UNIVERSITI PUTRA MALAYSIA

PRODUCTION OF RESISTANT STARCH TYPE III FROM NATIVE SAGO STARCH AS POTENTIAL PREBIOTIC

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PRODUCTION OF RESISTANT STARCH TYPE III FROM NATIVE SAGO STARCH AS POTENTIAL PREBIOTIC

By

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ABSTRACT

Resistant starch (RS) is a sum of starch that escaped gastrointestinal digestion and can be fermented by colonic microorganisms to produce short chain fatty acids (SCFA). Due to the similar properties, resistant starch is a potential prebiotic. Prebiotic is non-digestible food ingredients that selectively stimulate the growth and/or activity of gut microorganisms, thus promote the host health. Sago starch is abundance in Sarawak, usually used as main food ingredient such as in pancake, pearl and pudding. The production and utilisation of sago starch as functional food is currently limited. The main focus of this study is to create resistant starch type III from Sarawak's native sago starch as potential prebiotic. The resistant starch will be produced via retrogradation process and with the aid of enzyme, pullulanase. From this study, it showed that there are no significant different of resistant starch content among native sago starch and modified sago starches (RS3).

ABSTRAK

Kanji tahan (RS) adalah kanji yang terlepas proses pencernaan dan boleh difermentasikan oleh mikroorganisma kolon untuk menghasilkan asid lemak rantaian pendek (SCFA). Kanji Tahan mempunyai nilai-nilai yang berfungsi hampir sama dengan prebiotik. Prebiotik adalah bahan makanan yang tidak terhadam yang secara pilihan yang merangsang pertumbuhan dan / atau aktiviti mikroorganisma usus dengan itu menggalakkan kesihatan perumah. Kanji sagu adalah banyak dihasilkan di Sarawak, biasanya digunakan sebagai bahan makanan contohnya dalam pembuatan roti dan biskut. Pengeluaran dan penggunaan kanji sagu sebagai makanan kesihatan adalah terhad. Fokus utama kajian ini adalah mewujudkan kanji tahan jenis III daripada kanji sagu asli Sarawak untuk berfungsi sebagai prebiotik. Kanji tahan dihasilkan melalui proses retrogradasi dan juga enzim (pullulanase). Kajian ini menunjukkan tiada perbezaan kandungan kanji tahan yang signifikan antara kanji sagu asli dan kanji sagu yang telah diubahsuai (RS3).

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APPROVAL SHEET

I certify that this research project report entitled "Production of resistant starch type III from native sago starch as potential prebiotic" has been examined and approved as a partial fulfillment of the requirement for the degree of Bachelor Science Bioindustry in the Faculty of Agricultural and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus.

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CHAPTER 1

INTRODUCTION

1.1 Study Background

Malaysia is one of the largest sago starch producers in the world. However, the utilization of sago is still low compared to other sago producing countries, especially as functional food ingredients. Based on information gathered, the resistant starch content in native sago starch itself is very high compared to other starch sources, thus it has higher potential to produce more resistant starch as prebiotic when it is modified. Hence, this study is focused on production of modified resistant sago starch as potential prebiotic.

In normal starch food consumed, only relatively small percentage is able to escape digestion into small bowel. Resistant starch (RS) is a type of starch that is undigested in human small digestion system. It is transported to the lower gut for microbial anaerobic fermentation and act as potential prebiotic. RS have been classified into five types. Type I RS are referred to those physically inaccessible starches, such as partially milled grains and seeds. Type II RS are native starches with highly packed structure inside starch granules, such as potato starch. Type III RS are retrograded starches, which undergo heating and cooling procedures and resistant to amylase action. Type IV RS are chemically modified starches to decrease digestibility. Type V RS are fatty acid modified starches that have newly been proposed.

Resistant starches are substrates for microbial fermentation that produces few short chain fatty acids (SCFAs), for instances acetic, butyric and propionic acid where each of them have its own benefits for the host. The SCFAs are believed to have important roles in gastrointestinal health. Moreover, RS has the ability to regulate gut

microbiota favouring RS degradation process and SCFA production thus it can be considered as a prebiotic. Hence in this study, the RS type III or retrograded sago starch is produced through heating and cooling process. And some samples undergone retrogradation with the aid of enzyme; pullulanase.

1.2 Objective

The objective of this study is to produce RS type III from native sago starch as a potential prebiotic. Besides, it is also to determine and compare the RS content in the samples prepared.

