CHAPTER 2

LITERATURE REVIEW

2.1 Chloroplast in green plants

Chloroplast is a type of green plastid, found in green plants, that performs photosynthesis reactions by converting carbon dioxide and water into organic chemicals using light as a source of energy. The green pigment chlorophyll, mostly chlorophyll *a*, is the primary pigment used in the beginning process of photosynthesis by absorbing light energy. The chloroplast in the leaf is rich in essential bioactive lipophilic nutrients, including carotenoids, tocopherols, and galactolipids enriched in omega-3 fatty acids (Castenmiller, et al., 1999; Gedi et al., 2019; Soll et al., 1980; Wattanakul et al., 2019).

The main carotenoids in chloroplasts are β-carotene, lutein, violaxanthin, and neoxanthin (Gross, 2012; Konishi, et al., 1968; Serrano et al., 2005; Thornber, 1975). Carotenoids play major roles in the survival of plants. While plants have a photoprotection mechanism for better survival under high light stress, carotenoids play roles by scavenging reactive oxygen species, quenching the chlorophyll-excited states, and scattering excess energy into heat (Sun et al., 2018).

2.1.1 Composition of chloroplast

Chloroplast has an oval, or disk-shaped body, surrounded by an outer double membrane with a diameter of ~5 µm and a width of ~2.5 µm (Staehelin, 2003). The most conspicuous internal feature is the granum, made up of a stack of thylakoids. The thylakoid stacks, or granum, are connected by lamella in the stroma. About 50 % of the dry weight of a thylakoid is protein. Chloroplast envelopes, consisting of inner membrane, intermembrane space, and outer membrane have about 15 – 20 % of the total number of proteins in the chloroplast (Block et al., 2007). In the presence of protein molecules in the membrane, the mobility of lipids is diminished by hydrophobic interaction (Syamila, 2019). Figure 2.1 illustrates the structure of chloroplast.

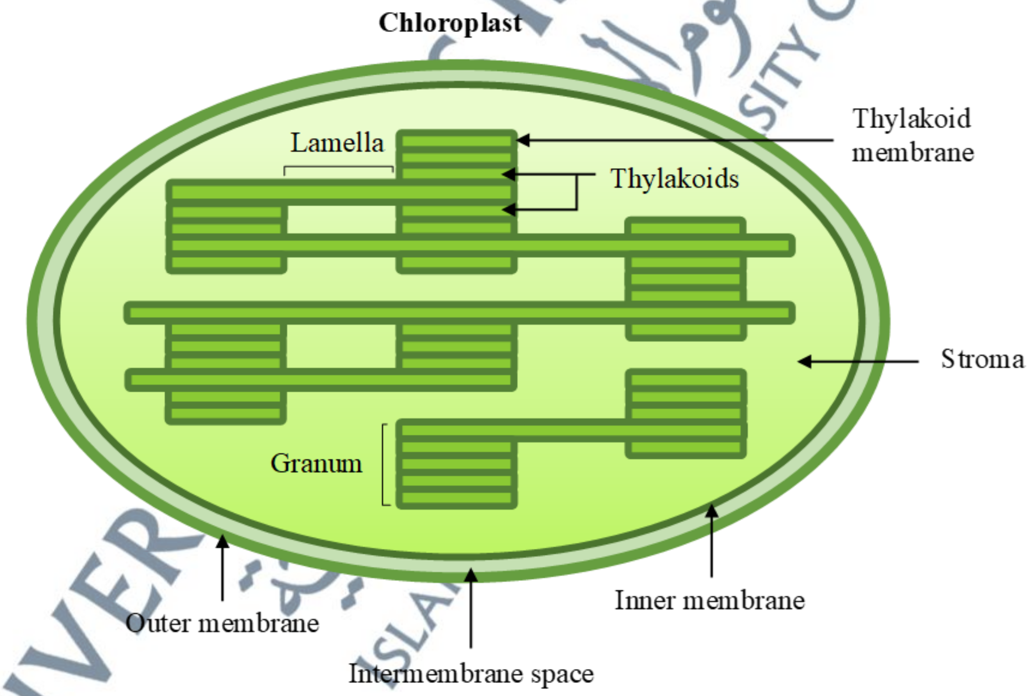


Figure 2.1: Structure of chloroplast

The inner space of thylakoids is filled with fluid called the lumen while thylakoid membranes are the layer that surrounds the thylakoids. The inner, outer, and thylakoid membranes are recognised as plastid membranes with a high proportion of galactolipids (70 %) and a low content of phospholipids (Syamila 2019). Monogalactosyldiacylglycerol (MGDG) is high in both thylakoids and the inner membrane, whereas digalactosyldiacylglycerol (DGDG) and phosphatidylcholine (PC) are high in the outer membrane (Block et al., 2007). The thylakoid membrane is the site where light-dependent photosynthesis reactions occur while light-independent reactions occur in the stroma. Carotenoids are located in thylakoid membranes for photosynthesis and photoprotection (Sun et al., 2018). The presence of carotenoids and the absence of chlorophylls reveal the yellow colour of chloroplast in higher plants (Block et al., 2007; Rodriguez-Amaya, 1999).

