UNIVERSITI PUTRA MALAYSIA

POTENTIAL OF NATIVE SAGO STARCH AS PREBIOTIC WITH AN ANTI-OBESITY FUNCTIONALITY

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By

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One hypothesis that has gained attention for the occurrence of obesity is due to the reaction of the microorganisms in our gut. It have been established that the food intake and dietary composition modulates the composition of the gut microbiota. Resistant starch has gained attention due to its benefits to host's health which resembles prebiotics. Sago starch has been reported containing 69% of RS. Sago starch is an important agricultural commodity in Sarawak; however, it is less exploited. This study aimed to determine the potential of sago starch as prebiotics and evaluating the range of low dosage that can confers health benefit in obese management which could be added as functional value to increase its competitiveness with other starches. A 48 male Sprague Dawley rats were fat-induced for 6 weeks prior to RS intervention. Resistant starch intervention was conducted for 8 weeks. Body weight and food intake were observed every week. Faecal samples were collected every 2 weeks interval. At the end of treatment, the rats were sacrificed and gastrointestinal tract were extracted for further analysis. Faecal sample subjected to bacteria profiling using the fluorescent in-situ hybridisation (FISH) technique. Both faecal and caecum sample were subjected to short chain fatty acids analysis using high-performance liquid chromatography. Hepatic lipid content were measured using Folch method. All dosage of sago starch showed a strong correlation of body weight loss, with reduction of food intake. This pattern suggests satiety properties. The increment of dosage RS in treatment showed an increment of *Bifidobacterium* spp. and *Lactobacillus* spp. regardless of phenotype when compared to 0% RS group. This pattern suggests the sago starch having bifidogenic factor. Short chain fatty acids analysis conducted in the faecal and caecum samples showed demonstrated a significant increase of the total SCFA production. Acetate, propionate and butyrate concentration are higher in sago starch group when compared to 0% RS group in both ceacum and faecal sample. Hepatic lipid analysis demonstrated sago starch group (4% SRS, 8% SRS, and 16% SRS) have lower fats accumulation in liver when compared to Hi-maize.

Body fats tissues also showed that RS-enriched diets group have lower fats than the low-fat diet and 0% RS group. The overall results show that sago starch elicits the similar effect as Hi-maize that can bring benefits to health. Sago starch at low dosage has the potential as a prebiotics with anti-obesity functionality with a consistent consumption.

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

POTENSI TEPUNG SAGU ASLI SEBAGAI PREBIOTIK YANG MEMPUNYAI FUNSGI ANTI-OBESITI

Oleh

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Satu hipotesis yang mendapat perhatian sebagai punca obesiti adalah reaksi mikroorganisma dalam usus kita. Pengambilan makanan dan komposisi makanan dipercayai boleh megubah komposisi mikrobiota usus. Kanji rintang telah mendapat perhatian kerana manfaatnya kepada kesihatan yang menyerupai prebiotik. Tepung sagu telah dilaporkan mengandungi 69% kanji rintang. Tepung sagu juga adalah komoditi pertanian penting di Sarawak; walau bagaimanapun, ia kurang dieksploitasi. Kajian ini bertujuan untuk menilaikan potensi tepung sagu sebagai prebiotik dan menilai pelbagai dos yang rendah yang dapat memberi manfaat kesihatan dalam pengurusan obes; yang mana laporan ini boleh digunakan untuk menambah fungsi tepung sagu bagi meningkatkan daya saingnya dengan tepung-tepung lain. Sebanyak 48 tikus jantan Sprague Dawley telah dipaksa obes selama 6 minggu. Intervensi kanji rintang dalam makanan dilakukan selama 8 minggu selepas minggu penggemukan. Berat badan dan kadar makanan dicatat setiap minggu. Sampel najis dikumpulkan setiap selang 2 minggu. Pada akhir rawatan, tikus dikorbankan dan saluran usus diekstrak untuk analisis selanjutnya. Sampel najis tertakluk kepada profil bakteria menggunakan teknik hibridisasi *in-situ* fluoresen (FISH). Kedua-dua sampel najis dan usus buntu tertakluk kepada analisa asid lemak rantaian pendek menggunakan kromatografi cecair berprestasi tinggi (HPLC). Kandungan lemak hati dikaji menggunakan kaedah Folch. Semua dos kanji rintang dalam tepung sagu menunjukkan korelasi yang kuat antara penurunan berat badan dengan pengurangan pengambilan makanan. Corak ini menunjukkan sifat kenyang. Peningkatan kanji rintang dalam rawatan menunjukkan kenaikan kiraan *Bifidobacterium* spp. dan *Lactobacillus* spp. tidak kira fenotip apabila dibandingkan dengan kumpulan 0% kanji rintang. Corak ini menunjukkan tepung sagu mempunyai faktor bifidogenik. Analisis asid lemak rantaian yang pendek yang dilakukan pada sampel najis dan usus buntu menunjukkan bahawa tepung sagu menunjukkan peningkatan yang ketara dalam