1.3 Significance of Study

The consumer request for high quality food products has led the growth of the use of new technologies and ingredients (Fuentes-Zaragoza *et al.*, 2010). There are reasons affecting changes in the consumer demand, such as: health concerns (cholesterol, cancer, obesity, etc.), changes in demographic characteristics (ethnics, population ageing, etc.), the need for convenience, alterations in delivery systems and expense (López and Pérez-Alvarez, 2008; Fuentes-Zaragoza *et al.*, 2010). The major reason for the increase in popularity of novel food with good nutritional properties is because of the increase in awareness of consumers towards the relationship of nutritious food, good health and well-being (Pérez-Alvarez, 2008; Sanz *et al.*, 2008). Resistant starch (RS) which is broadly used as a functional ingredient specifically in foods containing high levels of dietary fibre (Mikulíková *et al.*, 2008). Researches and studies have shown that individuals which consumed high in fibre have lowered risks of chronic diseases for instances cancer, coronary heart disease, obesity and probably diabetes (Canovas and Pérez-Alvarez, 2006). RS has been recommended

for use in probiotic compositions to stimulate the growth of beneficial microbiota such as *Bifidobacterium* (Brown *et al.*, 1996). As RS almost entirely escape through the digestion of small intestine, it can act as a growth substrate for probiotic microorganisms that is prebiotic (Sajilata *et al.*, 2006). Hence, in this study, I will focusing on producing resistant starch type III from native sago starch as a potential prebiotic.

CHAPTER 2

LITERATURE REVIEW

2.1 Prebiotic

Prebiotic is a non-digestible food ingredient that beneficial to the host by selectively stimulating the growth and/or activity of one or restricted numbers of bacteria in the colon, and thereby enhanced the host health (Roberfroid *et al.*, 2010). According to Buttriss and Stokes (2008), archetypal of prebiotics are inulin and oligofructose, which naturally existed in a sum of fruits and vegetables (e.g. bananas, chicory, Jerusalem artichokes, onions, garlic and leeks, and wheat), and other resistant oligosaccharides for instance inulin-type fructans. Roberfroid *et al.* (2010) has listed up three standards that can be used as guidelines in labelling prebiotics which are; (1) resistance to gastrointestinal acidity, hydrolysis by mammalian enzymes and gastric absorption; (2) fermented and consumed by the gut microbiota; (3) selectively stimulate activity and/or growth of one or restricted number of gut bacteria that contribute to host health and well-being.

2.2 Health Benefits of Prebiotic

Prebiotics will only be consumed by a specific group of gut microbiota when it reaches the colon (Zaman and Sarbini, 2015). For a prebiotic to be considered functional, it must specifically stimulates only the growth and activity of the beneficial bacteria such as *Bifidobacterium* and *Lactobacillus*, consequently creating metabolites like the short-chain fatty acids (acetate, butyrate and propionate) which afterwards contribute to the host (Sarbini *et al.*, 2014)).

Prebiotic reduces the risk of obesity type-2 diabetes by enhancing glucose tolerance and reduction in endogenous glucose production (Cani *et al.*, 2007a; Cani *et al.*, 2007b). According to Scholz-Ahrens *et al.* (2007), prebiotic increases the absorption of minerals and improves mineralization of bone. A significantly lower respiratory tract infection (RTI) incidents reported in infants being orally fed with prebiotics supplement (Luoto *et al.*, 2014). While increasing the stool frequency, it also decrease the faecal pH (Ben *et al.*, 2008). Complex fermentation samples of inulin-type fructan positively modulate expression of genes related to biotransformation in carcinoma cells thus decrease cancer risk and inflammatory bowel disease (Munjal *et al.*, 2012). It is suggested to consume prebiotic 5–8 g/day, yet beyond 20 g/day may perhaps cause intestinal uneasiness due to gas distension (Clausen and Mortensen, 1997).

2.3 Resistant Starch

Chemically, starches are polysaccharides composed of a-D-glucopyranosyl units linked together with a-D-(1–4) and/or a-D-(1–6) linkages, and are comprised of two molecular types: amylose, the straight chain polyglucan comprised of approximately 1000, a-D-(1–4) linked glucoses; and amylopectin, the branched glucan, comprised of approximately 4000 glucose units with branches occurring as a-D-(1–6) linkages (Haralampu, 2000; Sharma *et al.*, 2008).