2.1.2 Chloroplast-rich fraction (CRF)

Chloroplast can be recovered and concentrated by centrifugation method. A previous study had proven that concentrated chloroplasts, also called chloroplast-rich fraction (CRF) from green biomass are enriched in proteins, lipids, trace minerals and essential micronutrients like β-carotene, lutein, α-tocopherol, α-linolenic acid compared with the whole leaf materials (Gedi et al., 2017; Torcello-Gómez et al., 2019; Wattanakul et al., 2019, 2021, 2022). Juice extraction is a sustainable physical fractionation used to extract chloroplasts from plant material (Torcello-Gómez et al., 2019; Wattanakul et al., 2021). The liberation of chloroplasts from their cellular environment should result in more accessible nutrients as the digestion process would not be impeded by the cell wall present in the cell-bound chloroplasts (Gedi et al., 2017).

On top of that, the centrifugation method has been proposed in previous studies to recover CRF from green biomass (Torcello-Gómez et al., 2019; Wattanakul et al., 2021). The CRF pellet or dried CRF was collected by juicing and filtering the green biomass, followed by centrifugation to obtain the CRF layer, and then drying to remove any residual water (Syamila, 2019). The CRF layer consists of different chloroplast shapes including fully intact, sliced intact and burst chloroplasts. The intact chloroplasts can be observed as green, oval-shaped, or slightly concaved while green particles scattered in the background of the intact chloroplasts are considered the burst chloroplasts, floating the unstacked thylakoids (Syamila, 2019). Figure 2.2 shows a micrograph of CRF from sweet potato haulm under a light microscope (100x magnification). The intact chloroplast (red circle) in CRF from sweet potato haulm with floating thylakoid (yellow arrow) can be seen in the micrograph.

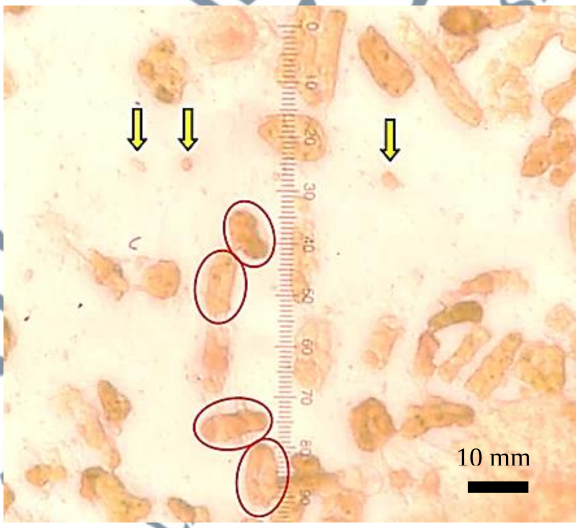


Figure 2.2: Micrograph of intact chloroplast (red circle) and thylakoid (yellow arrow) from sweet potato haulm under a light microscope (100x magnification)

2.2 Haulm

Agricultural activities generate a considerable amount of biomass waste in the form of four different sources; agro-industrial waste, aquaculture waste, livestock waste, and crop residue (Koul et al., 2022).

Haulm (from sweet potato, potato, and pea vine), straw (from barley, wheat, and rice), and stover (from corn, soybean, and sorghum) are examples of crop residues in agricultural fields. The non-harvested parts of the sweet potato plants are collectively called haulm. The haulm is used to feed livestock or plough back into the soil as a source of nitrogen, mulch, or burnt, still, most are treated as waste material (Zhang et al., 2019). Only a small portion of corn, wheat, and rice crop residues is utilised in the production of bioethanol or animal fodder, while the rest is discarded or burned (Koul et al., 2022).

To accomplish sustainable agriculture and environment, agricultural waste management is being implemented including animal feeding, roof thatching, surface mulch, composting, fertilisers, direct combustion, pyrolysis, bio-bricks, and bioconversion of biomass waste into various marketable products (Koul et al., 2022). Still, improper and minimal crop residue management practices could create substantial environmental pollution and raise public health concerns.

