74 %, respectively. The BA % of lutein was highest in CRF of SP, followed by WB, fresh, and CP. Even so, the highest nutrient accessible for uptakes (NA mg/g dw) of lutein was in CRF of WB (0.073 mg/g dw), followed by SP (0.051 mg/g dw), fresh (0.047 mg/g dw), and CP (0.016 mg/g dw). A similar trend between β-carotene and lutein accessibility (BA % and NA %) was shown in this study for CRFs treated by SP and WB.

The BA % of β-carotene and lutein was significantly increased after heat treatments excluding CRF treated with CP where no significant difference was noticeable for both nutrients. No significant difference in BA % of β-carotene and lutein was observed between SP and WB treatment. This was in agreement with findings from Arumsari et al. (2020), where higher BA % of β-carotene was presented in boiled (38 %) and microwaved (26 %) sweet leaves compared to raw (22 %).

In addition, the bioaccessibility of β-carotene and lutein improved significantly (15 and 72 folds, respectively) in spinach after hydrothermal cooking showing that cooking improves the release of carotenoid (Courraud et al., 2013). In a recent study, heat treatment on pea vine haulm CRF increased β-carotene and lutein accessible for uptake by at least 2 folds (Wattanakul et al., 2022). Cooking enhances nutrient bioaccessibility via a particle reduction mechanism, enhancing the release of phytochemicals after digestion (Hornero-Méndez & Mínguez-Mosquera, 2007).

Hayes et al. (2021) reported a significant decrease in carotenoid bioaccessibility for thermally treating spinach explained by the changes in free mineral content after the thermal treatment, hindering the bioaccessibility of lipophilic nutrients. The accessible divalent minerals released from the processing and digestion could reduce the micellarisation of carotenoids through interaction with fatty acids, bile salts or other constituents, limiting their solubility in the aqueous phase (Wattanakul et al., 2022). Inhibitory effects of divalent ions (calcium and magnesium) on carotenoid micellarisation and uptake were observed in the *in-vitro* digestion of spinach (Biehler et al., 2011a, 2011b).

In our study, nutrient accessible for uptake (NA %) for lutein was reduced significantly in the CRF treated by CP, compared to SP and WB. This could be triggered by the considerable concentration of divalent minerals (calcium, magnesium, iron, zinc, copper, manganese) retained (or even increased) in the CRFs after the heat treatments – see Section 5.5.4, Table 5.6. The retained minerals might convert into divalent ions, which are the cofactors in inhibiting the micellarisation of lipophilic compounds, resulting in lower bioavailability in the CRF.

Generally, the accessibility of lutein (BA % and NA %) was slightly higher than β-carotene in the CRFs of SPH. It has been stated that lutein is more easily released from the food matrix than β-carotene attributable to the lower polarity and greater hydrophilicity of xanthophylls compared to carotenes (Castenmiller et al., 1999; Chitchumroonchokchai et al., 2004; Garrett et al., 1999). This was supported by a recent study, which reported a greater release of lutein (about 4 folds greater) compared to β-carotene in CRF from fresh pea vine haulm juice (Wattanakul et al., 2022).

Food processing through the application of heat treatments showed a positive impact on nutrient bioaccessibility (BA %) from SPH (except CP). However, only WB treatment improved the nutrient accessible for uptake (NA %) in the CRFs. The lower temperature and cooking time in WB treatment could give the best conditions to promote bioaccessibility and nutrient accessible for uptake in the CRF. Modification in heat treatment conditions involving heating temperature and heating time is recommended to enhance the accessibility of nutrients present in the CRF of SPH.

In our findings, water blanching (WB) appears to be the best heat treatment compared to conventional pasteurisation (CP) and steam pasteurisation (SP). It is an economical method that destroys enzymatic activity and removes pesticide residues in vegetables or fruit. Although WB seemed to have a great loss in phenolic content and antioxidant activity (due to the leaching of water-soluble and heat-sensitive nutrients) – see Section 5.5.2, Figure 5.4, the amount of nutrients accessible for absorption was successfully recovered in the CRF.