Naturally occurring RS is frequently found in cereal grains, seeds and heated starch and starch-containing foods (Charalampopoulos *et al.*, 2002). Although the main source of carbohydrate in the human diet is starch, nevertheless some starch in the diet is undigested and could not be absorbed in the small intestine (Ratnayake and

Jackson, 2008). RS can be well-defined as the amount of starch and its digestion product that resists digestion in the small intestine of a healthy human (Englyst *et al.*, 1992).

RS has a tremendously expansive range of constituents. Currently, there are different types exist (RS1–4), these are commonly classified based on physical and chemical characteristics (Nugent, 2005).

2.4 Type of Resistant Starch

2.4.1 RS1: Physically Inaccessible Starch

RS1 is entrapped in the protein matrix or cell wall material and is physically inaccessible for enzyme hydrolysis. For example, starch granules in the vitreous endosperm of cereal grains are embedded in a dense protein matrix. The protein matrix is relatively water-impermeable, which retards starch swelling during cooking and acts as a barrier for amylase hydrolysis (O'Dea *et al.*, 1980; Rooney and Pflugfelder, 1986). Grinding of raw grains or seeds increases the susceptibility of starch to enzyme hydrolysis because the starch granules are free from the protein matrix (O'Dea *et al.*, 1980; Jenkins *et al.*, 1988). RS1, however, is not observed in the floury endosperm of cereal grains because the protein matrix is weak and discontinuous and the starch is more accessible for enzyme hydrolysis. Starch granules in pasta are also embedded in protein matrix, which is attributed to the greater protein content of durum wheat than that of other wheat species. The protein matrix forms a barrier and prevents the diffusion of water into the centre of the pasta (Cunin *et al.*, 1995). As the result, the starch granules in the centre of the pasta are not fully gelatinized during boiling and are less susceptible to amylase hydrolysis

than the starch in white bread (Granfeldt *et al.*, 1991; Barkeling *et al.*, 1995). The digestibility of starch in pasta increases when protein is removed using proteases (Fardet *et al.*, 1998; Zhang and Hamaker, 1998). The RS1 in legume seeds are surrounded by cell wall. The rigid cell wall retards the penetration of water into the legume seeds, and thus prevents the starch granules from getting enough water to swell and to gelatinize during cooking (Kon *et al.*, 1971; Würsch *et al.*, 1986). Thus, the starch granules in the cooked whole bean are not completely gelatinized and less digestible than those in the cooked ground bean. Although RS1 showed benefits for human health, its presence in the cereal grains is not desirable as feed for small animals such as young chicks. The entrapped starch is not available to provide energy for the growth and development of the animal. It is also not desirable in the ethanol production because it reduces the final ethanol yield. Heat processing has been used to reduce the RS1 content in the grains through protein denaturation and starch gelatinization (Nnanna and Phillips, 1990). Milling of the grains increases starch digestibility by reducing the particle size, disrupting the protein matrix, and releasing the starch granules (O'Dea *et al.*, 1980; Weurding *et al.*, 2001). Other treatments, suchas protease incubation and steam flaking, can also weaken the protein matrix (Zhang and Hamaker, 1998; Zinn *et al.*, 2002). Endogenous proteases and amylases in the grains can be activated through germination, which increases the hydrolysis of both protein and starch (Nnanna and Phillips, 1990; Kataria *et al.*, 1992).

2.4.2 RS2: Native B-Type Semi-Crystalline Starch

Isolated uncooked starch granules with the B- and some C-type polymorphs (RS2), such as uncooked potato starch, green banana starch, and high-amylose maize starch,

are resistant to enzyme hydrolysis (Jane *et al.*, 2003). This is attributed to the homogenous internal structure of the B-type polymorphic starch granules (Jane, 2006; Ao and Jane, 2007). The branch chains of the B-type polymorphic amylopectin are also longer than those of the A-type counterpart and the branching points of the B-type polymorphic amylopectin are mostly located at the amorphous regions, which results in a more perfect crystalline structure (Jane *et al.*, 1997). The RS2, in general, loses its enzyme resistance after heat processing. Starch gelatinization during heat processing converts semi-crystalline structure (RS2) to highly digestible, amorphous structure (Knutson *et al.*, 1982). The high-amylose maize starch with an amylose content above 50%, however, has high gelatinization temperature above 100°C (Li *et al.*, 2008). This is attributed to that the *amylose-extender* mutation increases the contents of amylose and long branch-chain of amylopectin (Jane *et al.*, 1999). Thus a fraction of the native crystalline structure (RS2) remained in the high-amylose maize starch after heating at the water-boiling temperature (Li *et al.*, 2008). The heat stability and the enzyme resistance of the granular starch (RS2) can be enhanced using mild acid hydrolysis and hydrothermal treatments (Brumovsky and Thompson, 2001). Acid hydrolysis of the amorphous regions in the starch granules results in a higher percentage of crystalline regions. Hydrothermal treatments allow the starch crystallites to rearrange to a more perfect crystalline structure. The increase in the crystallinity and the perfection of the starch crystallites results in an increase in the RS content of the granular starch.