2.2.1 Component of haulm

Haulm is a post-harvest field residue, which is characterised by the plant parts that are left after harvesting the crops. In sweet potato plantations, the leaves, stems, and stalks (collectively called haulm) remain in the fields after sweet potato harvesting (Zhang et al., 2019). Similarly, it turns out that the stems, tops, and foliage of the potato plant are considered haulms after collecting the tubers. Harvesting peas (seeds) will leave behind other parts of the plant such as stalks, leaves, pods, and vines, which are typically called pea vine waste (or haulm) to rot on the land (Xia et al., 2016). In addition, the corn stover, which consists of stalks, leaves, tassels, cobs, and husks is left in the fields after the corn harvest (Koul et al., 2022). Straw or hay from crops such as rice, barley, oat, and wheat also are recognised as haulms, and their main purpose is usually for livestock feed or livestock bedding. Hence, the haulms can be identified as the collection of leaves, stems, stalks, or tops of various cultivated plants, left after harvesting the crops.

2.2.2 Research on haulm

Despite its profusion and attainability, haulm possesses great physicochemical quality and nutritional value (Hanifah et al., 2022a; Kaplan et al., 2018; Lamidi & Ingweye, 2020; Xia et al., 2016). The sweet potato (*Ipomoea batatas* L.) haulm juice powder (SPHJP) contains a considerable amount of carbohydrate, protein, ash, fibre, and fat with values ranging from 39.33 to 42.18, 35.23 to 35.26, 10.57 to 13.24, 7.72 to 8.44, and 2.42 to 2.68 g/100 g dw, respectively (Hanifah et al., 2022a). The study recorded that the pasteurisation process had decreased the carbohydrate content and increased the ash content in the SPHJP. High protein content reveals the SPHJP has the potential to be converted into a sustainable source of nutrients and an innovative plant-based protein. Pea vine (*Pisum sativum*) waste contains a high amount of holocellulose and lignin, starch, crude protein, and ash with values of 43, 22, 18, and 15 % dw, respectively (Xia et al., 2016). Holocellulose (native cellulose) is better than chemically modified cellulose grades in terms of eco-friendly characteristics and recyclability (Yang & Berglund, 2021).

Potato (*Solanum tuberosum* L.) haulm has high feed quality values considering its numerous minerals content present in the biomass. The potato haulm contains iron, manganese, copper, zinc, lead, nickel, cadmium, and cobalt with values varied from 47.35 to 180.07, 28.14 to 85.15, 10.84 to 15.35, 4.14 to 15.60, 4.14 to 15.60, 6.74 to 9.80, 3.40 to 8.60, 1.02 to 1.55, and 0.43 to 1.13 mg/kg dw, respectively (Kaplan et al., 2018). Lamidi and Ingweye (2020) reported that both fresh groundnut haulm and ensiled corn stover had higher crude protein content (12.18 and 11.67 %, respectively) compared to the cereal residues (range of 2 to 8 %).

On top of that, the groundnut haulm and ensiled corn stover were high in iron (164.46 and 159.78 mg/kg, respectively) and zinc (34.15 and 31.29 mg/kg, respectively) along with a certain amount of calcium, phosphorus, potassium, sodium, and magnesium with a range between 0.18 and 0.49 % (Lamidi & Ingweye, 2020). Green biomass has a variety of nutritional components that are potentially beneficial for dietary purposes and may provide significant contributions towards agricultural sustainability.

2.3 Antiquity and characteristics of sweet potato (*Ipomoea batatas* L.)

The sweet potato (*Ipomoea batatas* L.) is a creeping dicotyledonous plant belonging to the Convolvulaceae family or the morning glory family (Bovell-Benjamin, 2007). Plants in this family are vines, shrubs, or reptiles, which produce rhizomes and roots, and some species have latex and alkaloids (Cartabiano-Leite et al., 2020). It is an herbaceous perennial vine with white and purple sympetalous flowers, palmately lobed leaves or alternate heart-shaped and large nutritious storage roots (Mu & Li, 2019).

The stems cultivate in crawling and prostrate to the ground to promote cyclic sprouting, generating roots by making internodes in contact with the soil (Cartabiano-Leite et al., 2020). Sweet potato plant has a root with smooth skin whose colour ranges between beige, yellow, orange, red, purple, and brown while its flesh ranges from beige to white, pink, red, yellow, orange, purple and violet (Mu & Li, 2019). The starchy, large, and sweet-tasting roots are undoubtedly the most extraordinary part of the plant. A single sweet potato plant may produce about 40 to 50 roots, ranging from a few to 30 cm in length with a weight between 100 and 1,000 g (Bovell-Benjamin, 2007).

