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PRODUCTION OF RESISTANT STARCH TYPE III AND IV AND THEIR IN VIVO CHARACTERISATION USING BALB/c MICE MODEL

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By

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Sago is an important agricultural commodity for Sarawak. The potential of sago is highlighted in the Malaysia's 11th plan as part of the poverty eradication programme. Sago starch indigestibility provides a new perspective of sago utilisation as a prebiotic. The indigestible portion of starch is termed as resistant starch (RS). In the present study, RS type III and IV were produced through retrogradation and chemical modifications respectively. Retrogradation was done on different starch paste concentration of 10, 18 and 30%. The resultant RS type III was analysed for solubility, swelling power, amylose and RS content. Resistant starch type IV was produced through hydroxypropylation, acetylation, cross-linking and double modification combining acetylation and cross-linking. The resultant RS was subjected to the same analysis as RS type III. Native sago starch, retrograded starch at 18% initial starch paste concentration, 2% acetylated starch and double modified starch combining 1.5% cross-linking and 2% acetylation were further analysed for prebiotic evaluation through *in vivo* study using BALB/c mice model. All RS was supplemented into a standardised AIN93-M feed formulation. Feeding treatment was conducted for 4 weeks. The daily feed intake and body weight were recorded. Faeces samples were collected on the eighth day and on the final three consecutive days of feeding treatment. All faeces samples were subjected to short chain fatty acid analysis using high-performance liquid chromatography. All starch modifications showed an increase in solubility and swelling power. Native sago showed a high content of RS at 69%. Retrograded starch showed a significantly low amylose and RS content than native starch ($P < 0.05$). Only 1.5 and 2% acetylation, as well as double modification of 1.5% cross-linking and 2% acetylation showed an increase in RS content than native sago starch. Cross-linking and double modified starch showed an increase in amylose content at all level of modification. Mice fed diet supplemented with RS type III and acetylation showed a better overall growth performance with an increase of body weight with a decrease in feed intake. Mice fed diet supplemented with double modified and native sago starch showed a weight loss with a decrease in feed intake. This pattern supported satiety

properties. Short chain fatty acid analysis showed the highest lactate production in mice fed diet supplemented with double modified starch at 43.57%. The highest propionate concentration was shown in mice fed diet supplemented with RS type III and double modified starch at 10.4%. The results showed that double modified sago starch has the potential as a prebiotic candidate ingredient. The weight reduction potential shown in a diet supplemented with double modified starch could also be further implemented in a weight management diet.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

