

**PHYSICOCHEMICAL VARIATION AND *IN-VITRO*  
BIOACCESSIBILITY OF CHLOROPLAST-RICH  
FRACTION (CRF) FROM SWEET POTATO  
(*IPOMOEA BATATAS L.*) HAULM**

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**UNIVERSITI SAINS ISLAM MALAYSIA**

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Thesis submitted in partial fulfilment for the degree of  
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UNIVERSITI SAINS ISLAM MALAYSIA

July 2024

## AUTHOR DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged.

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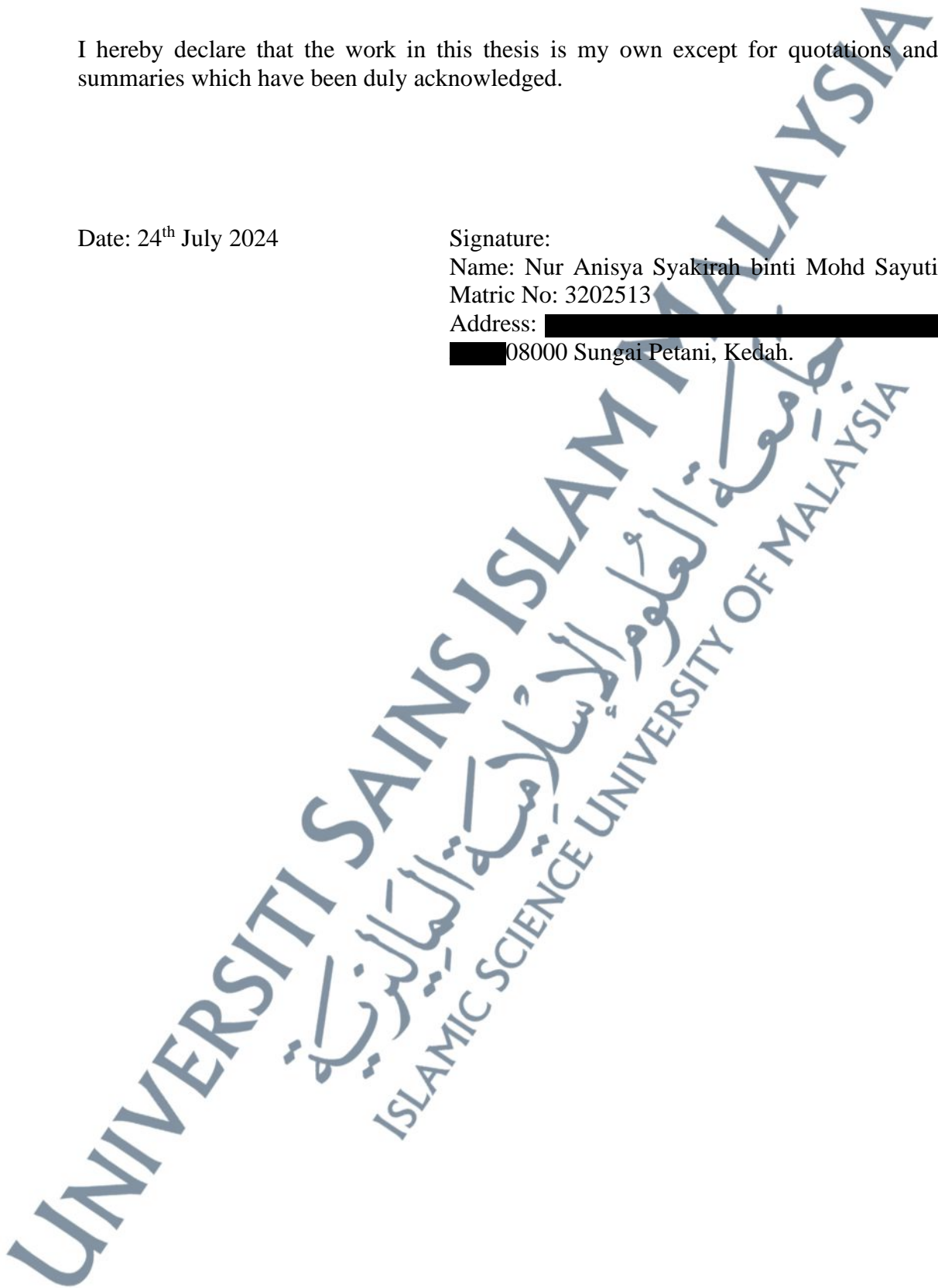
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## ABSTRAK