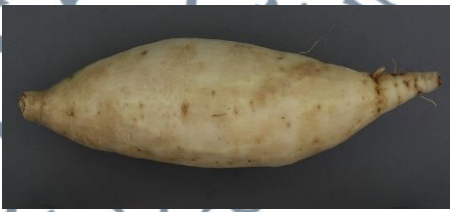
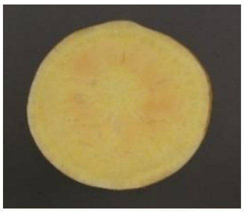
Besides its tuberous roots, other parts such as shoots, leaves and stems are all edible and nutritious. Consequently, this plant has been emphasized as the crop that managed to support more people per unit hectare than any other food (Bovell-Benjamin, 2007). The length of the stem ranges from 1 to 5 m, and the thickness ranges from 3 to 10 mm with internodes growing along the stem 2 to 20 cm apart (Cartabiano-Leite et al., 2020). The leaves are slightly pubescent or glabrous and the shape varies between ovate, elliptical, orbicular, sagittal, and have petiolate by the entire edges (Cartabiano-Leite et al., 2020).

The sweet potato is a versatile plant, due to its high-yielding tuber crops, drought-tolerant, and wide adaptability to various climates and farming systems (Bovell-Benjamin, 2007). This plant also has a higher tolerance to diseases and pests compared to other leafy vegetable plants grown in tropical countries (Islam, 2006). Figure 2.3 shows the sweet potato plantation located in Kangar, Perlis.

Figure 2.3: Sweet potato plantation located in Kangar, Perlis

In Malaysia, numerous varieties of sweet potatoes can be found such as Banting, Biru Jepun, Biru Johor, CH purple, CH Red, Gendut, Guan, Kuala Bikam, Vitato, 57 Tainung and others (Adzhar et al., 2023; Tan, 2015; Yusoff et al., 2018). The varieties are mainly recognised by colour differences in the skin and flesh of roots besides the disparity in shape and colour of their leaves and vines. Table 2.1 shows the varieties and characteristics of some sweet potatoes commonly found in Malaysia. The sweet potato plant used in the recent study was purple-skin yellow-fleshed sweet potato (Japanese Yellow variety) as shown in Table 2.1 (e). The sweet potato roots are commonly consumed as steamed or boiled sweet potato or else as ‘traditional’ snacks such as ‘keria’ and crisps. The roots are also consumed as ingredients in Malaysian desserts like curry puff and porridge.

Table 2.1: Characteristics of sweet potato varieties

Variety	Sweet potato skin	Sweet potato flesh
a) Biru Jepun		
b) CH purple		
c) Vitato		
d) 57 Tainung		
e) Japanese Yellow		

Source: Characteristics of Sweet Potatoes (*Ipomoea batatas*) Varieties. Retrieved from Plant Variety Protection Malaysia website: http://pvpbkkt.doa.gov.my.

The sweet potato is Malaysia's second largest cash-crop area, after sweet corn with around 2,776 hectares of plantation area in 2022 (Department of Agriculture Malaysia, 2023). In 2022, the yield of sweet potato root production was 16.098 tonnes per hectare (Department of Agriculture Malaysia, 2023). After three months of growing, the sweet potato roots are matured and harvested, and the next cycle of sweet potato plantation will immediately take place once it is cleared up. This allows the plantation and harvest cycle to be executed at least 3 times a year. Haulm which consists of stems, stalks, leaves, and shoots are left behind on the field as soon as the crops are collected.

2.4 Nutritional content of sweet potato stems and leaves

Aside from the excellent consumption of sweet potato tubers, other parts of the sweet potato plant such as shoots, leaves, stems, and stalks are possibly and effectively utilised as valuable foodstuffs attributable to their richness in nutrient contents. The vine, shoots, and young leaves of plants are good sources of vitamins, minerals, antioxidant molecules, and dietary fibre (Mu & Li, 2019). Moreover, part of the sweet potato haulm, specifically the leaves, is high in protein, phenolic compounds, antioxidant activity, ascorbic acid, and tocopherol (Suárez et al., 2020; Zhang et al., 2019). The nutritional value of sweet potatoes varies depending on the harvesting period, harvesting practices and production methods (Johnson & Pace, 2010).

2.4.1 Proximate composition

Moisture value is the highest composition among other proximate compositions found in sweet potato crop residue. Ishida et al. (2000) stated that the stalks had the highest value of moisture (88.9 – 94.4 g/100 g fw) compared to the leaves and stems with values reaching from 84.9 to 87.1 g/100 g fw and from 79.2 to 83.7 g/100 g fw, respectively. The moisture contents for three types of sweet potato leaves studied by Iyaka et al. (2015) had similar values ranging from 83.75 to 85.75 g/100 g fw. Sun et al. (2014b) obtained about 16.69 to 31.08 g/100 g dw of protein content and Tang et al. (2021) recorded comparable results ranging between 16.2 and 30.3 g/100 g dw. However, protein contents recorded by Johnson and Pace (2010) and Iyaka et al. (2015) had different values varied from 26 to 30 and from 21.85 to 24.53 g/100 g dw, respectively.