jumlah pengeluaran SCFA. Kepekatan asetat, propionat dan butirat lebih tinggi berbanding dengan kumpulan 0% kanji rintang dalam usus buntu dan sampel najis. Analisis lipid hepatik menunjukkan kumpulan tepung sagu (4% SRS, 8% SRS dan 16% SRS) mempunyai pengumpulan lemak yang lebih rendah dalam hati berbanding dengan kumpulan *Hi-maize*. Tisu lemak badan juga menunjukkan bahawa kumpulan diet yang diperkaya kanji rintang mempunyai lemak badan yang lebih rendah berbanding diet rendah lemak dan kumpulan 0% kanji rintang. Hasil keseluruhan menunjukkan bahawa tepung sagu mempunyai ciri-ciri yang sama dengan kanji rintang komersial iaitu *Hi-maize* yang mana mampu membawa manfaat kepada kesihatan. Tepung sagu pada dos yang rendah mempunyai potensi sebagai prebiotik yang mempunyai funsgi sebagai anti obesiti dengan penggunaan yang konsisten.

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'Blessed are the merciful for they shall receive mercy." (Matthew 6:7)

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LIST OF ABBREVIATIONS

RS	Resistant starch
SCFA	Short chain fatty acid
HPLC	High performance liquid chromatography
m	Meter
kg	Kilogram
µm	Micrometer
g	Gram
DNA	Deoxyribonucleic acid
min	Minute
rpm	Rotation per minute
GOPOD	Glucose oxidase / peroxidase
nm	Nanometer
AMG	Amyloglucosidase
M	Molar
mm	Millimetre
mM	mill molar
ANOVA	Analysis of variance
GIP	Gastric inhibitor polypeptide
PYY	Peptide YY
NAFLD	Non-alcoholic fatty liver disease
OP	Obese prone
OR	Obese resistant

CHAPTER 1

INTRODUCTION

The occurrence of obesity is becoming common among adults and children. Worldwide statistic showed 39% of the adult is overweight and obesity in 2014 (World Health Organization, 2017) and estimated to cause 3 to 4 million deaths (Lim *et al.*, 2013). Obesity which predominantly occurs in a developed country has drastically increased in developing country, influenced by modern lifestyles that lack physical activity and unhealthy food choices (Hedley *et al.*, 2004). It has been suggested as a result of increased high simple sugar and high fats diet consumption such as cake, pizza, pastries and etc has been stated as the main culprit of this epidemic (Manz, Amann, Ludwig, Vancanneyt, and Schleifer, 1996). Obesity is a constitutes to the risk factor for type-2 diabetes, liver malfunction, hypertension and cardiovascular diseases (Kyrou and Tsigos, 2009). Realising this, prevention of obesity development is very much essential.

One hypothesis that has gained some attention for the occurrence of obesity is due to the reaction of the microorganisms in our gut. It has been determined that obesity and insulin resistance are associated with low-grade chronic systemic inflammation by the action of bacterial lipopolysaccharide (LPS) (Hotamisligil, 2006). These bacterial LPS is continuously produced in the gut by gram-negative bacteria and transported into intestinal capillaries (Neal *et al.*, 2006). Such LPS is transported from the intestine toward target tissues by a mechanism facilitated by lipoproteins, freshly synthesized from epithelial intestinal cells in response to a high-fat diet (Vreugdenhil *et al.*, 2003) thus triggering the secretion of pro-inflammatory cytokines (Sweet and Hume, 1996; Wright, Ramos, Tobias, Ulevitch, and Mathison, 1990). These cytokines which are the key inducers to insulin resistance will promote excessive fat accumulation thus leading to development of obesity (Cani, 2007; Cani *et al.*, 2007). Therefore, alteration of the gut microbiota, towards more 'positive and beneficial' environment and prevention of dysbiosis may be the key factors to reduce obesity.

It is well known that composition of gut microbiota can be modifying by changes in diet. The modulation of gut microbiota is strongly influenced by dietary intake (Conlon and Bird, 2014) as well as genetics and immunology factor (Rodríguez *et al.*, 2015). There are some food ingredient showed positive influence towards the obese gut microbiota in several studies through both *in-vivo* and *in-vitro* experimentation (Hildebrandt *et al.*, 2009; Sarbini, Kolida, Deaville, Gibson, and Rastall, 2014). The imbalance of these composition *i.e* dysbiosis is believed leading to the rising of many diseases. Due to this findings, few studies have been conducted to focus more on gut microbiota potential combating metabolic disease through the fermentation of dietary sources (DiBaise, Frank, and Mathur, 2012).