In addition, the fortification of edible oil could increase the micellarisation of carotenoids and subsequently improve their bioaccessibility (Chitchumroonchokchai et al., 2004; Garrett et al., 1999; Hornero-Méndez & Mínguez-Mosquera, 2007). As low accessibility of β-carotene and lutein was seen in the fresh and heat-treated CRFs from SPH, the carotenoid accessibility can be optimised by the inclusion of palm oil (2.5 %) during the digestion.

6.3.3 Effect of oil addition

Palm oil was chosen in this study since it is a locally produced oil, commonly used in cooking. Besides being economical, palm oil is also a good source of free fatty acids and does not contain a measurable number of carotenoids.

Overall, palm oil addition (2.5 % of wt/wt) improved the accessibility of both β-carotene and lutein in all heat-treated CRFs (Table 6.3). Although there was a significant reduction in nutrient retention (%), the bioaccessibility of micronutrients (BA %) in the heat-treated CRFs was recovered and a significant increment ($p < 0.05$) was observed with the presence of dietary lipids. The increment trend in BA % of β-carotene was comparable to lutein, with percentage increase ranging from 25 to 42 %, and from 21 to 31 %, accordingly.

The β-carotene reported a slightly higher percentage increase of BA %, suggesting that edible oil contributed more effect on the micellarisation of β-carotene in comparison to lutein. This was supported by the latest finding where in the presence of oil, the amount of β-carotene in the micellar phase was higher than lutein in the CRF of heated pea vine haulm juice (Wattanakul et al., 2022). Dietary lipids assist the micellarisation process by two mechanisms: i) facilitate the transfer of carotenoids from the food matrix to the lipid droplets during the gastric phase, and ii) formation of small oil droplets by emulsifiers (pancreatic lipase and bile salts) which incorporate into the mixed micelles and further absorbed in the intestine (Arumsari et al., 2020).

Table 6.3: The concentration of β-carotene and lutein accessible for uptake after *in-vitro* digestion with palm oil in heat-treated chloroplast-rich fractions (CRFs) from sweet potato haulm

Nutrient concentrations	**CP**	**CP + O**	**SP**	**SP + O**	**WB**	**WB + O**
β-carotene						
Initial concentration (mg/g dw)	0.23 ± 0.01^{a}	0.22 ± 0.00^{a}	0.28 ± 0.02^{a}	0.18 ± 0.02^{b}	0.26 ± 0.05^{a}	0.31 ± 0.00^{a}
Nutrient retention (%)	88.25 ± 5.08^{a}	81.50 ± 7.22^{a}	41.15 ± 3.86^{b}	78.00 ± 7.19^{a}	79.90 ± 20.50^{a}	54.13 ± 1.83^{b}
Nutrient bioaccessibility (BA%)	11.90 ± 0.29^{b}	44.50 ± 7.29^{a}	17.30 ± 2.03^{b}	59.60 ± 6.67^{a}	15.75 ± 1.11^{b}	40.38 ± 0.01^{a}
Nutrient accessible for uptake (NA%)	10.49 ± 0.37^{b}	35.92 ± 3.17^{a}	7.07 ± 0.38^{b}	46.20 ± 2.04^{a}	12.47 ± 2.75^{b}	21.87 ± 1.66^{a}
Nutrient accessible for uptake (NA mg/g dw)	0.024 ± 0.00^{b}	0.080 ± 0.01^{a}	0.020 ± 0.00^{b}	0.084 ± 0.01^{a}	0.032 ± 0.00^{b}	0.067 ± 0.01^{a}
Lutein						
Initial concentration (mg/g dw)	0.25 ± 0.01^{a}	0.13 ± 0.00^{b}	0.30 ± 0.02^{a}	0.18 ± 0.02^{b}	0.31 ± 0.04^{a}	0.21 ± 0.00^{b}
Nutrient retention (%)	76.34 ± 7.30^{a}	86.10 ± 6.27^{a}	59.33 ± 8.13^{a}	73.00 ± 8.78^{a}	94.21 ± 10.21^{a}	80.59 ± 2.70^{a}
Nutrient bioaccessibility (BA%)	8.45 ± 2.85^{b}	39.01 ± 3.56^{a}	29.23 ± 2.16^{b}	50.16 ± 3.77^{a}	25.35 ± 2.12^{b}	46.75 ± 3.98^{a}
Nutrient accessible for uptake (NA%)	6.34 ± 1.68^{b}	33.44 ± 0.75^{a}	17.29 ± 2.28^{b}	36.49 ± 3.62^{a}	23.74 ± 0.72^{b}	37.61 ± 2.14^{a}
Nutrient accessible for uptake (NA mg/g dw)	0.016 ± 0.00^{b}	0.043 ± 0.00^{a}	0.051 ± 0.00^{b}	0.065 ± 0.00^{a}	0.073 ± 0.01^{a}	0.080 ± 0.00^{a}

