Faculty of Resource Science and Technology

Phenolic compounds, Antioxidant Capacity and Compound Profiles of Extracts from Raw Sago Starch Fermentation with Endophytes

Nurul Aida Qarina binti Mohd Razali

Master of Science
2024

Phenolic compounds, Antioxidant Capacity and Compound Profiles of Extracts from Raw Sago Starch Fermentation with Endophytes

Nurul Aida Qarina binti Mohd Razali

A thesis submitted

In fulfillment of the requirements for the degree of Master of Science

(Biochemistry)

Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2024

DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

……………………………………

Signature

Name: Nurul Aida Qarina binti Mohd Razali

Matric No.: 19020134

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

Date: 30th April 2024

ACKNOWLEDGEMENT

All praises to Allah S.W.T and our beloved Prophet Muhammad S.A.W. Without His blessings and love, I might never finish my master's and this thesis. I would like to take this opportunity to those who have contributed directly or indirectly to this thesis.

Many days were carried out to complete the study and many have given their supports throughout my journey until today. My sincere gratitude to my supervisors, Prof Awang Ahmad Sallehin for his super encouragement and guides for my masters. My co-supervisor, Dr. Nurashikin, your papers and questions have been a great help in filling the gap that I overlooked. My fellow batchmates, Alexandra and Nick Laurence, thank you for the great help and spent time. A special gratitude to Dr. Ngieng for his advice and special classes on experimental as well as fellow lab mates.

To my uncles and aunties who had been supporting me morally and financially, a big shoutout for you and thank you so much for all the encouragement. To my Pak Uda, Safry Kamal and Wan Chu, Noraini Jaafar who has been supporting me financially ever since COVID-19 hits, thank you for everything. My aunt, Nor Hany and beloved cousins, thank you for giving me moral support and encouragement despite everything that I have been through. My besties, Emmaryna and Nur Irdina, thank you very much for being there through my ups and downs and hearing my woes and midnight troubles.

Finally, I would like to thank the management of the Universiti Malaysia Sarawak for making it possible for me to complete my study here in Sarawak. Thank you all.

May Allah bless and repay all your good deeds.

ABSTRACT

Escalating prevalence of free radicals in the modern environment, driven by factors such as pollution and unhealthy lifestyles, poses a significant health concern. There is a need to explore the potential benefits and applications of antioxidants in mitigating the oxidative damage caused by free radicals and their role in promoting overall well-being. In the last decade, an increasing number of research findings and studies have addressed strategies to combat oxidative stress. This surge in interest stems from concerns regarding the potential health risks associated with synthetic drugs, which can contribute to silent yet perilous conditions like heart disease, hypertension, and cancer. As a result, there has been a growing exploration of natural-based materials as potential alternatives. Endophytic fungi, residing within the tissues of various plant species, have emerged as a promising alternative source of antioxidants that exhibit analogous properties to their host plants. This unique symbiotic relationship holds immense potential for the extraction of antioxidant compounds, offering new avenues for harnessing nature's own defences against oxidative stress. To implement this, one of the alternative ways is through value-added food. Antioxidant-enriched value-added food products achieved via innovative fermentation techniques have gained prominence as a sustainable approach to enhance both nutritional content and shelf-life, offering promising prospects for healthier dietary choices and reduced food waste. Despite an annual production of 14,000 tonnes of underutilized sago starch, this valuable resource remains largely untapped, presenting a pressing challenge for sustainable utilization and economic development, mainly in the region of Sarawak and in some small areas in Johor. This study aims to investigate innovative approaches to maximize the utilization of sago starch through fermentation, thereby unlocking its full potential for various applications in the food and industrial sectors. In this study, traditionally processed sago starch is being used rather than industrially processed sago starch. The latter often loses crucial nutritional factors during its processing, whereas the former, known as raw sago starch (RSS) *"lemantak"* holds promise as a more nutritionally valuable option. Consequently, this research aims to explore the presence of potential phenolic compounds with antioxidant properties in fermented raw sago starch, offering an alternative avenue for extracting nutraceuticals from this resource. Additionally, employment of microbial fermentation with fungal endophytes to further enhance the production of phenolic compounds in traditionally processed RSS *"lemantak"*. Also, to fully maximise the potential of the fermented RSS *"lemantak"*, optimisation process was also carried out by manipulating several parameters such as effect of fungal endophytes consortia, effect of fermentation time, effect of extraction time and effect of solvent extraction. It is crucial that these parameters were manipulated to maximise the potential for phenolic compound production from fermented RSS *"lemantak"*. All selection was made based on the total phenolic and antioxidant profiles. It was found out that the best fungal consortia were consortium of *Aspergillus niger* and *Penicillium chermesinum* (AP) under solid-state fermentation condition. It was also found out the best fermentation period was on Day 7 while the best extraction time was 24 hrs of incubation under water bath extraction method at 50°C. Nevertheless, the best extraction solvent was methanol as compounds were more soluble in a more polar solvent. Extractives were also analysed through GC-MS method to identify the contributed bioactive compounds. Compounds such as Eicosane and 1-Eicosanol were found to be the most dominant in the extracts reported to have both antioxidant and antimicrobial activity. This opens the potential of fermented RSS into becoming new value-added food, harnessing the inherent nutritional benefits of sago starch and its newfound antioxidant capacity. As exploration in diverse applications of endophytic fungi continues in food science and nutrition, our findings contribute to a deeper understanding of sustainable food processing and functional ingredient discovery.