2.4.3 RS3: Retrograded Amylose

Gelatinized and amorphous starch molecules tend to retrograde into a double helical structure during storage. The orderly aligned crystallites of the retrograded amylose

(RS3) are enzyme resistant and have a melting temperature above 120°C, thus they remain in food products after heat processing (Sievert and Pomeranz, 1989, 1990; Gruchala and Pomeranz, 1993; Klucinec and Thompson, 1999). Starch with large amounts of amylose and/or long branch chains of amylopectin, such as legume and high-amylose cereal starches, has been used to produce RS3 (Sievert and Pomeranz, 1989, 1990; Vasanthan and Bhatty, 1998). The long-branch chains of amylopectin have properties similar to amylose, which increase the apparent amylose content of the starch. On the contrary, the short-branch chains of amylopectin form double helices that are not long enough to produce stable crystallites. Extrusion cooking has been used recently to produce RS3 from flours and starches (Vasanthan *et al.*, 2002; Faraj *et al.*, 2004; Agustiniano-Osornio *et al.*, 2005). Extrusion is commonly used in food industry to make breakfast cereals, snack foods, and many other similar food products. The RS content of the extruded foods depends on the screw speed, extrusion temperature, and moisture content. The optimum RS3 content is achieved when the starch is cooked by extrusion at low screw speed (higher residence time), which allows the amylose chains to retrograde (Agustiniano-Osornio *et al.*, 2005). The fresh extrudate, however, usually contains RS3 below 10% because it is highly amorphous. The RS3 content in starch can be optimized by altering the conditions for nucleation andpropagation of amylose retrogradation (Slade and Levine, 1988; Jang and Pyun, 1997). Repeated cycles of autoclaving and cooling of starch have been used to optimize the nucleation and propagation of retrograded amylose crystallites (RS3) (Sievert and Pomeranz, 1989).

Enzyme debranching and mild acid hydrolysis of starch can increase the linear chains for greater amylose retrogradation (Vasanthan and Bhatty, 1998; Guraya *et al.*,

2001). Debranching enzyme, such as isoamylase and pullulanase, hydrolyses α-D-(1-6) glycosidic branching of amylopectin and produces linear molecules. Acid hydrolyses the amorphous regions of the starch granules, where branching points of amylopectin are mostly located, producing linear chains.

2.4.4 RS4: Chemically Modified Starch

Esterification of starch using acetic anhydride, propylene oxide, or octenyl succinicanhydride has been used to increase the resistance of starch granules to enzyme hydrolysis (RS4) (Chung *et al.*, 2008; Oh *et al.*, 2008). The hydrophobic moieties, such as acetyl group, hydroxypropyl group, and octenyl group, increase the water insolubility of the starch granules and decrease the accessibility of enzyme to hydrolyse starch molecules. The hydrophobic moieties also prevent the starch molecules from fitting to the enzyme binding site and being hydrolysed. Crosslinking of starch granules using sodium trimetaphosphate (Woo and Seib, 2002; Sang and Seib, 2006), phosphorus oxychloride (Han and BeMiller, 2007), or citric acid (Unlu and Faller, 1998; Xie and Liu, 2004) also increases the RS4 content of starch. Crosslinked starch molecules have reduced flexibility to fit into the enzyme binding site (Shin *et al.*, 2004). In addition, crosslinking holds the starch molecules together because it limits the swelling of starch granules and prevents the dispersion of starch molecules during cooking (Shin *et al.*, 2003; Singh *et al.*, 2003).