Sisa pertanian telah menjadi kebimbangan pada masa kini kerana kuantitinya yang banyak dihasilkan setiap tahun. Ubi keledek (*Ipomoea batatas* L.) merupakan tanaman kontan kedua terbesar di Malaysia. Ia dituai selepas tiga bulan penanaman, meninggalkan bahagian tanaman yang lain (batang, daun, dan tangkai) yang secara kolektifnya dipanggil "sisa pertanian", di ladang. Sisa pertanian ubi keledek (SPH) merupakan sumber kloroplas yang mengandungi nutrien lipofilik, iaitu  $\beta$ -karotena dan lutein. Projek ini bertujuan untuk mengumpul data mengenai pecahan yang kaya dengan kloroplas (CRFs) dari SPH dengan, i) mengkaji kesan perbezaan batas (CB) terhadap variasi fizikokimia CRF, ii) mengkaji kesan rawatan haba (pasteurisasi konvensional [CP], pasteurisasi stim [SP], dan penceluran air [WB]) terhadap variasi fizikokimia CRF, dan iii) mengkaji kestabilan pencernaan dan bioaksesibiliti  $\beta$ -karotena dan lutein dalam CRF. Sisa pertanian ubi keledek dari tiga CB (CB 1, CB 2, dan CB 3) mempunyai variasi dalam komposisi proksimat, kandungan total klorofil, dan aktiviti antioksidan (ujian DPPH dan FRAP). CB 2 menunjukkan kandungan klorofil tertinggi (7.65 mg/g dw) dan aktiviti antioksidan (86.57  $\mu$ g/ml [IC<sub>50</sub>] dan 155.67 mmol Fe [II]/g dw). Semua CB menunjukkan kandungan anti-nutrien yang rendah, dari 1.89 hingga 2.40 g/100 g dw (asid oksalik) dan dari 55.62 hingga 71.70 mg/100 g dw (asid fitik). Penyelidikan ini mendapati bahawa semua CRF yang dirawat dengan haba mengalami pengurangan klorofil, fenolik keseluruhan (TPC), dan kandungan asid oksalik, serta peningkatan keterlarutan ( $p < 0.05$ ). Sampel yang dirawat dengan haba mengekalkan TPC (24 – 50 %) dan FRAP (36 – 84 %), di samping meningkatkan DPPH (20 – 30 %). Yang paling penting, rawatan haba CP adalah yang terbaik berbanding SP dan WB dalam mengekalkan TPC dan aktiviti antioksidan. Akhir sekali, penyelidikan ini mendapati bahawa rawatan haba (SP dan WB) dan penambahan minyak dapat meningkatkan nutrien bioakses (BA) dan nutrient boleh diakses (NA). SP mempunyai BA tertinggi untuk  $\beta$ -karotena (17.30 %) dan lutein (29.23 %) manakala WB mempunyai NA tertinggi untuk  $\beta$ -karotena (12.47 %) dan lutein (23.74 %). Penyertaan minyak meningkatkan BA  $\beta$ -karotena (2 hingga 4 kali ganda) dan lutein (1 hingga 2 kali ganda). Kajian ini menawarkan sumber nutrien penting yang mampan daripada sisa pertanian, dengan potensi manfaat dalam inovasi bahan baru untuk penggunaan manusia atau haiwan, manakala keluaran sampingan daripada sisa pertanian boleh dicipta menjadi aplikasi makanan dan bukan makanan.

