PRELIMINARY INVESTIGATION ON PROCESSING TREATMENTS OF CHLOROPLAST-RICH FRACTION (CRF) FROM SWEET POTATO HAULM

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Abstract

Modernization of agro-food sector through the application of innovation and technologies is one of the new directions envisioned from the Ministry of Agriculture and Agro-based Industry (MOA). This involves food-based plant being grown as a buffer for better profit returns. In every harvest of sweet potato’s root (Ipomoea batatas L.), the rest of the plant parts (stems, leaves, and stalks) which is collectively called “haulm” will be leaved behind on the field. Haulm is a potential source of chloroplasts that contain high nutrients and phytochemicals. In the present study, proximate analysis, water activity and colour analysis of chloroplast-rich fraction (CRF) from sweet potato haulm treated with conventional pasteurisation (CP), steam pasteurisation (SP) and water blanching (WB) were performed. The different treatments on sweet potato haulm significantly affect the crude fat and ash content in the CRF with values ranged between 1.99-3.86\% and 12.82-13.67\%, respectively. There was no significant difference measured in water activity for all treated CRF. Total colour difference between CRF from CP and SP was the lowest (\(\Delta E = 2.88\)) while total colour difference between CRF from CP and WB, and CRF from SP and WB were higher with values of \(\Delta E = 8.93\) and 7.31, respectively. Hence, the colour difference was easily distinguish with naked eye for CRF from WB compared to CRF from CP and SP. This study offers a sustainable source of nutrients, and alternative usage of agriculture waste in the circular economy by upcycling the essential nutrients in the haulm.

Keywords: Processing treatments, chloroplast-rich fraction, sweet potato haulm, physicochemical analysis.

INTRODUCTION

Agriculture solid waste has become a concern nowadays as its amount keeps growing by caused of the increased agricultural production and the need to feed the ever-expanding human population. In Malaysia, there are around 3,623 hectares of sweet potato (Ipomoea batatas L.) farming in 2020 (Jabatan Pertanian Semenanjung Malaysia, 2020). A cycle of sweet potatoes plantation can give up to 8.42 tonnes of dry haulm waste per hectare and the cycle can be repeated three times a year on the same piece of land. The haulms contain stem, leave, and stalk which has a high amount of protein, ascorbic acid, tocopherol, phenolic compounds, and antioxidant activity (Chengcheng et al., 2020; Suárez et al., 2020). To date, sweet potato haulm is mainly used to feed livestock, returned directly to the soil, mulching, or burnt (Chengcheng et al., 2020).
Sweet potato or Ipomoea batatas L. is a creeping dicotyledonous plant, comes from the Convolvulaceae or morning glory family. The leaves are glabrous or slightly pubescent which the shape varies between ovate, sagittal, orbicular, elliptical, and have petiolate by the entire edges. The stem length can vary from 1 to 5 m, thickness between 3 to 10 mm with internodes developed in 2 to 20 cm apart along the stem. The stems cultivate in crawling, prostrate to the ground while promoting repetitive sprouting to generate roots by way of the internodes present contact with the soil. Sweet potato is a versatile type of plant judging from its drought tolerant, high yielding tuber crops and wide adaptability to various climates and farming systems (Bovell-benjamin, 2007; Cartabiano-leite et al., 2020). Moreover, it has higher tolerance of pests and diseases compared to many other leafy vegetables grown in the tropical countries (Islam, 2006).

Heat treatment needs to be implemented to avoid degradation of physicochemical quality due to native enzymatic activity. Cooking process like boiling, steaming, microwaving, roasting or frying could modify physical characteristics, chemical compositions, phytochemical contents, and the chemical nature of specific compound (Kourouma et al., 2019). Blanching and pasteurisation are the most efficient and practical thermal process to inactivate enzyme reactions, stabilise texture and flavour as well as to extend the shelf life of the nutrients in chloroplast-rich fraction from plants (Wattanakul et al., 2019; Wattanakul et al., 2021). Blanching is necessary before drying to inhibit the browning caused by polyphenol oxidase and peroxidase, as well as removing pesticide residues and enhancing drying rate (Luo et al., 2020).