2.5 Pullulanase

Pullulanase (EC 3.2.1.41, pullulan 6-glucanohydrolase) was first stated by Bender and Wallenfels (1961) in a mesophilic bacterium, *Klebsiella pneumonia* (formerly known as *Aerobacter aerogenes* or *Klebsiella aerogenes*). Later Plant *et al.* (1987)

reported the new class of pullulanases which split pullulan at a-1, 6 linkages and amylose at a-1, 4 linkages. Reliant on their inability or ability to hydrolyze a-1, 4-glycosidic linkages in other polysaccharides, pullulanases are categorized into two based on the substrate specificity, type I and type II pullulanases, respectively (Antranikian and Winkelmann, 1992).

Type I pullulanase (*a-Dextrin 6-Glucanohydrolase, EC 3.2.1.41*) specifically hydrolyzes a-1, 6 linkages in branched oligosaccharides, such as pullulan, starch, amylopectin, and glycogen, forming linear a-1, 4-linked oligomers. It is used in combination with other starch-hydrolyzing enzymes for the complete conversion of polysaccharides to low molecular weight compounds. The enzyme from plant sources was earlier called R-enzyme (Lee and Whelan, 1971), which causes complete conversion of pullulan to maltotriose. Its action on pullulan is commonly in a random endo-fashion. The smallest substrate that is attacked by pullulanase is 6, 2 - a-maltosyl maltose (Marshall, 1973). Pullulanase type I also exhibits the reverse reaction of condensation. On incubation of high concentrations of pullulanase with maltotriose or maltose, various condensation products with a-1, 6-glycosidic linkages are synthesized (Abdullah and French, 1970).

Type II pullulanase (EC 3.2.1.1/41), unlike type I enzyme, is capable of hydrolysing a -1, 4-glycosidic linkages in polysaccharides in addition to the a-1, 6-glycosidic linkages in pullulan and branched substrates (Bertoldo *et al.*, 2004). It cleaves pullulan at a-1, 6 linkages and amylose at a-1, 4 linkages (Plant *et al.*, 1987). The enzyme causes the complete conversion of polysaccharides to small sugars without the requirement of other enzymes like a -amylase or b -amylase (Antranikian and Winkelmann, 1992). Due to its action pattern, this enzyme is also referred to as a–

amylasepullulanase (Melasniemi, 1988; Kim and Kim, 1995) or amylopullulanase (Saha and Zeikus, 1989). Both type I and type II pullulanases do not hydrolyze substrates such as dextran, isomaltose, and isomaltotriose, which are solely linked by a -1, 6-glycosidic bonds. The inability of these enzymes to hydrolyze these substrates suggests the requirement of a-1, 4 linkages in the vicinity of a-1, 6 bonds for enzymatic activity (Abdullah and French, 1970; Antranikian and Winkelmann, 1992).

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Material and Treatments

The study was conducted at Universiti Putra Malaysia Bintulu Sarawak Campus. The control sample used in this experiment is Fibersym, a commercialized RS type 4. The native sago starch was obtained from local market in Bintulu, Sarawak. While other samples were prepared by gelatinization and retrogradation of the sago starch with and without the aid of enzyme, pullulanase. Six treatments are used to compare the resistant starch content. All treatments are triplicates.

The treatments are :

Table 3.1 Details of treatment use in resistant starch content

Treatment	Description
T1	Fibersym (Control)
T2	Native sago starch
T3	Debranched at 4 h sago starch
T4	Debranched at 8 h sago starch
T5	Debranched at 12 h sago starch
T6	Retrograded sago starch

3.2 Preparation of 30 PUN mL^{-1} Pullulanase

50 ml of water is autoclaved for sterilisation. The enzyme pullulanase is diluted with steriled water at the amount of 0.5 ml pullulanase: 19.5 mL water. 30 PUN mL^{-1} of the diluted pullulanase solution are to be directly used or stored in fridge.

3.3 Preparation of retrograded resistant starch from native sago starch without pullulanase

Sago starch (10 g) was dispersed in 40 ml of water, and then pressure-cooked in an autoclave at 121°C for 20 min. The autoclaved starch paste was allowed to cool to room temperature and then stored at 4°C for 24 h. The prepared starch paste was re-exposed to the autoclaving–cooling cycle twice, and then oven dried at 50°C for 24 hours and then milled to produce fine particles. RS content in all prepared samples was analysed. Each preparation was carried out in triplicate.