Based on a study conducted by Sun et al. (2014b), sweet potato leaves had lipid values in the range from 2.08 to 5.28 g/100 g dw, but another study found slightly lower ranging from 1.50 to 1.93 g/100 g dw (Iyaka et al., 2015). The crude fibre content in leaves varied among cultivars, ranging from 9.15 to 14.26 g/100 g dw (Sun et al., 2014b), and the findings were considered in range by the values (10.38 – 11.13 g/100 g dw) stated by Iyaka et al. (2015). Compared to earlier studies, recent findings recorded lower crude fibre content in the range of 5.18 to 10.38 g/100 g dw (Tang et al., 2021). Sweet potato leaves had the highest amount of soluble dietary fibre in comparison with stems and stalks (Johnson & Pace, 2010). Still, Ishida et al. (2000) found the highest fibre value in stems compared to leaves and stalks with average values of around 10, 6, and 7 g/100 g dw, correspondingly from two types of sweet potato cultivars.

The average carbohydrate content of sweet potato leaves was 51.0 g/100 g dw (Sun et al., 2014b). Conversely, higher values of carbohydrate contents recorded by Iyaka et al. (2015) ranged between 53.67 and 58.51 g/100 g dw leaving the leaves to have a profound contribution to the energy requirement for human adults. Ishida et al. (2000) found considerably low ash values in leaves, stems, and stalks of sweet potatoes with values ranging from 1.53 to 1.88, 0.84 to 1.30 and 0.94 to 1.65 g/100 g dw, respectively. In other findings, it was reported that ash contents for *Ipomoea batatas* L. leaves ranged from 7.75 to 10.25 g/100 g dw (Iyaka et al., 2015) while the earlier study found greater amounts of ash ranging from 7.39 to 14.66 g/100 g dw (Sun et al., 2014b). A high amount of ash indicates the presence of minerals abundantly in the plant. It was reported that a wide range of variations in the nutritional content of sweet potatoes harvested in different months were probably due to different locations of harvest fields (Suárez et al., 2020).

2.4.2 Total phenolic content and antioxidant activity

Due to their redox properties, phenolic compounds in plants could act as antioxidants. Antioxidants derived from plants are beneficial in protecting the human body from various health issues, including inflammation, oxidative damage, ageing, arthritis, cirrhosis, cancer, and Alzheimer's disease (Ghasemzadeh et al., 2012; Tang et al., 2021). Total phenol in the leaf extract of sweet potato varied between 4.47 and 8.11 mg GAE/g dw while its antioxidant activity varied between IC_{50} value of 184.3 to 450.46 μg/ml (Ghasemzadeh et al., 2012). In another study, sweet potato leaves from harvest period one recorded the highest content of total polyphenol and antioxidant activity with values of 9.1 g/100 g and 7.4 g VCE/100 g dw, respectively between the three different harvesting periods (Suárez et al., 2020). Subsequently, antioxidant activity was strongly correlated with total phenolic content estimated by DPPH radical scavenging activity (Cioloca et al., 2021; Ghasemzadeh et al., 2012).

Carotenoids, ascorbic acid, and tocopherols function as antioxidants in lipid phases by reacting with free radicals or singlet molecular oxygen (Sies & Stahl, 1995).

The carotenoid content of sweet potato tops is mainly from β-carotene and lutein. Islam (2016) reported carotenoid content in leaves ranged from 0.9 to 23.4 g/100 g dw which was higher than another finding from Ishiguro et al. (2004) with an average value of 34.7 mg/100 g dw for total carotene. The leaf tips of sweet potatoes studied by Tang et al. (2021) recorded higher carotenoid value for the purplish-green type (215 mg/100 g dw) compared to the pure green type (171 mg/100 g dw). It was stated that β-carotene and lutein contents in sweet potato leaves and stalks ranged from 19.01 to 28.85 and from 35.21 to 52.01 mg/100 g dw, respectively (Li et al., 2017).

β-carotene content published by Nguyen et al. (2021) varied from 273 to 400 μg/100 g dw, and they stated that the leaves held the highest value compared to stems and stalks. Similarly, the lutein content was highest in leaves followed by stems and petioles with average values of 36.8, 1.8, and 1.6 mg/100 g fw, respectively (Ishiguro & Yoshimoto, 2006). In different states of leaves, Krishna et al. (2018) reported lutein content with average values of 442.3, 179.5, and 14.7 mg/g for fresh, frozen, and dried leaves, respectively. In short, carotenoids were found higher in leaves than in petioles and stems of sweet potatoes.