In plants, chloroplasts synthesized most of the valuable nutrients like fatty acids, amino acids, vitamins and pigments. Concentrating chloroplasts as chloroplast-rich fractions (CRF) can be a potential source of essential micronutrients for human or animal consumptions. Liberation of CRF from the plant’s cell wall are expected to allow higher digestibility and absorption of available nutrients from plant materials (Gedi et al., 2019; Syamila, 2019). The lipophilic nutrients from non-heat-treated chloroplast-rich fraction (CRF) from spinach has limited bioaccessibility for body uptake compared to its blanched version (Gedi, 2017; Syamila, 2019; Wattanakul et al., 2019). This led to a suggestion for a down-stream processing involving heat treatment to assist micellarisation of lipid soluble nutrients and to increase bioaccessibility. The CRF from sweet potato haulm could produce new ingredient innovations.
METHODOLOGY

Raw Materials Preparation
Sweet potato haulm was collected from a farm (Perlis). The haulm was cleaned with tap water to remove dirt and soil. Stems from the haulm were cut into smaller pieces (about 2-3 cm). Then, haulm was divided into three batches for three different downstream processing treatments.

Down-stream Processing Treatments
a) Conventional pasteurisation (CP)
The haulm was juiced using a twin gear juicer (SAVTM JE-31) and was conventionally pasteurised (85 °C, 5 min). The, the haulm was immersed in an ice-water bath to rapidly cool the juice down to room temperature before isolation of the chloroplast-rich fraction (CRF).

b) Steam pasteurisation (SP)
The haulm was packed into a vacuum sealed bag for steam pasteurisation. The sealed bags were placed in the rack of a Retort (Lagarde RP362), sealed, vented and heated over 5 min to reach a temperature of 105 °C and 1 bar. Then, it was immersed in an ice-water bath to rapidly cool the haulm down to room temperature before juicing using a twin gear juicer (SAVTM JE-31) before isolation of the chloroplast-rich fraction (CRF).

c) Water blanching (WB)
The haulm was water-blanced in hot water (85 °C, 3 min). Then, it was immersed in an ice-water bath to rapidly cool the haulm down to room temperature before juicing using a twin gear juicer (SAVTM JE-31) before isolation of the chloroplast-rich fraction (CRF).

Isolation of Chloroplast-Rich Fraction (CRF) and Freeze-Drying
The CRF from the processed haulm-juiced was isolated according to method from Wattanakul et al. (2021) with slight modification. The juice was centrifuged (Thermo Jouan CR3i) at 4000 rpm for 15 min at 4 °C. The supernatant was decanted off from the chloroplast pellet and centrifuge again under the same conditions for at least 3 times (or until the supernatant was clear or light green). The pellets containing the CRF were freezeed at -18 °C prior to freeze-drying. Then, frozen CRF was freeze-dried (Freeze Dryer FD-550, Eyela) at 3 Pa, 20 °C for 24 hr. The dried CRF was weighed, before ground to homogeneous powder (< 250 μm) using mortar and pestle under dim light. and. The dried CRF was stored in a vacuum-sealed aluminium pouches at -80 ± 1 °C for further analyses.
Total Soluble Solid (TSS)
Sweet potato haulm juice (SPHJ) after undergo down-stream processing treatment was analysed for total soluble solid (TSS) before being centrifuged and freeze-dried. The TSS content was determined by using digital handheld refractometer and expressed as °Brix. The °Brix is based on the quantity of dissolved solids in a liquid via specific gravity. The refractometer was calibrated with distilled water before the analysis.

Total Solid Content (TSC) and Water Content
Total solid content, also called as dry matter includes both the suspended solids and dissolved salts. The TSC was determined by calculating the weight of dry sample divided by the weight of the wet sample. Then, the percentage of TSC was subtracted from 100 to obtain water content (%) of CRF.