3.4 Preparation of debranched retrograded resistant starch from native sago starch with pullulanase.

Debranching of gelatinized or retrograded sago starch was carried out by the method reported by Ozturk *et al*. (2009). 10 g of sago starch was dispersed in 40 ml water. The mixture was pressure-cooked in an autoclave at 121°C for 20 minutes, and then cooled to 60°C. One millilitre of pullulanase solution (enzyme activity 30 PUN mL^{-1}) was added to the gelatinized starch paste. During debranching, the gelatinized sago starch was incubated at 60°C for 2, 4, 8 or 12 hours respectively under continuous agitation. The starch paste was then heated to 100°C `for 10 minutes to inactivate the enzyme, and then cooled to room temperature, stored at 4°C for 24 hours, before retreated with two autoclaving–cooling cycles. The starch paste was dried and milled. RS content in all prepared samples was analysed. Each preparation was carried in triplicate.

3.5 Isolation of resistant starch

In a 2 ml microcentrifuge tube, 20 mg of starch sample was washed with 1.8 ml of 90% ethanol and warmed to 60°C for 5 minutes with occasional vortexing. It was

then centrifuged at 1500 xg for 10 minutes. The supernatant was decanted and the washing was repeated twice. Next, 1.8ml of distilled water was added and vortexed. The solution was heated on a boiling water bath for 5 minutes and then centrifuged at 1500 xg for 10 minutes to obtain the pellet which was the isolated resistant starch.

3.6 Total resistant starch content

In a universal bottle, suspended pellet was mixed with 2.0 ml of 10 N KOH and heated on boiling water bath for 5 minutes. 2.0 ml of 10 M of H_3PO_4 was added slowly to neutralize the solution. 5 µl of aqueous extract of the flour was added to 500 µl (4%) phenol and 2.5 ml (96%) H_2SO_4. The concentration of the sample was extrapolated from a standard curve obtained by serially diluting 1mg/ml glucose standard to varying concentrations (0.05-0.5 µg/ml) in 0.05 increment which then further re-diluted with 1 ml of water each. The absorbance was read at 490 nm. The result will be expressed in percentage.

3.7 Statistical Analysis

Data were analyzed statistically using analysis of variance (ANOVA) to detect treatment effect. Means of treatments were compared by using Tukey's Test ($p \leq 0.05$). The statistical software used was Statistical Analysis System (SAS) version 9.3 (SAS 2009).

CHAPTER 4

RESULTS

4.1 Concentration of Resistant Starch Content

Table 4.1 Mean of resistant starch (RS) concentration (%) in each treatment (mean values and standard deviation, n3)

Treatment	Concentration of RS (%)
T1 (Fibersym)	1.720 ± 0.100^{a}
T2 (Native Sago Starch)	1.220 ± 0.000^{b}
T3 (Retrograded Sago Starch)	1.053 ± 0.104^{b}
T4 (Debranched 4 hours)	1.070 ± 0.173^{b}
T5 (Debranched 8 hours)	1.137 ± 0.153^{b}
T6 (Debranched 12 hours)	1.070 ± 0.132^{b}

Note: ^{a,b} Mean value with unlike superscript letters were significantly higher/lower in comparison among fermentation periods in the same substrate fermentation system ($p \leq 0.05$).

From Table 4.1, it is showed that T1 or Fibersym is the only significantly difference among the treatments. Fibersym contained higher percentage of RS compared to others which is 1.72%. Statistically there are no significant difference among native sago sample (T2), retrograded sample (T3), and debranched retrograded starch samples (T4, T5 and T6). However, there are slight differences among the treatments.

4.2 Comparison of RS Content among Debranched Retrograded Sago Starches

Statistically, there are no significant differences among three debranched retrograded sago starch for all 4, 8, and 12 hours (Figure 4.1). Yet by percentage, T5, which is the retrograded starch debranched for 8 hours shows slightly higher concentration of 0.067% than those debranched for 4 hour and 12 hours. While for T4 and T6, there is not much different in RS content (%) between each other.