Furthermore, the sweet potato tops, notably their leaves, are good sources of ascorbic acid (vitamin C) and tocopherol (vitamin E). The vitamin C content in the leaves of a Japanese cultivar was 72.0 mg/100 g dw (Ishiguro et al., 2004), those of South China cultivars ranged from 99 to 511 mg/100 g dw (Tang et al., 2021), and those of Taiwanese cultivars ranged from 62.7 to 81 mg/100 g dw (Nguyen et al., 2021). Suárez et al. (2020) reported the highest content of vitamin C (104.6 mg/100 g dw) and vitamin E (5.8 mg/100 g dw) in sweet potato leaves harvested during the third period (September 21) compared to the first and second harvest period (August 22 and

September 6). A higher content of vitamin E in leaves was recorded by Ishiguro et al. (2004) as compared to the previous study by Nguyen et al. (2021) with average values of 15.8 and 2.12 mg/100 g dw, respectively. Hence, harvesting in a particular period could also affect the concentration of particular nutrients present in sweet potato leaves.

2.4.3 Mineral composition

Minerals are generally classified into two groups: macrominerals and microminerals. Macrominerals such as calcium, magnesium, phosphorus, and potassium are required in greater amounts in the diet due to their essential roles in the human body. In contrast, microminerals, so-called trace minerals, are essential as well, but they are required in tiny amounts for our bodies (Sui et al., 2019). Some examples of microminerals are iron, zinc, manganese, and copper. Minerals help the body develop, grow, and stay healthy by performing various functions. Sweet potato plants particularly the leaves are good sources of mineral nutrients, mainly calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P), zinc (Zn), iron (Fe), manganese (Mn), sodium (Na) and copper (Cu), (Iyaka et al., 2015; Sun et al., 2014b; Tang et al., 2021).

The most abundant macroelement found in sweet potato leaves was K, followed by P, Ca, Mg and Na with an average content of 1625.1, 1248.2, 744.9, 405.2 and 159.98 mg/100 g dw, respectively (Sun et al., 2014b). Similarly, high amounts of essential minerals K, P, Ca, Mg and Na are found in the leaf tips in the range from 4,546 to 5,966, 431 to 592, 500 to 1,068, 200 to 280, and 14.0 to 544.1 mg/100 g dw, respectively (Tang et al., 2021). Other findings also recorded K, P, Ca, Mg and Na as minerals with high content in the leaves ranging from 479.3 to 4280.6, 1,311 to 2639.8, 229.7 to 1958.1, 220.2 to 910.5 and 8.06 to 832.31 mg/100 g dw, respectively (Nguyen et al., 2021). From other findings, some major essential elements such as Mg, Ca and Fe were found with lower values compared to the previous studies with values varied from 23.72 to 25.21, 33.17 to 35.42, and 17.05 to 18.03 mg/100 g dw, respectively in the leaves (Iyaka et al., 2015). The variety of crops, soil types, and preservation methods could probably contribute to the broad disparities of minerals amount found in sweet potato leaves (Iyaka et al., 2015).

Furthermore, the amplest microelement in *Ipomoea batatas* L. leaves stated by Sun et al. (2014b) was Fe, followed by Mn, Zn, and Cu with average values of 8.15, 4.10, 2.27, and 1.28 mg/100 g dw respectively. The contents of Fe, Mn, Zn, and Cu ranged from 8.82 to 18.44, 3.43 to 12.84, 2.80 to 4.84, and 1.25 to 1.93 mg/100 g dw, respectively, recorded by Tang et al. (2021) were comparable with the previous study exhibited by Sun et al. (2014b). However, a previous study reported lower values of Fe, Mg, Zn and Cu in the range from 1.9 to 21.8, 1.7 to 10.9, 1.2 to 3.2 and 0.7 to 1.9 mg/100 g dw, respectively (Nguyen et al., 2021). The purplish-green leaf tips recorded a maximum K/Na ratio, which is 3.9 times higher than pure green leaf tips, making them suitable for preventing atherosclerosis, hypertension, and cardiac arrhythmias (Sun et al., 2014b; Tang et al., 2021). The K/Na ratio plays a role in plant growth, photosynthesis activity, and preventing oxidative damage from reactive oxygen species (Hasanuzzaman et al., 2018).

2.5 Antinutritional content in plants

Oxalic acid and phytic acid are phytochemicals commonly classified as antinutritional factors in plants. They are predominantly found in salt forms (oxalates and phytates), which can chelate metal ions and interfere with the mineral's bioavailability and assimilation of proteins (Abong' et al., 2021).