Keywords: Fermentation, endophytes, raw sago starch, antioxidant, optimisation

Penghasilan dan Pengekstrakan Sebatian Fenolik daripada Penapaian Pati Sagu Mentah dengan Kulat Endofit

ABSTRAK

Kemunculan radikal bebas yang semakin meningkat dalam persekitaran moden, yang dipacu oleh faktor-faktor seperti pencemaran dan gaya hidup yang tidak sihat, menjadi kebimbangan kesihatan yang signifikan. Terdapat keperluan untuk meneroka manfaat dan aplikasi potensi antioksida dalam mengurangkan kerosakan oksidatif yang disebabkan oleh radikal bebas dan peranannya dalam meningkatkan kebahagiaan keseluruhan. Mutakhir ini, peningkatan penyelidikan dan kajian yang telah menganalisis pelbagai strategi untuk melawan tekanan oksidatif. Pertumbuhan minat ini timbul daripada kebimbangan mengenai risiko kesihatan yang mungkin berkaitan dengan antioksida yang diperbuat secara sintetik, yang boleh menyumbang kepada keadaan berisiko seperti penyakit jantung, hipertensi, dan kanser. Lantaran daripada itu, kajian dan penyelidikan menggunakan bahan berasaskan semula jadi sebagai sumber alternatif yang berpotensi semakin meningkat atas isu keselamatan. Kulat endofit, yang menetap dalam tisu pelbagai spesies tumbuhan, muncul sebagai sumber alternatif yang menjanjikan sumber antioksida yang mempunyai sifat analog dengan tumbuhan inang mereka. Hubungan simbiotik unik ini mempunyai potensi besar untuk penghasilan sebatian antioksida, menawarkan jalan baru untuk memanfaatkan pertahanan semula jadi alam terhadap tekanan oksidatif. Untuk melaksanakan ini, salah satu cara alternatif adalah melalui makanan berharga tambah. Produk makanan berharga tambah yang diperkayakan dengan antioksida melalui teknik fermentasi inovatif telah mendapat perhatian sebagai pendekatan mampan untuk meningkatkan kandungan pemakanan dan tempoh simpan, menawarkan prospek yang menjanjikan untuk pilihan pemakanan yang lebih sihat dan mengurangkan pembaziran makanan. Walaupun terdapat