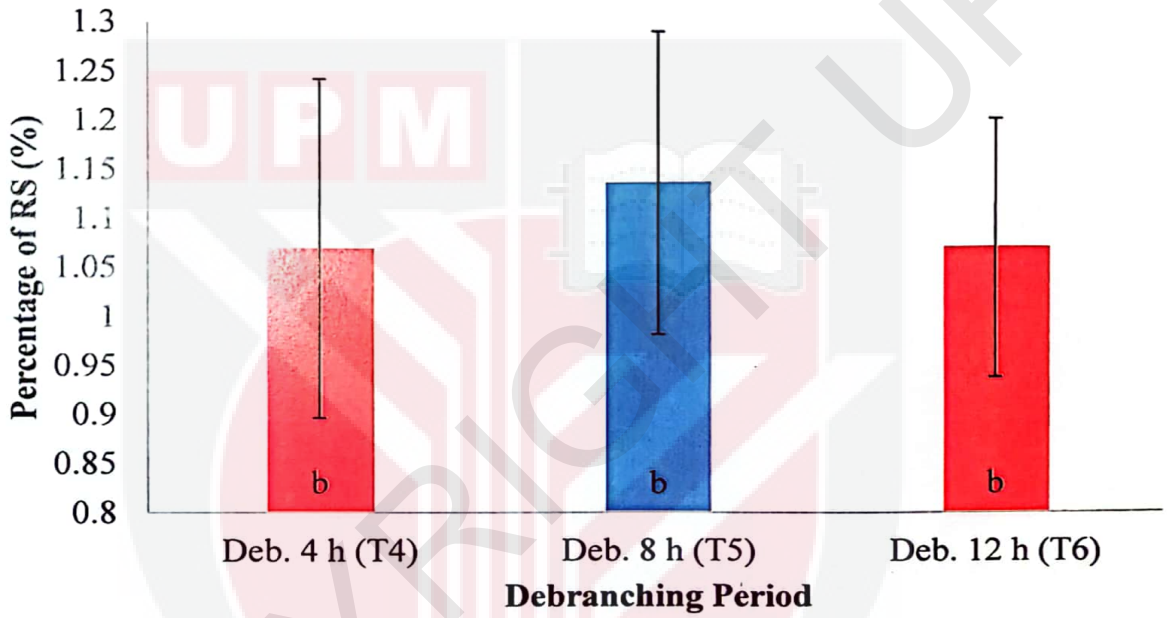

Figure 4.1 Comparison of total RS Concentration (%) among debranched sago starch at different debranching period (4, 8 and 12 hour). Values are means with standard deviation and the same letters indicates that there were no significant difference among treatment ($P \leq 0.05$)

4.3 Comparison of RS Content between Retrograded and Debranched Retrograded Sago Starch

Statistically based on Table 4.1, there are no significant differences between retrograded sago starches debranched for 8 hours (T5) and retrograded sago starch (T3). In which, T5 has the highest RS content among the debranched retrograded sago starches (Figure 4.2). Yet by percentage, retrograded sago starch debranched for 8 hours (T5) show higher concentration of RS than the retrograded sago starch at 0.084% of RS content.