Product Recovery Yield
The dried CRF from each down-stream processing was collected to determine the product recovery yield. The yield of dried CRF was calculated using the ratio between the weight of dry matter collected after drying and the weight of total solid in the sample.

Proximate Compositions
AOAC methods were applied for moisture, crude protein, crude fibre, crude fat and ash analysis on a dried CRF. Moisture content was determined by using moisture analyser (A&D MX-50, Malaysia). Crude protein was determined using Kjeldahl method, whereby the percentage nitrogen was calculated on a dry matter basis. The amount of nitrogen was then multiplied by a nitrogen-protein conversion factor of 6.25 to get the crude protein content of the sample. Crude fibre was determined using dilute acid and dilute alkali hydrolysis in Fibretherm. Total lipids was determined by Soxhlet continuous extraction method while total ash content was determined by charring 1 g of sample on electric hot plate until the smoke ceased, followed by incinerating the charred sample in a muffle furnace at 550 °C for overnight.

Water Activity
Water activity of the dried CRF was analysed using Aqualab water activity meter (Model Series 4TE, Decagon Devices, Inc USA) based on the chilled-mirror dewpoint technique.

Colour Analysis
Colour of dried CRF was measured by colour analyser (Labscan XE Hunterlab). The sample was spread in an optical glass with 6.4 mm diameter diaphragm. Then, the colorimeter was calibrated with a white and black reference plate before measuring.
the \( L^* \) (\( L^* \)=0 [blank] and \( L^* \)=100 [white], \( a^* \) (-\( a^* \)=greenness and +\( a^* \)=redness) and \( b^* \) (-\( b^* \)=blueness and +\( b^* \)=yellowness)" for colour expression. The total colour difference, Delta E of dried CRF between the down-stream processing treatments was measured using the following equation:

\[
\text{Total colour difference (} \Delta E \text{)} = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2}
\]

**Statistical Analysis**

All experiments were conducted in triplicate. All collected data were analysed statistically to analysis of variance (ANOVA) using the Minitab version 17 software (Stat Inc, USA). One way of analysis was applied according to Tukey-test with 95% confidence interval. The results were expressed as means ± standard deviation (M ± SD) and the difference in means at \( p < 0.05 \) were considered significant.

**RESULTS AND DISCUSSION**

**Total Soluble Solid (TSS)**

The total soluble solid (TSS) content represents strong positive correlation with sugar content followed by vitamins and amino acid. TSS content was analysed before the juice being centrifuged and freeze-dried. Sweet potato haulm juice (SPHJ) treated with conventional pasteurisation (CP) recorded higher amount of sugar content compared to the other juices treated with steam pasteurisation (SP) and water blanching (WB), with TSS content of 4.10, 2.60 and 2.13 °Brix \( (p < 0.05) \) respectively.

There was a significant difference between the TSS content in SPHJ treated with CP and with both SPHJ treated with SP and WB. High TSS content was always found at higher heating temperature and time (Jafari et al., 2017; Hajar-Azhari et al., 2018). However, as the haulm was heated first before juiced, some moisture might loss during the process. For juices treated with SP and WB, the haulm was treated first before being juiced. Hence, some water from the steam pasteurisation treatment as well as the water from the water blanching treatment may contribute to the higher water content in SPHJ, resulted in higher TSS content in both treatments.

**Product Recovery Yield, Total Solid Content and Water Content of Dried CRF**

Product recovery yield, total solid content (TSC) and water content were determined after the juice being centrifuged producing chloroplast-rich fraction (CRF), and the CRF was then being dried. Table 1 showed the product recovery yield, total solid content and water content of the dried CRF.
The product recovery yield of sample CP was much higher than sample SP and WB. Low yield of CRF might be due to the insufficient centrifugation method as the supernatant collected was quite viscous and quite dark in colour compared to the supernatant collected by Syamila (2019). Hence, the researcher suggested to apply continuous centrifugation method with higher centrifugation force and longer centrifugation time in order to maximise the product recovery yield of CRF collected from the SPHJ.