pengeluaran tahunan sebanyak 14,000 tan, kanji sagu yang tidak dimanfaatkan menyebabkan sumber berharga ini masih belum digunakan sepenuhnya, lantas mengakibatkan cabaran yang mendesak dalam penggunaan yang mampan dan pembangunan ekonomi, terutamanya di Sarawak dan beberapa kawasan kecil di Johor. Kajian ini bertujuan untuk menyiasat pendekatan inovatif untuk memaksimumkan penggunaan kanji sagu melalui fermentasi, dengan itu membuka potensi penuhnya untuk pelbagai aplikasi dalam sektor makanan dan industri. Dalam kajian ini, kanji sagu yang diproses secara tradisional ditekankan sebagai subjek utama dan bukannya kanji sagu yang diproses secara industri. Hal ini demikian kerana, kanji sagu yang diproses secara industri sering kehilangan faktor pemakanan penting semasa pemprosesannya, manakala kanji sagu yang diproses secara tradisional yang juga dikenali sebagai 'kanji sago mentah (RSS) "lemantak",' menjanjikan sebagai pilihan yang lebih bernilai dari segi nutrisi pemakanan. Oleh itu, penyelidikan ini bertujuan untuk meneroka kehadiran sebatian fenolik berpotensi dengan sifat antioksidan dalam kanji sagu mentah yang diproses secara tradisional telah difermentasikan, menawarkan jalan alternatif untuk mengekstrak nutraseutikal daripada sumber ini. Selain itu, penggunaan fermentasi mikrob dengan kulat endofit untuk meningkatkan pengeluaran sebatian fenolik dalam RSS lemantak yang diproses secara tradisional. Selain itu, untuk memaksimumkan potensi sepenuhnya daripada RSS "lemantak" yang telah difermentasikan, proses pengoptimuman juga dilakukan dengan memanipulasi beberapa parameter seperti kesan konsortium kulat endofit, kesan tempoh fermentasi, kesan tempoh pengekstrakan, dan kesan pengekstrakan pelarut. Adalah penting bahawa parameter-parameter ini dimanipulasi untuk memaksimumkan potensi pengeluaran sebatian fenolik daripada RSS "lemantak" yang telah difermentasikan. Pemilihan kesemua ini dibuat berdasarkan profil fenolik keseluruhan dan profil antioksida. Didapati konsortium

kulat terbaik adalah konsortium Aspergillus niger *dan* Penicillium chermesinum *(AP) di bawah keadaan fermentasi pepejal. Juga didapati tempoh fermentasi terbaik adalah pada Hari ke-7 manakala tempoh pengekstrakan terbaik adalah 24 jam di bawah kaedah pengekstrakan air panas pada suhu 50°C. Walau bagaimanapun, pelarut pengekstrakan terbaik adalah metanol kerana sebatian-sebatian lebih larut dalam pelarut yang lebih polar. Ekstrak juga dianalisis melalui kaedah GC-MS untuk mengenal pasti sebatian bioaktif yang menyumbang. Sebatian seperti Eicosane dan 1-Eicosanol didapati menjadi yang paling dominan dalam ekstrak yang dilaporkan mempunyai aktiviti antioksida dan antimikrob. Ini membuka potensi RSS yang telah difermentasikan menjadi makanan berharga tambah baharu, memanfaatkan manfaat pemakanan asli kanji sago dan kapasitinya yang baru ditemui untuk antioksida. Seiring dengan penjelajahan dalam pelbagai aplikasi kulat endofit dalam sains makanan dan pemakanan, penemuan kami menyumbang kepada pemahaman yang lebih mendalam tentang pemprosesan makanan yang mampan dan penemuan makanan berfungsi.*

Kata kunci: *Penapaian, kulat endofit, kanji sagu mentah, antioksidan, pengoptimuman*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