PENGHASILAN KANJI RINTANG JENIS III DAN IV DAN PENCIRIAN *IN VIVO* MENGGUNAKAN MODEL TIKUS BALB/c

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Sago ialah komoditi pertanian yang penting di Sarawak, kebanyakkannya disebabkan oleh potensi sago untuk berkembang subur di tanah gambut. Perladangan sago telah dibesarkan untuk membangunkan sebahagian daripada 1.5 hektar tanah gambut yang tidak mempunyai system perparitan yang berkualiti. Potensi sago telah ditekankan di dalam pelan Malaysia ke-11 sebagai sumber untuk program penghapusan kemiskinan. Ketidakhadaman kanji sago memberi perspektif baru dalam penggunaan kanji sago sebagai prebiotik. Bahagian kanji yang tidak dihadam digelar sebagai kanji rintang. Kanji rintang jenis 3 dan 4 dihasilkan melalui proses retrogradasi dan pengubahsuaian secara kimia. Proses retrogradasi telah dilakukan ke atas adunan kanji sago dengan kepekatan yang berbeza iaitu 10, 18 dan 30%. Kanji rintang jenis 3 yang terhasil telah dianalisa untuk kelarutan, kuasa pembengkakan, kandungan amilosa dan kanji rintang. Kanji rintang jenis 4 telah dihasilkan melalui proses hidrosipropilasi, pengasetilan, penghubungan silang dan dwi modifikasi yang mencamtumkan proses pengasetilan dan penghubungan silang. Kanji rintang jenis 4 yang terhasil telah menjalani analisa yang sama seperti kanji rintang jenis 3. Kanji sago asli, kanji retrogradasi pada 18% permulaan kepekatan adunan kanji, 2% kanji asetilan dan dwi modifikasi yang menggabungkan 1.5% , penghubungan silang dan 2% pengasetilan telah dianalisa dengan lebih jauh untuk penilaian sebagai prebiotik melalui kajian in vivo menggunakan model mencit BALB/c. Kesemua kanji rintang ditambah kedalam rumusan makanan AIN93-M yang telah diseragamkan. Rawatan pemakanan dilaksanakan selama 4 minggu dan pengambilan pemakanan dan berat badan telah direkodkan secara harian. Sampel najis telah dikumpulkan pada hari ke-8 dan 3 hari terakhir rawatan pemakanan. Kesemua sampel najis telah dianalisis melalui kromatografi cair berprestasi tinggi untuk analisa asid lemak rantaian pendek. Kesemua modifikasi telah menunjukkan peningkatan kelarutan dan kuasa kebengkakan. Kanji sago asli menunjukkan kandungan kanji rintang yang tinggi pada 69%. Kanji retrogradasi menunjukkan kandungan amilosa dan kanji rintang yang nyata rendah berbanding kanji asli ($P < 0.05$). Hanya pengasetilan pada tahap 1.5 dan 2% dan juga dwi modifikasi menglibatkan 1.5% penghubungan silang dan 2% pengasetilan menunjukkan peningkatan kandungan kanji rintang berbanding kanji asli. Kanji yang dihubung silang dan di dwi modifikasi menunjukkan peningkatan kandungan amilosa di semua tahap modifikasi. Mencit yang diberi makan diet

pemakanan yang ditambah kanji rintang jenis 3 dan kanji asetilasi menunjukkan prestasi pembesaran keseluruhan yang lebih baik dengan peningkatan berat badan diiringi penurunan kadar pengambilan makanan. Mencit yang diberi makan dengan rumusan pemakanan yang ditambah dengan kanji dwi modifikasi dan kanji sago asli menunjukkan penurunan berat badan dengan diiringi penurunan kadar pengambilan makanan. Corak pemakanan dan berat badan ini menyokong sifat kekenyangan. Analisis asid lemak rantaian pendek menunjukkan jumlah penghasilan laktat yang paling tinggi oleh mencit yang diberi makan diet makanan yang ditambah dengan kanji dwi modifikasi pada kadar 43.57%. Kepekatan propionat tertinggi ditunjukkan oleh mencit yang diberi makan diet makanan yang ditambah dengan kanji rintang jenis 3 dan kanji dwi modifikasi pada kadar 10.4%. Data menunjukkan kanji dwi modifikasi mempunyai potensi sebagai calon prebiotik secara *in vivo*. Potensi penurunan berat badan yang ditunjukkan di dalam diet yang ditambah rumusan kanji dwi modifikasi juga boleh selanjutnya digunakan di dalam diet pemakanan untuk pengurusan berat.

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LIST OF ABBREVIATIONS

RS	Resistant starch
SCFA	Short chain fatty acid
ha	Hectare
HPLC	High performance liquid chromatography
m	Meter
kg	Kilogram
µm	Micrometer
RVU	Rapid visco unit
FOS	Fructo-oligosaccharide
GOS	Galacto-oligosaccharide
g	Gram
DNA	Deoxyribonucleic acid
min	Minute
rpm	Rotation per minute
GOPOD	Glucose oxidase / peroxidase
nm	Nanometer
AMG	Amyloglucosidase
M	Molar
mm	Millimeter
ANOVA	Analysis of variance
FDA	Food and drug administration
VAS	Visual analogue scale
GIP	Gastric inhibitor polypeptide