The TSC recorded for CRF treated with CP was significantly higher than CRF treated with SP and WB. In CRF treated with CP, 89.52 % removal of water was calculated while higher sublimation of water was recorded in CRF treated with SP and WB with values of 91.83 and 91.53 % respectively. Conventional pasteurisation treatment removed higher moisture content from CRF by converting water into water vapour in gaseous phase. Hence, the evaporation of water caused by the heat treatment might lead to denser concentration of SPHJ (Kumar et al., 2017).

Table 1: Product recovery yield, total solid content and water content of dried CRF.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conventional pasteurisation (CP)</th>
<th>Steam pasteurisation (SP)</th>
<th>Water blanching (WB)</th>
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</thead>
<tbody>
<tr>
<td>Product recovery yield (%)</td>
<td>98.11 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96.02 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>96.43 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Total solid content (%)</td>
<td>10.48 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.17 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.47 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>89.52 ± 0.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>91.83 ± 0.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>91.53 ± 0.24&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

Means with similar letters are not significantly different (Tukey’s test, <i>p</i> < 0.05).

**Proximate Analysis of dried CRF**

The effect of different down-stream processing treatments on moisture, crude protein, crude fibre, crude fat and ash content of dried CRF was illustrated in Table 2.

Table 2: Proximate analysis of dried CRF.

<table>
<thead>
<tr>
<th>Proximate analysis</th>
<th>Down-stream processing treatments</th>
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<tbody>
<tr>
<td></td>
<td>Conventional pasteurisation (CP)</td>
<td>Steam pasteurisation  (SP)</td>
<td>Water blanching (WB)</td>
<td></td>
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<tr>
<td>Moisture (%)</td>
<td>9.61 ± 0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.36 ± 0.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.10 ± 0.24&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Crude protein (%)</td>
<td>35.00 ± 0.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.61 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.29 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Crude fibre (%)</td>
<td>6.85 ± 1.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.43 ± 1.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.36 ± 1.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Crude fat (%)</td>
<td>2.68 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.99 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.86 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Ash (%)</td>
<td>13.67 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.41 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.82 ± 0.11&lt;sup&gt;c&lt;/sup&gt;</td>
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</table>

Means with similar letters are not significantly different (Tukey’s test, <i>p</i> < 0.05).

The moisture content for CRF ranged between 8.10 to 9.61%. Crude protein content in the processed-CRF varied between 31.61 to 35.29%, whereby the SP-CRF recorded significantly lower than CP and WB. The protein values reported by this study were in range with values collected in 13 different cultivars of dried sweet potato leaves from China (Hong et al., 2020), but were higher than values stated for
dried leaf by Tang et al. (2020). On the other hand, lower protein values were mentioned by Suárez et al. (2020) for sweet potato leaves harvested in three different periods. The content of crude fibre was highest in CRF from SP, followed by WB and CP with an average of 12.43, 8.36 and 6.85%, respectively. The crude fibre contents found in this study were consistent with those reported by previous studies (Hong et al., 2020; Tang et al., 2020).

There were significant differences in crude fat and ash content among the three down-stream processing treatments for CRF. The crude fat contents of CRF (1.99 - 3.86 g/100 g dw) were slightly comparable with fat content found in sweet potato dried leaves (2.49 – 4.28 g/100 g dw) located in China (Hong et al., 2020). The dried CRF of SPHJ showed significant difference (p < 0.05) in ash contents among the three down-stream processing treatments ranging from 12.82 to 13.67 %. However, the ash contents of CRF in our study were relatively higher than the dried sweet potato leaves harvested at all three different periods ranged from 10.39 to 11.30 g/100 g dw (Suárez et al., 2020). The CRF obtained from spinach juice exhibited smaller ash content (5.10 %) (Syamila et al., 2019). Differences in harvesting time, maturity stages and sweet potato cultivars and varieties are some factors that may be relatable to the variation values in proximate analysis (Hong et al., 2020; Suárez et al., 2020; Tang et al., 2020).

