

CHAPTER 7

CONCLUSION AND RECOMMENDATION

7.1 General conclusions

The first objective of this study is the determination on the effect of different crop borders on the physicochemical variation of chloroplast-rich fractions (CRFs) from sweet potato haulm (discussed in Chapter 4). This research concludes that the CRFs of SPH harvested from different crop borders (CBs) had variations in proximate compositions (moisture, ash, and crude protein), chlorophyll content, and antioxidant activity (DPPH and FRAP assays). Total chlorophyll content was highest in CRF from Crop Border 2 (CB 2) with a value of 7.65 mg/g dw. CRF from CB 2 also had the most potent antioxidant compounds among the CBs. The CRF showed remarkably greater antioxidant activity (86.57 $\mu\text{g/ml}$ [IC₅₀] and 155.67 mmol Fe [II]/g dw) than both varieties of *Ipomoea aquatica* (water spinach and land spinach). A moderate and strong correlation of total phenolic contents against the antioxidant activity was shown in IC₅₀ DPPH ($r = -0.5634$) and FRAP ($r = 0.8776$) assays, demonstrating that polyphenols in the CRF were accountable for the antioxidant activity. The composition of almost all elements in CRF was comparable between the CBs ($p > 0.05$). The CRF contains essential minerals including magnesium, iron, zinc, copper, manganese, potassium, calcium, and phosphorus. This research supports that centrifugation of CRF has contributed to a significant level of nutritional properties, especially antioxidant compounds. The CRF from SPH had desirable properties of powder; low water activity

(0.24 – 0.45 a_w) and low dispersibility (38 – 41 %), which results in good shelf life and great flowability behaviour. In addition, CRF exhibited a comparable low concentration of antinutritional factors between CBs ($p > 0.05$); oxalic acid (1.89 – 2.40 g/100 g dw) and phytic acid (55.62 – 71.70 mg/100 g dw), suggesting that the CRF from SPH is a safe ingredient to be exploited and fortified in health-promoting products such as dietary supplement and animal feed.

For the second objective of this study, which is to emphasise the effect of heat treatments on the physicochemical variation of chloroplast-rich fractions (CRFs) from sweet potato haulm (discussed in Chapter 5), it is found that heat treatments proposed in this study: conventional pasteurisation (CP), steam pasteurisation (SP), and water blanching (WB) greatly influenced the physicochemical properties of the CRF. SP treatment exhibits an impact on the proximate composition: crude protein, crude fat, and carbohydrate contents. Conversely, CP treatment succeeded in retaining the entirely proximate composition present in CRF while WB treatment was effective in minimising the loss of total chlorophyll concentration (35 %) in CRF, compared to CP (65 %) and SP (64 %). Total phenolic content (TPC) retention in heat-treated CRFs ranged from 24 to 50 % and higher in antioxidant (FRAP assay), ranging from 36 to 84 %. Antioxidant activity (DPPH assay) increased with about 20 to 30 % increment. CP treatment preserved TPC and antioxidant activity greatest, followed by SP, and WB. Heat treatments have successfully diminished at least half of the antinutrient oxalic acid (from 50 to 61 % reduction) and increased several important minerals (potassium, sulphur, magnesium, iron, and manganese) to a greater extent. In physical analysis, heat treatments improved the solubility of CRF by the formation of porous and amorphous surfaces (seen at 1000x and 3000x magnifications of scanning electron microscopy

images). No intact chloroplast was shown in all heat-treated CRFs, implying that the chloroplast had a cell wall breakage, releasing the thylakoids from the membrane. The highest retention in the green colour of CRF was observed after WB, proven by the least loss in chlorophyll pigments. Concerning the nutritional and quality of CRF, both WB and CP treatments excellently preserved nutrients by different approaches. WB treatment is competent in minimising chlorophyll degradation, resulting in the best green colour preserved by the CRF. CP treatment is alternatively expert in retaining the amount of total phenolic compounds and antioxidant activity (DPPH and FRAP assays) of CRF, besides efficiently reducing antinutrient (oxalic acid) present at a lower concentration.