Food ingredient such as inulin and oligofructose has proven its ability positively influence the gut microbiota through several studies (Ramirez-Farias *et al.*, 2008; Wang, X. and Gibson, 1993). The food ingredient that plays important role in positively modulating gut microbiota can also be known as prebiotic. Prebiotic is defined as an indigestible starch fraction that beneficially affects the host by selectively stimulating the growth and/or activity of one or limited number of gut microbiota particularly, *Bifidobacteria* sp. and *Lactobacilli* sp. which subsequently improves the host health (Roberfroid, 2010). Benefits of prebiotics have been proven which include improvement in intestinal permeability and host immunity, reduction of potentially pathogenic bacteria and improved SCFA production (Slavin, 2013).

One of the potential candidates of prebiotics which is gaining attention is resistant starch (RS). The starch is named as resistant starch due to its ability to escape digestion in the small intestine and passes into the colon (Xie, Liu, and Cui, 2006). Consumption of resistant starch is coming to attention due to its ability to confer health benefits to the consumer which resemble prebiotics, and are abundant in many common diets (Zaman and Sarbini, 2015). The beneficial effect of resistant starch has been extensively reviewed and this includes glycaemia response, glucose reduction, fat oxidation, insulin sensitivity and satiety (Belobrajdic, King, Christophersen, and Bird, 2012).

In this study, *Metroxylon sagu* also known as sago is used as tested substrate. Sago starch is one of the most important agricultural commodities in Malaysia, particularly Sarawak where 96% yield are particularly from Sarawak (Uthumporn, Wahidah, and Karim, 2014; Zi-Ni, Rosma, Karim, and Liong, 2015). Previous study reported sago starch containing 69% of resistant starch which was considered high compared to other commercial resistant starch such as Hi-maize®, Novelose, and Fibersym (Zaman, 2015). The amylose content and resistant starch were claimed influencing the digestibility of substrate which contributing factor to confer health benefits to it consumer (Bajury, Rawi, Sazali, Abdullah, and Sarbini, 2017; Falony *et al.*, 2009; Slavin, 2013).

Currently, Malaysia is the largest sago starch exporter with annual extracted starch at a range of 40,000 to 51,000 tonnes per year from 2004 to 2013 (den Besten *et al.*, 2013; Olano-Martin, Mountzouris, Gibson, and Rastall, 2000; Uthumporn *et al.*, 2014). Sago starch extracted per unit area is significantly higher than that of rice, corn, wheat, and cassava which could produce up to 25 tonnes per hectare of starch per year (Silvi, Rumney, Cresci, and Rowland, 1999). However, the consumption of sago only takes up to 3% of other starch resources globally when compared to other dominated starch such as tapioca, potato, and corn which can go up to 300,000 tonnes annually (Zaman, 2015). In order to increase the competitiveness with other sources, a functional value should be added as a marketing strategy and directly contribute the growth of sago-farming. In present study, we are comparing sago starch with the commercial resistant starch type II, Hi-maize. This is due to the similar intrinsic factor of sago starch with Hi-maize (type II RS) which the starches was extracted and occur in its natural granule form (Nugent, 2005). Moreover, Hi-maize has been extensively studied for it beneficial properties towards human's health which has been reviewed for it prebiotics effect (Zaman and Sarbini, 2015).

Appropriate amount of resistant starch is necessary to confer its beneficial physiological effects. Previous studies reported positive impact towards host health such as promotes satiety, increase short chain fatty acids production and reduce body weight (Le Leu, Hu, Brown, Woodman, and Young, 2009; Raben *et al.*, 1994; Zenel and Stewart, 2015). However, high dose of resistant starch was applied in these studies. Generally, food ingredient has low resistant starch (Zi-Ni *et al.*, 2015). Belenguer *et al.* (2006) suggested the daily resistant starch consumption should be approximately 20 g for it to confer health benefits. However, the daily intake of an individual differs for the different region where American, the range of 3 g to 8 g (Demigné *et al.*, 1995); European, approximately 4 g (Dysseler and Hoffem, 1994) and Serbian, approximately 6 g (Byrne, Chambers, Morrison, and Frost, 2015). Hence, in this study, we are evaluating the ability of low dosage of the resistant starch from sago starch which within the range of recommendation daily consumption to confer health benefit particularly to combat obesity or as a weight loss regime.

In addition, obese-prone and obese-resistant phenotype was used in the study to investigate the response of gut microbiota and it fermentation product which could be used as prebiotic potential screening upon the consumption as well as its ability as obese management regime. The diet-induced obese rats mimicking closely to obese human which only some rats developed insulin resistance and dyslipidemia (Belobrajdic *et al.*, 2012; Levin, Hogan, and Sullivan, 1989). The resulted changes can be comparable to the human body rather than using genetic-modified obese rats. This study aims to evaluate the impact of different low dosage of the resistant starch from sago starch that could confer benefits health through *in-vivo* experimentation. The specific objectives of this study as follows:

1. To evaluate the anti-obesity Property of Sago Starch using Fat- Induced Rats.
2. To investigate the response of gut microbiota and it fermentation product upon the consumption of sago starch for its prebiotic potential.

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