µl	Microlitres
BHA	Beta hydroxy acid
BHT	Butylated hydroxytoluene
°C	Degree Celsius
Consortia A	Fungal endophyte *Aspergillus niger*
Consortia AF	Fungal endophytes consortia of *Aspergillus niger* & *Fusarium equiseti*
Consortia AFP	Fungal endophytes consortia of *Aspergillus niger*, *Fusarium equiseti* & *Penicillium chermesinum*
Consortia AP	Fungal endophytes consortia of *Aspergillus niger* & *Penicillium chermesinum*
Consortia F	Fungal endophyte of *Fusarium equiseti*
Consortia P	Fungal endophyte of *Penicillium chermesinum*
Consortia PF	Fungal endophytes consortia of *Penicillium chermesinum* & *Fusarium equiseti*
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
g	Gram
g/L	Gram per litre
GAE	Gallic acid equivalent
GCMS	Gas chromatography mass spectrometry
GMM	Glucose minimal media
HPLC	High performance liquid chromatography
hrs	Hours

MEA	Malt extract agar
MEB	Malt extract broth
mg GAE/ g	Milligram of gallic acid equivalent per gram
min	Minutes
ml	Millilitres
mM	Millimolar
MS UV SPECTRO	Mass spectrometer ultraviolet spectrometer
nm	Nanometre
Na_2CO_3	Sodium carbonate
OFAAT	One factor at a time
PFRAP	Potassium ferric reducing power
pH	Potential of hydrogen
RNA	Reactive nitrogen species
ROS	Reactive oxygen species
rpm	Rotation per minute
RSS	Raw sago starch
SMF	Submerged fermentation
SSF	Solid state fermentation
TBHQ	Tert-butylhydroquinone
TPC	Total phenolic content
UV-VIS Spectrometer	Ultraviolet-visible light spectrometer
w. b.	Wet basis
w/v	Weight per volume

CHAPTER 1

INTRODUCTION

1.1 Study Background

Natural products from plants have gathered significant attention due to the recent evidence suggesting their capacity to improve oxidative stress and other related diseases. Since ancient times, plants have been a reservoir of cures and treatments to many diseases and ailments. From different parts of plants, various kind of ailments were treated either applied externally or through consumption of the parts of the plants. Due to these values, plants were harvested for their benefits especially medicinal plants. However, when major harvesting of medicinal plants was carried out, over-harvesting should be considered and observed, as overharvesting will not only endanger the species of the medicinal plants and herbs, but this will also contaminate the gene pool of the species leading to a loss of genetic diversity. Although gene diversity can be restored through mutations, but it takes long time for it to recover. Moreover, habitat loss due to over-harvesting will also be occurred. Therefore, rather than fully utilising the medicinal plants, another organism which lives symbiotically, endophytic fungi, are being utilised instead.

This research utilised the endophytic fungi isolated from the host plant to help produce beneficial bioactive compounds as it was proven by studies that the bioactive compounds found in the endophytes and its host plant are within the same spectrum (Singh et al., 2016 & Kusari et al., 2012). Moreover, majority of endophytic fungi are non-pathogenic in nature making most of the derived metabolites are suitable for human usage due to the non-toxic property to mammalian cells (Ancheeva et al., 2020). It is well-known that the active compounds that have been isolated from fungal endophytes include antibiotics, anticancer, antifungal, and antiviral. With the discovery of paclitaxel, an anticancer drug derived from the bark of *Taxomyces andreanae* from *Taxus brevifolia*, the surge of finding endophytic fungi with useful metabolites have been trending over the decades. These metabolites which was derived from the secondary metabolites produced in plants are biologically active compounds which comprises of phenolics, flavonoids and terpenoids classes, playing a major role in inhibiting the activity of the reactive oxygen species or free radicals which are harmful to the human body.

Oxidative stress can be associated to many human diseases such as inflammation leading to the pathogenesis of cancer (Maeda & Omata, 2008), cardiovascular diseases (Montetucco et al., 2011) and diabetes (Sell & Eckel, 2009). According to Gülçin (2020), oxidative stress can be defined as an imbalance between production reactive oxygen species and antioxidant defences.