**Kata kunci:** *Sisa pertanian ubi keledek, pecahan yang kaya dengan kloroplas, rawatan haba, fizikokimia, bioaksesibiliti in-vitro*

## ABSTRACT

Agricultural waste has become a concern nowadays due to the remarkable quantity produced per year. Sweet potato (*Ipomoea batatas* L.) is the second largest cash crop in Malaysia. It is harvested after three months of growing, leaving the rest of the plant parts (stems, leaves, and petioles) which are collectively called “haulm”, behind on the field. Sweet potato haulm (SPH) is a potential source of chloroplast that contains highly lipophilic nutrients, namely  $\beta$ -carotene and lutein. This project aimed to establish data on the chloroplast-rich fractions (CRFs) from SPH by, i) investigating the effect of different crop borders (CBs) on the physicochemical variation of CRF, ii) investigating the effect of heat treatments (conventional pasteurisation [CP], steam pasteurisation [SP], and water blanching [WB]) on the physicochemical variation of CRF, and iii) investigating the digestive stability and bioaccessibility of  $\beta$ -carotene and lutein of CRF. The haulm harvested from three CBs (CB 1, CB 2, and CB 3) had variations in proximate compositions, total chlorophyll content and antioxidant activity (DPPH and FRAP assays). CB 2 exhibited the highest chlorophyll content (7.65 mg/g dw) and antioxidant activity (86.57  $\mu$ g/ml [IC<sub>50</sub>] and 155.67 mmol Fe [II]/g dw). All CBs showed low antinutrients, ranging from 1.89 to 2.40 g/100 g dw (oxalic acid) and from 55.62 to 71.70 mg/100 g dw (phytic acid). This research found that all heat-treated CRFs had a reduced chlorophyll, total phenolic (TPC), and oxalic acid content, and enhanced solubility ( $p < 0.05$ ). Heat-treated samples retained TPC (24 – 50 %) and FRAP (36 – 84 %), while increasing the DPPH (20 – 30 %). Most importantly, CP heat treatment was best compared to SP and WB in preserving TPC and antioxidant activity. Lastly, this research found that heat treatments (SP and WB) and the inclusion of oil can improve nutrients' bioaccessibility (BA) and accessible for uptake (NA). SP had the highest BA for  $\beta$ -carotene (17.30 %) and lutein (29.23 %) while WB had the highest NA for  $\beta$ -carotene (12.47 %) and lutein (23.74 %). Oil inclusion further increased the BA of  $\beta$ -carotene (at least 2 to 4 folds) and lutein (at least 1 to 2 folds). This study offers a sustainable source of essential nutrients from agricultural waste, potentially benefiting in new ingredient innovations for human or animal consumption while by-products from the waste may be invented for food and non-food applications.

**Keywords:** *Sweet potato haulm, chloroplast-rich fraction, heat treatment, physicochemical, in-vitro bioaccessibility*