2.5.1 Oxalic acid

Oxalic acid is mostly found in leafy vegetables and plants. It will build a strong bond with nutrients in the gastrointestinal tract, rendering them inaccessible to the body (Gemede & Ratta, 2014). When oxalates bind with calcium, insoluble calcium oxalates (salts) will form, interfering with the absorption of calcium from the diets. Incessant precipitation of insoluble calcium oxalates in the kidneys or urinary tract will form sharp-edge calcium oxalate crystals, leading to the formation of stones when acid is excreted in the urine (Gemede & Ratta, 2014). Thus, oxalic acid and its salts affect human nutrition and human health by causing the formation of kidney stones in the urinary tract (Issa et al., 2020). Moreover, oxalic acid builds strong bonds with other minerals such as magnesium, potassium and sodium resulting in the formation of oxalate salts (Gemede & Ratta, 2014).

2.5.2 Phytic acid

Phytic acid is the primary storage for phosphorus and inositol in leafy vegetables, plant seeds and grains. The salt form of phytic acid is called phytate, also known as inositol hexakisphosphate (Gemede & Ratta, 2014). It has a strong binding affinity for minerals such as magnesium, calcium, zinc, iron, copper, and molybdenum to form insoluble complexes which are not readily absorbed in the gastrointestinal tract (Akande et al., 2010; Issa et al., 2020). The phosphorus bound to phytate is not bio-available to humans and non-ruminant animals due to the absence of specific enzymes located in the first stomach chamber of ruminant animals, which is necessary to process the phosphorus in phytates (Gemede & Ratta, 2014). Too much phytate in the diet may result in mineral deficiency associated with nutritional deficiency diseases such as rickets, osteoporosis or osteomalacia (Issa et al., 2020).

2.5.3 Effect of processing methods on antinutrients

Antinutrient content in sweet potato leaves may vary depending on the plant variety, cooking, and preservation techniques. Oxalic acid was significantly reduced in sun-dried and cooked sweet potato leaves with a reduction of 9 and 54 %, respectively (Mwanri et al., 2011). Issa et al. (2020) stated that the amount of oxalic and phytic acids was reduced by boiling and wet frying cooking techniques in four types of vegetables (sweet potato leaves, green pigweed, drumstick tree and jute mallow) probably as a result of thermal degradation and dissolution in water.

In other studies, processing methods involving boiling, dehydration, and fermentation diminished the antinutrients (oxalic and phytic acids) in sweet potato leaves to different extents (Abong' et al., 2021). The boiling and dehydration processes break plants' cell walls, resulting in the leaching and degradation of antinutrients, whereas the fermentation process breaks down antinutrients through microbial action (Abong' et al., 2021). To sum up, processing and preservation methods such as boiling, frying, dehydration and fermentation could reasonably lessen antinutrients (oxalic and phytic acids) in sweet potato leaves.

2.6 Effect of heat treatments on nutrients

Implementation of heat treatment not only reduces the concentration of antinutrients naturally found in plants, but the approach also can inhibit the physicochemical quality degradation by the action of enzymatic activity (peroxidase and polyphenol oxidase). Cooking processes such as boiling, steaming, frying, roasting or microwaving could modify the physical characteristics, chemical compositions, and phytochemical contents depending on the processing conditions, chemical nature of the specific compounds, and structure of the food matrix (Kourouma et al., 2019).

Thermal treatment: blanching and pasteurisation are the most practical post-harvest treatments in inhibiting enzyme reactions, extending the shelf life of nutrients, and stabilising the texture and flavour of plant materials (Wattanakul et al., 2019, 2021). Blanching is necessary before the drying process to remove pesticide residues, enhance the drying rate, and inhibit browning effects by inactivating peroxidase and polyphenol oxidase present in plants (Luo et al., 2020). Heat treatment of biomass will inactivate endogenous enzymes that might cause lower-quality pea vine haulm (PVH) upon storage (Wattanakul et al., 2019, 2021, 2022). Heat treatment of juice recovered from the PVH is an effective way to preserve nutrients in the CRF powder (Wattanakul et al., 2019, 2022).

It was approved that the peroxidase and polyphenol oxidase activities were reported to decline by 85.7 and 87.64 %, respectively, in blanched sweet potato leaves (Luo et al., 2020). Enzyme inactivation was found to be proportional to the cooking treatment time of steaming, hot water, and microwave blanching (Severini et al., 2016).