**Water Activity and Colour Analysis of dried CRF**

Water activity measures the availability of water to react with any compounds and it is used to determined the stability and safety of food with respect to microbial growth, deteriorative reaction rates as well as the chemical and physical properties of food products. Meanwhile, colour is an important quality attribute of food products which can influence the preference of consumer. It plays a vital role as an indicator for both visual and nutritional quality of food products. The water activity and colour analysis of dried CRF values were illustrated in Table 3.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Water Activity ($a_w$)</th>
<th>Colour Analysis</th>
<th>Total Colour Difference ($\Delta E$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lightness ($L$)</td>
<td>Redness ($a$)</td>
</tr>
<tr>
<td>CP</td>
<td>0.33 + 0.03</td>
<td>32.46 + 0.17</td>
<td>7.10 + 0.21</td>
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<tr>
<td></td>
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</tr>
<tr>
<td>SP</td>
<td>0.36 + 0.03</td>
<td>34.30 + 0.63</td>
<td>5.25 + 0.03</td>
</tr>
<tr>
<td>WB</td>
<td>0.36 + 0.02</td>
<td>29.71 + 0.49</td>
<td>1.64 + 0.13</td>
</tr>
</tbody>
</table>

Means with similar letters are not significantly different (Tukey’s test, p < 0.05).

Water activity of dried CRF was insignificantly different among all the downstream processing treatments. Hence, the difference in temperature of treatments did not affect the water activity of dried CRF. The dried CRF of SPHJ in our study had
higher water activity (0.33-0.36 a_w) compared to CRF from freeze-dried spinach juice (0.18 a_w) reported by Syamila (2019). This could be probably due to the higher content of moisture in our dried CRF.

Figure 1 showed the coloured-image of dried CRF treated with CP, SP and WB. The values for lightness (L), redness (a) and yellowness (b) of dried CRF showed significant difference between the three down-stream processing treatments (Table 3). The total colour difference (ΔE) of dried CRF between CP-SP was much lower than CP-WB and SP-WB, with values of ΔE 2.88, 8.93 and 7.31, respectively. Total colour difference with value more than ΔE 5 indicates that the difference in colour can be recognised easily by our naked eye (Obón et al., 2009). Hence, the colour of dried CRF treated with WB could be certainly distinguished from dried CRF treated with CP and SP (Figure 1). In contrast, the total colour difference between dried CRF treated with CP and SP was lower than ΔE 5, showing that the colour difference between both treated dried CRF could not visibly distinguished. The higher factor of L and b, and lower factor of a are inversely proportional with the browning and discolouration, indicates better quality of the dried product.

Figure 1: Dried CRF treated with CP, SP and WB.

Suggestions
Further study on determination of antioxidant activity, mineral contents, bioactive compounds and antinutrient contents in the dried CRF of SPHJ must be conducted to understand more on the valorisation potential of this huge agricultural waste. Furthermore, the bioaccessibility of bioactive compounds of the dried CRF need to be investigated to explore the digestibility and absorption of available nutrients from the plant materials after exposed to several down-stream processes.

CONCLUSION
In this study, nutritional contents, water activity and colour analysis of dried CRF for the three down-stream processing treatments were determined. For proximate analysis, only crude fat and ash contents were significantly different between
conventional pasteurisation (CP), steam pasteurisation (SP) and water blanching (WB) of dried CRF. Colour analysis showed significant difference in dried CRF treated with WB compared to SP and CP samples. Further study on determination of antioxidant activity, mineral contents, bioactive compounds, antinutrient contents, and bioaccessibility of the dried CRF must be conducted. This agriculture solid waste can be a potential source of essential nutrients which can be utilised as an alternative ingredients in the food/feed industries.

ACKNOWLEDGEMENT
This paper is part of a research project supported by the Malaysian Ministry of Higher Education Fundamental Research Grant Nos. (USIM/FRGS/FST/KPT/53320).

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