## الملخص

أصبحت المخلفات الزراعية مصدر قلق في الوقت الحاضر بسبب الكميات الملحوظة المنتجة سنويًا. البطاطا الحلوة (*Ipomoea batatas* L.) هي ثاني أكبر محصول نقدي في ماليزيا. يتم حصادها بعد ثلاثة أشهر من النمو، مما يترك بقية أجزاء النبات (السيقان والأوراق) التي تُسمى بشكل مجمع "بقايا"، خلفًا على الحقل. البقايا من البطاطا الحلوة (SPH) هو مصدر محتمل للكلوروبلاست يحتوي على مواد غذائية قليلة الليبوفيلية بشكل كبير، وهي  $\beta$ -كاروتين واللوتين. يهدف هذا المشروع إلى تأسيس بيانات حول الكسرة الغنية بالكلوروبلاست (CRFs) من SPH من خلال، (i) دراسة تأثير الحدود المختلفة للمحصول (CBs) على التباين الفيزيوكيميائي لـ CRF، (ii) دراسة تأثير معالجات الحرارة (التحليل الكهربائي التقليدي [CP]، التحليل الكهربائي البخار [SP]، والغليان بالماء [WB]) على التباين الفيزيوكيميائي لـ CRF، و (iii) دراسة الاستقرار الهضمي والوصول الحيوي لـ  $\beta$ -كاروتين واللوتين لـ CRF. كانت المخلفات التي تم حصادها من ثلاثة CBs (CB 1، CB 2، و CB 3) لها تفاوتات في التكوينات القريبة، ومحتوى إجمالي للكلوروفيل والنشاط المضاد للأوكسدة (اختبارات DPPH و FRAP). أظهرت CB 2 أعلى محتوى للكلوروفيل (7.65 mg/g dw) ونشاط مضاد للأوكسدة (86.57  $\mu$ g/ml [IC<sub>50</sub>] و 155.67 mmol Fe [II]/g dw). أظهرت جميع CBs تركيزات منخفضة لمضادات العناصر الغذائية، تتراوح بين 1.89 إلى 2.40 g/100 g dw (حمض الأوكساليك) ومن 55.62 إلى 71.70 mg/100 g dw (حمض الفايثيك). أظهرت هذه الدراسة أن جميع CRFs المعالجة بالحرارة كان لديها تقليل في محتوى الكلوروفيل والفينولات الإجمالية (TPC) وحمض الأوكساليك، وزيادة في الذوبانية ( $p < 0.05$ ). العيقات المعالجة بالحرارة حافظت على حوالي 24 - 50% من TPC و 36 - 84% من FRAP، مع زيادة في DPPH بنسبة 20 - 30%. والأهم من ذلك، وجد أن علاجات الحرارة CP كانت الأفضل مقارنة بـ SP و WB في الحفاظ على TPC ونشاط المضادات الأوكسدية. وأخيرًا، وجدت هذه الدراسة أن علاجات الحرارة (WB و SP) وإضافة الزيت يمكن أن تحسن إمكانية الوصول إلى العناصر الغذائية (BA) والمتاحة للاستيعاب (NA). كان لـ SP أعلى BA لـ  $\beta$ -كاروتين (17.30%) ولوتين (29.23%) بينما كان لـ WB أعلى NA لـ  $\beta$ -كاروتين (12.47%) ولوتين (23.74%). أدى تضمين الزيت إلى زيادة نسبة BA في  $\beta$ -كاروتين (على الأقل 2 إلى 4 أضعاف) واللوتين (على الأقل 1 إلى 2 أضعاف). تقدم هذه الدراسة مصدرًا مستدامًا للعناصر الغذائية الأساسية من المخلفات الزراعية، مما قد يستفيد منه في الابتكارات

الجديدة للمكونات للاستهلاك البشري أو الحيواني بينما قد يتم اختراع النواتج الفرعية من النفايات للاستخدامات الغذائية وغير الغذائية.

الكلمات الرئيسية: مخلفات من البطاطا الحلوة، الكسرة الغنية بالكلوروبلاست، معالجة حرارية، فيزيائية كيميائية، إمكانية الوصول البيولوجي في المختبر

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## LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BHT	Butylated hydroxytoluene
CB	Crop border
CP	Conventional pasteurisation
CRF	Chloroplast-rich fraction
DPPH	1,1-diphenyl-2-picrylhydrazyl
dw	Dry weight
FRAP	Ferric reducing antioxidant potential
fw	Fresh weight
GAE	Gallic acid equivalent
HPLC	High performance liquid chromatography
IC <sub>50</sub>	Half maximal inhibitory concentration
PTFE	Polytetrafluoroethylene
rpm	Revolutions per minute
SEM	Scanning electron microscopy
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
SP	Steam pasteurisation
SPH	Sweet potato haulm
SSF	Simulated salivary fluid
TEA	Triethylamine
TPC	Total phenolic content
TSC	Total solid content
TSS	Total soluble solid
UV	Ultraviolet
VCE	Vitamin C equivalent
WB	Water blanching
WSI	Water solubility index