Finally, the third objective on the digestive stability and bioaccessibility of β -carotene and lutein of chloroplast-rich fractions (CRFs) from sweet potato haulm (discussed in Chapter 6) has shown an improvement after implementing heat treatments. Steam pasteurised CRF had the highest bioaccessibility (BA) while water blanched CRF had the highest nutrient accessible for uptake (NA) for both micronutrients. Water blanched CRF also had the highest NA (0.073 mg/g dw), highlighting that water blanching is an excellent treatment for stabilising lipophilic nutrients of CRF during *in-vitro* digestion. Moreover, the inclusion of edible oil (2.5 % palm oil) further increased the BA of β -carotene (at least 2 to 4 folds) and lutein (at least 1 to 2 folds), with the highest NA (0.084 mg/g dw) in CRF from SP. This implies that CRF with steam pasteurisation (SP) treatment in combination with edible oil addition, had the best accessibility of lipophilic nutrients for absorption.

7.2 Recommendations for future works

The liberation of chloroplast from its cell wall is supposed to assist the release of nutrients from the plant's cellular environment. Yet, low carotenoid content was reported in the chloroplast-rich fraction (CRF) from sweet potato haulm (SPH), ranging from 0.23 to 0.38 mg/g dw and from 0.25 to 0.41 mg/g dw for β -carotene and lutein, respectively. In addition, the carotenoids accessible for uptake (NA %) were low (22 – 46 %), even with the combined application of heat treatment and oil inclusion – see Section 6.3.3, Table 6.3.

Therefore, there are 3 recommendations on the fundamental scientific works (Section 7.2.1) regarding 1) Nutrient bioaccessibility, 2) Carotenoid isomerisation, and 3) Latex production issues. On top of that, 2 recommendations on applied research and development (Section 7.2.2) relating 1) Nutrient bioaccessibility, and 2) *In-vitro* digestion model issues. Last but not least, 2 suggestions for farm operation (Section 7.2.3) involving 1) Drying process and 2) Heat treatment.

7.2.1 Fundamental scientific

Reduction in particle size is a major factor that influences the positive effect on bioaccessibility (Lemmens et al., 2010). Since our study did not determine the particle size of CRF powder, its larger size could be the reason for the low nutrients present. Hence, the identification of particle size is suggested. Fractionation of the CRF powder using different particle size ranges is also recommended to study the water solubility index of powder. Besides, modifications in preparing CRF powder (to obtain smaller particle size) are proposed, as a way to enhance the bioaccessibility of nutrients.

Determination of soluble fibre in CRF is suggested in future studies as a higher amount of dietary fibre will result in a lower micellar carotenoid level (Aschoff et al., 2015). Since our CRF is sourced from sweet potato haulm (consisting of leaves, petioles, and stalks of sweet potato plants), dietary fibre could present in high amounts which is the key factor for minimal carotenoid bioaccessibility.

Heat treatment induced isomerisation of carotenoids and degradation of chlorophylls to pheophytins and pyropheophytins (Hayes et al., 2021). Our investigation did not discuss the carotenoid's isomer, so the interpretation of data on the carotenoid content might be limited. Thus, phytochemical conversion to the isomeric forms should be identified to better comprehend the phytochemical behaviour in thermal processed CRFs. Besides, using a typical reversed-phase column such as C18 could be difficult to separate trans- β -carotene from its cis isomers. Hence, a C30 column is a better option as it provides high shape selectivity for separating hydrophobic structurally related isomers and unique selectivity complementary to other reversed-phase columns (Jing et al., 2012).