1.2 Endophytic Fungi Produced Similar Bioactive Compounds to Host Plant

Years of research have studied the relationship between the isolated endophytic fungi and its host plant. Theoretically, the isolated endophytes may or may not produce similar compounds as its host plants. Despite of the numerous studies that claimed to produce compounds similar to its host plant, these isolated fungi required a certain stress or an activation of cluster of genes to induce the metabolite pathway via *in vitro* conditions to produce similar bioactive metabolites as their host plant (Sharma et al., 2021). Endophytes are microbes which colonize living, internal tissues of plants without causing any harm or symptoms of disease to their host plants as they live in symbiosis (Gouda et al., 2016). They are well known to produce secondary metabolites that are similar to the host which are beneficial to industries such as agriculture, cosmeceutical, and medical uses. Reports have indicated that endophytes contribute substances that have various bioactivity reactions such as antimicrobial, antioxidant and enzyme production. As of now, only endophytic bacteria isolated from sago palm tissue culture were reported to possess antimicrobial activity (Labrador et al., 2014). No reports for endophytic fungi detected in sago palm were ever reported. Leveraging endophytic fungi-derived bioactive compounds in research is crucial for unlocking their untapped potential to combat drug-resistant pathogens, chronic diseases, and environmental sustainability, fostering innovation and progress in various scientific fields.

1.3 Problem Statement

The human body harbours defence mechanism against oxidative stress induced by free radicals. Apart from the innate defence system, synthetic drugs are also available to improve the capacity of the body defence mechanism against oxidative stress. But owing to harmful side effects of the synthetic drug, research on natural products has taken leap in recent years. From food technological view, antioxidants served as a preservative to prevent food from spoilage through oxidation, thus reducing the loss of nutrients, and maintaining the texture, colour pigment, taste, freshness, functionality, and aroma (Carocho & Fereira, 2013).

Sago starch is one of the staple foods derived from the underutilized crop *Metroxylon sagu* Rottb., the sago palm. The sago palms are commonly found in peat swamp areas widely distributed in Malaysia, Indonesia, and Papua New Guinea. In Malaysia, this palm is majorly found in the state of Sarawak and a few places in the state of Johor. Production of sago starch has long been utilised decades ago through different methods with one of it using the traditional way of processing it. Traditionally processed sago starch is commonly known as sago *"lemantak"* among locals. They are the most common native sago starch sold in the wet markets of Sarawak and is an infamous local produce among the Sarawakian. The potential of sago starch is limitless. Studies reported that it contains high in amylopectin ranging from 69 to 78.8% making it as a potential bioplastic with high tensile properties (Arshad et al., 2019). Resistant starch is a selective starch that stimulates the growth and/or activity of one or more number of beneficial bacteria including *Lactobacilli* and *Bifidobacterial* and thus influencing positively of the host health which is known as the prebiotic effect (Arshad et al., 2019). Currently, there are no reports on the functional properties of sago *"lemantak"*, nor studies carried out to investigate the antioxidant ability of the sago starch *"lemantak"*. To further investigate on the functional properties of sago starch *"lemantak,"* this study also utilized fermentation techniques as a method to compare of the properties between unfermented sago starch and the fermented sago starch.

Fermentation is an old food processing technique since ancient times. This technique is known to make the food lasts longer by having a longer shelf life. Not only that, but it can also improve the texture, palatability and enhances the nutritional value of the food itself (Zhao et al., 2019). For instance, the *tempeh* and *tapai* production. Depending on the source of microorganism, fermentation can be classified as natural fermentation whereby the natural microflora in the substrates itself was used as the starter inoculum while seed fermentation is an artificially insemination of the microorganism upon the substrate. In general, the technique of fermentation is divided into solid phase fermentation and liquid phase fermentation which is also called submerged fermentation depending on the amount of water in the system. Studies have found out microorganisms used in fermentation feeds on carbohydrates to produce gases, acids, and enzymes which in turn modified the starch structure into a looser form which then generates delightful taste and tastes slightly sour (Yang & Tao, 2008). Therefore, this study focuses on the phytochemical properties,