CHAPTER 1

INTRODUCTION

Metroxylon sagu or commonly known as sago is one of the most important commodities in Malaysia, particularly Sarawak, with an annual export of extracted starch at 60,000 to 70,000 tonnes worth up to RM 90 million (Department of Statistics, 2012). This makes Sarawak the world's largest exporter of the starch (Awg-Adeni, Bujang, Hassan, & Abd-Aziz, 2012; Singhal et al., 2008). Aside from being an economical and important agricultural commodity in Sarawak, the main advantage of this starch crop is that it is the only commodity that can withstand and grow on peat soil, which covers 75% of the coastal plains and lowland river basins in Sarawak (Bujang, 2014). However, the consumption of sago starch globally stands at only 3% in the starch market dominated by corn, potato and tapioca, at 200,000 to 300,000 tonnes per annum. In order to increase its competitiveness among other industrial starches, the development of new product and marketing strategy is important. Realising its potential, the Malaysian government has emphasised on the sago industry in the 11th Malaysia Plan (the year 2016 to 2020) as the source in poverty eradication program (Uggah, 2013). In the present study, we are looking for a new perspective of sago starch utilisation as a type of functional food *i.e.* prebiotic and resistant starch.

Prebiotics are non-digestible food ingredients that benefit the host by stimulating the growth or activity of one or a limited number of bacteria in the colon. Present study on prebiotic is limited as most only focuses on acquiring sufficient data for inulin-type fructans and oligosaccharides to be confirmed as functional food. Nevertheless, that did not stop the market of prebiotic from blooming. The prebiotic market in the US and Europe is projected to reach USD 1.17 billion and USD 225.31 million respectively by 2015 (Global Industry Analyst, 2010). Another report by the Global Industry analyst predicted that the global sales of prebiotic could reach as high as USD 4.8 billion by 2018 (Spinner, 2013). Many more prebiotic products have been continuously introduced to the market, with its nutritional benefit at the forefront of its promotion. Therefore, the study of various food ingredients such as resistant starch (RS) as a prebiotic may provide more variant in the market.

Resistant starch can be classed and categorise into four types based on their origins and characteristics. Molecular structure and starch granules structure confers the resistance to RS type I and II. Both are mostly naturally occurring RS. However, RS type II can be developed via strategised breeding and genetic engineering. Resistant starch type III is retrograded starch while RS type IV is chemically modified resistant starch. Resistant starch type II and III of different sources and origin have been successfully marketed in Europe. Resistant starch type IV which is chemically modified starch has to comply with regulations that limit the amount of chemical allowed in food ingredients.

The physical and chemical modifications have been used extensively on starch to improve their physicochemical properties. Both modifications have also been shown to increase the resistant starch content of various starches. The physical modification through retrogradation process has increases RS content in starch by increasing the molecular order of the starch structure, thus inhibiting the entrance of digestive enzymes. Chemical modification introduces a functional group into the starch structure, changing their overall reactivity towards enzymes and reorganising their molecular structures.

Generally, resistant starch could fulfil the first criterion of a prebiotic, which is their resistance against upper intestinal digestion. However, to further test the potential of RS as prebiotic, subsequent validations through *in vivo* and/or *in vitro* analysis need to be conducted. A majority of *in vivo* studies are conducted using rodent and pig model while *in vitro* analysis are conducted through controlled batch culture fermentation to mimic colonic environment. A strategised human trial study can be further conducted to fully qualify RS as a prebiotic.

This study aims to extract, modify and quantify RS content from *Metroxylon sagu* and to evaluate the potential of the extracted and modified RS as a prebiotic. The specific objectives of this study are as follows;

1. Production of resistant starch type III from native sago starch through retrogradation process.
2. Production of resistant starch type IV from native sago starch through chemical modifications.
3. *In vivo* evaluation of native and modified sago starches using BALB/c mice model as a prebiotic.

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