Statistical analysis was run and compared between the same sample with and without oil. Different letters mean significant differences (Tukey’ s test, $p < 0.05$). CP, SP, WB, and + O mean CRF treated with CP (conventional pasteurisation), SP (steam pasteurisation), WB (water blanching), and + O (with oil addition).

The addition of palm oil significantly increased the β-carotene accessible for uptake (NA mg/g dw) by at least 2 to 4 folds for the heat-treated CRFs with CRF of WB having the lowest concentration. Moreover, CRFs of CP and SP exhibited a substantial increase in lutein accessible for uptake (NA mg/g dw), while CRF of WB reported no significant difference. It explains that the addition of oil had the least influence on the CRF of WB in comparison to the other heat treatments. Still, WB treatment appears to have the highest lutein accessible for uptake among the treatments.

All heat treatments appear to have a positive impact on the β-carotene and lutein accessible for uptake after *in-vitro* digestion with palm oil. However, only little to no difference in nutrient accessibility was demonstrated between heat treatments, suggesting that nutrients present in the CRF from SPH react to the treatments proposed in our study in a similar approach. Both lipophilic nutrients were accessible in a comparable range with and without oil, but still low compared to other findings on CRFs (Syamila, 2019; Wattanakul et al., 2022).

The increment in the proportion of micellarised carotenoids was established with the increment of dietary lipids (Hornero-Méndez & Mínguez-Mosquera, 2007; Tan et al., 2020). Hence, enhancement in nutrient bioaccessibility is proposed for future works by; i) increasing the amount of oil inclusion (2.5 to 10 % of oil addition) to the CRF material, or ii) blending CRF material with edible oil to create an emulsion for better encapsulation and hydrophobic compound delivery, thus aiding the micellarisation process. Micelle formation (micellisation) creates water-soluble compounds, enabling lipid digestion products that are hydrophobic in nature (such as lipophilic nutrients) to be transported to the small intestinal surface for absorption.

6.4 Conclusions

This study provides information on the stability and accessibility of β-carotene and lutein sourced from chloroplast-rich fractions (CRFs) of sweet potato haulm (SPH). The variations in retention and bioaccessibility of nutrients were investigated with the implementation of heat treatments (conventional pasteurisation [CP], steam pasteurisation [SP], and water blanching [WB]) and oil inclusion (2.5 % palm oil).

It is concluded that;

1. Food processing through the application of heat treatment showed a positive impact on nutrient retention, nutrient bioaccessibility (BA %) and nutrient accessible for uptake (NA %) in the CRFs.
2. CRF from SP had the highest BA (29.2 %) while CRF from WB had the highest NA (23.7 %) and nutrient retention (94.2 %). Besides, CRF from WB had the highest nutrient accessible for uptake (0.073 mg/g dw), suggesting that WB is an excellent treatment for stabilising lipophilic nutrients during digestion.
3. The inclusion of oil increased the BA of β-carotene (at least 2 to 4 folds) and lutein (at least 1 to 2 folds), presenting the highest nutrient accessible for uptake (0.084 mg/g dw) in CRF from SP.
4. This implies that SP treatment in combination with oil addition, gives the best accessibility of β-carotene and lutein for absorption.
5. Implementation of heat treatment (concerning low heating time and temperature) with edible oil addition is recommended to optimise nutrient uptake of CRF from SPH.