However, steam blanching treatment required the shortest time (30 sec) to reduce about 90 % of the peroxidase enzyme in broccoli (*Brassica oleracea* L., var. *Italica*) compared to microwaving blanching (50 sec) and hot water blanching (90 sec) treatment (Severini et al., 2016). Furthermore, Wattanakul et al. (2019) suggested treatment of steam sterilisation (100 °C, 4 min) and hot water blanching (85 °C, 3 min) on the post-harvest residue to prevent loss of galactolipid content, further oxidation of polyunsaturated fatty acids and the release of free fatty acid.

In addition, steaming and boiling were preferred over microwaving, roasting, and frying to preserve carotenoids, phenolic compounds, and vitamin C in orange-fleshed sweet potato (Kourouma et al., 2019). The researchers reported higher retention of β-carotene after boiling (15 – 45 min) and steaming (15 – 45 min) with values ranging from 57.46 to 82.19 % and from 60.15 to 76.46 %, respectively.

For total phenolic content, about 91.37 % retention was found in orange-fleshed sweet potato after steaming for 35 min. Conversely, steaming for 25 min had the greatest antioxidant activity (65.86 %) than steaming for 15, 35, and 45 min with values of 55.96, 62.15 and 58 %, respectively in DPPH radical scavenging activity. Meanwhile, the retention rate of lutein content in sweet potato leaves was 118.9 % in stir-frying, 91.7 % in steaming, 79.7 % in simmering, and 75.9 % in boiling (Sugawara et al., 2011). As for petioles, the retention of lutein was 100.0 % in stir-frying, 75 % in simmering, 75 % in boiling and 62.5 % in steaming (Sugawara et al., 2011).

In summary, heat treatments could lessen the peroxidase and polyphenol oxidase activities, in addition to preserving carotenoids, phenolic compounds and other chemical constituents present in plants.

2.7 Bioaccessibility of lipophilic nutrients

Bioaccessibility is part of compounds released from its food matrix in the gastrointestinal tract, contributing to their availability for intestinal absorption (Cilla et al., 2018). Carotenoids and soluble vitamins (A, D, E, K) found in chloroplasts are lipophilic nutrients, or ‘lipid-loving’ molecules attracted to lipids. The cell wall of chloroplasts is a physical barrier for carotenoid release, and its polysaccharide composition cannot be degraded by human digestion. Only a few polysaccharides can be hydrolysed by the gut microbiota (Sriwichai et al., 2016).

The foremost step in the digestion of lipophilic nutrients is the liberation of the components from the food matrix. This step includes the reduction of particle size in addition to the cellular breakage to emancipate the carotenoids (Eriksen et al., 2017). The liberation of intact chloroplasts from their cellular environment offers a novel way to give more nutritional impacts on the consumer since the digestion process would not be impeded by the presence of a cell wall (Wattanakul et al., 2019, 2021, 2022). It allows better micellarisation of lipid-soluble vitamins, leading to a higher accessibility and absorption of nutrients (Gedi et al., 2019; Syamila, 2019).

Food matrix composition, food preparation (physical or mechanical treatments), and food processing (thermal or non-thermal treatments) are the factors that may have a great impact on the nutrients' bioaccessibility (Bohn et al., 2015). Food preparation such as juicing, chopping, cutting, slicing, trimming, grating, and mashing will have different nutrient bioavailability depending on the disruption of the plant matrix. Thermal treatments such as steaming, boiling, baking, stir-frying, deep-frying, and microwaving could upsurge the bioavailability of micronutrients by destroying the cell wall integrity and organelle membranes where the carotenoids were found, allowing the digestive enzyme to efficiently release them from the food matrix (Bengtsson et al., 2010; Chandrika et al., 2010; Cilla et al., 2018; Veda et al., 2006; Wattanakul et al., 2019).

The bioaccessibility of carotenoids in plant foods is widely accepted to be influenced by their physicochemical state within the plant matrix, the type and degree of heat processing, interactions between carotenoids and the presence of additional components in the meal such as fat and fibre (van het Hof et al., 2000). It is established that fortification of dietary lipids could increase micellarisation of carotenoids and subsequently improve their bioaccessibility (Chitchumroonchokchai et al., 2004; Garrett et al., 1999; Hornero-Méndez & Mínguez-Mosquera, 2007).

The formation of carotenoid-rich mixed micelles during the nutrient absorption process is crucially dependent on the presence of dietary fat in the intestine (van het Hof et al., 2000). The β-carotene bioaccessibility enhanced with the addition of 2.5 to 10 % dietary fat due to the increased solubilisation capacity of the mixed micelles, but then decreased when the dietary fat was raised to 20 % due to precipitation and sedimentation of the β-carotene (Tan et al., 2020). Dietary fat plays a major role in facilitating the solubilisation and transferring the carotenoids from the food matrix to the tissues (Shilpa et al., 2020).