In our study, latex was rapidly released from the stem and petiole parts of SPH upon wounding (data not presented). Plants produce latex as a natural defence against herbivores. Latex in sweet potato plants exhibits oxidase activity by polyphenol oxidase and peroxidase enzymes, affecting the physicochemical quality of plants after harvesting (Gracz-Bernaciak et al., 2021). Hence, a study on latex production is recommended for future works, to comprehend more about the quality and bioaccessibility of nutrients in CRF from SPH. Variations in genotypes and environmental conditions (e.g., light levels and cloudy weather) influenced latex production in sweet potato plants, and young parts of vines and leaves usually produced more latex than older and mature parts (Data et al., 1996).

7.2.2 Applied research and development

This study found that even though the nutrient accessible for uptake (NA) in the CRF is increased with 2.5 % of oil inclusion, the NA could be more if a higher amount of oil is incorporated into the incubation mixture. This is due to the higher formation of carotenoid-rich mixed micelles leading to an increase in the total amount of carotenoids available for absorption (Tan et al., 2020). Furthermore, the application of rabbit gastric extract (RGE) is suggested as it could increase bioaccessibility by fostering the emulsification of nutrients before their incorporation into mixed micelles (Iddir et al., 2021). The tensioactivity of rabbit gastric lipase is high, allowing it to penetrate phospholipid layers easily and become more resistant to bile salts during the intestinal passage (Iddir et al., 2021).

The involvement of higher mechanical forces during digestion will give better emulsion of human digestion and improve the release of nutrients (Wattanakul et al.,

2022). The motion mixes and shifts the chyme back and forth in the peristalsis mechanism aiding the bloodstream to efficiently absorb nutrients. With these considerations, the use of an impeller to mimic peristaltic movement in the digestive tract is recommended for future *in-vitro* digestion experiments.

The comparison between *in-vitro* digestion models; static, semi-dynamic, and dynamic with *in-vivo* studies (Mackie et al., 2020; Mulet-Cabero et al., 2020) in terms of bioaccessibility performance of nutrients should be understood properly. Although bioaccessibility is hard to measure *in-vivo*, a previous study reported that fish fed with CRF diets positively influenced their growth and carotenoid profile (Gedi et al., 2019). This justifies that *in-vivo* studies on supplements or innovative foods with CRF can be proposed in future work to study the physiological behaviour of the lipophilic nutrients generated *in-vivo*. Besides, a more harmonised *in-vitro* digestion model should be used (involving gastric lipase and peristaltic motion) to establish a better interpretation of CRF nature, specifically from sweet potato haulm, to maximise the lipophilic nutrients release.

7.2.3 Suggestions for farm operation

Even though the vacuum freeze-drying process efficiently preserves plant nutrients through its rapid and cold drying mechanisms, it appears uncommercial for farmers. Conventional drying such as the sun-drying process is cost-effective and environmentally friendly. It is suitable for application in small scale farming where electricity or other fuel sources may not be affordable or very limited. Another example of conventional drying is the oven-drying process, which is carried out at a lower cost in the agriculture industry. In addition, solar drying technology is efficient and practical

for application in medium and large scale agro-industrial due to its availability, low operation and maintenance cost system, reliability, and environmental friendly (Ortiz-Rodríguez et al., 2022). The solar drying process runs in the most hygienic way and enables farmers to preserve agricultural green waste even under damp conditions. Thus, sun drying, oven drying, and solar drying are considered better options for farmers in sustaining food security and agricultural economics.

Conventional pasteurisation (CP) is a simple heating process, commonly applied at home. However, heating biomass juice on a large scale will be more challenging since the heating time tends to be longer and the heating temperature might be difficult to control. This may influence the nutrient stability of the green biomass. Steam pasteurisation (SP) demands specific heating equipment (autoclave or steamer) and involves additional steps and materials such as packing the biomass into a plastic bag before the steaming process. Conversely, water blanching (WB) requires no specific equipment or materials and can be easily practiced on either small or large scale biomass. Moreover, WB treatment operates in a lower heating time compared to CP and SP treatments. Concerning the economic viability of farm operations, water blanching appears the most convenient post-harvest treatment for the sweet potato haulm.