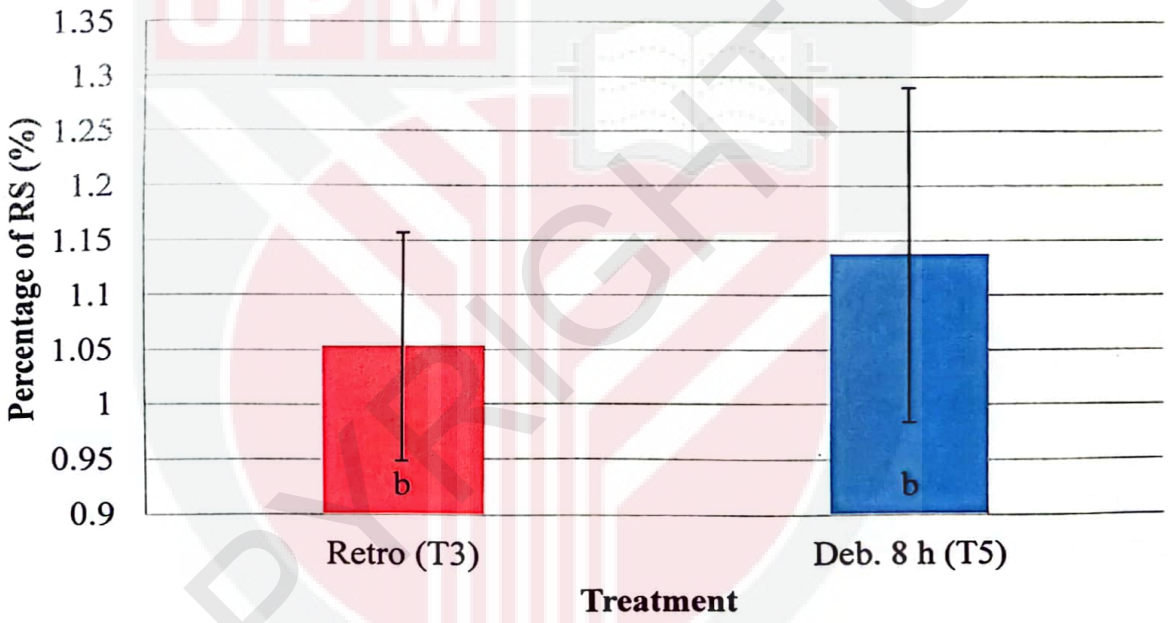

Figure 4.2 Comparison of total RS Concentration (%) between retrograded and debranched sago starch 8 hour. Values are means with standard deviation and the same letters indicates that there were no significant difference between treatment ($P \leq 0.05$)

CHAPTER 5

DISCUSSION

5.1 Concentration of Resistant Starch Content

Based on Table 1 in the last chapter, the positive control of the experiment which is the commercial released RS4, Fibersym (T1), contained the highest percentage of RS. According to Mun and Shin (2006), RS4 is the most resistant due to its cross-linked structure.

There is no significant different among the sago starches (native or modified). Maybe there is no modification needed to increase the amount of resistant starch in sago starch. Gelatinization greatly increases the digestibility of starch, presumably due to both granular disorganization and increased porosity of the substrate (Ring *et al.*, 1988). Thus, instead of making the starch more resistant, the extra processing would upsurge its digestibility.

Numerically, the native sago starch (T2) and retrograded starch (T3) contained higher RS content as in the debranched sago starch at 4 h (T4), 8 h (T5) and 12 h (T6). This may be due to T1, T2 and T3 did not undergo the same heating treatment. Whereas for retrograded debranched samples, the samples undergone heating and cooling cycles after the gelatinization process. In which, they are heated to 60°C to initiate the debranching process and further heated up to 100°C to stop the pullulanase activity. Under these conditions, Leong *et al.* (2007) stated that the starch was gelatinized again and again (gelatinization temperature of sago starch is around 72°C). Based on Englyst and Cummings (1987), the amount of RS basically

depends on the degree of gelatinization and amylose retrogradation during cooling of cooked food.

Study by Ring *et al.* (1988) shown that gelatinization greatly increases the digestibility of starch, presumably owing to both granular disorganization and increased porosity of the substrate. Thus, it is proven that addition heating treatment in T4, T5 and T6 cause the starch to be more digestible and easily loss throughout the process.

5.2 RS Comparison among Debranched Retrograded Sago Starch

As shown in Table 4.1, there is no significant difference among debranched starches (T4, T5 and T6). Yet based on the data, T5 showed a slightly higher percentage of RS than other debranched samples. The RS content increased (0.067%) from 4h to 8h of debranching and decreased (0.067%) at 12h debranching (Figure 4.1). The rise of RS content from 4h to 8h indicated that the debranching process has released medium to small chains that were able to form RS (Leong *et al.*, 2007). The branches produced during the period of 4h to 8h are long enough to form RS. As described by Eerlingen *et al.* (1993), the yield of RS varied with average chain length of the amylose and the yield of RS formed in amylose with short chains can be low. Therefore, the lower RS acquired for 12 h debranched sample was most likely due to the accumulation of short chains that were unable to form RS structure.

5.3 Comparison of RS content between retrograded and debranched retrograded sago starch

According to Table 4.1, statistically there are no difference between retrograded starch (T3) and debranched retrograded starch (T4, T5, and T6). However, there are still slight differences of RS content percentages in comparing the samples. Among the debranched samples T5 has the highest RS percentage compared to T3 (Figure 4.1). By using T5, the highest RS content among debranched retrograded samples for comparison, Figure 4.2 showed that T5 contained 0.084% higher RS than T3. This difference is most probably due to the debranching by pullulanase which might promote association of free linear chain segments and their close packing. Subsequently, the number of perfect starch crystals would increase. Without disentanglement from the amylopectin molecule, the association of the linear segments of starch units and subsequent crystallite formation may be tough due to lesser flexibility of the starch chain segments owing to their closer proximity to the branching points (Leong *et al.*, 2007).

CHAPTER 6

CONCLUSION

RS type III can be produce through the process of retrogradation or enzymatic hydrolysis. Sago starch when subjected to enzymatic hydrolysis by pullulanase could trigger the formation of RS. There are several methods can be apply to determine RS content and in this study, chemicals are used to isolate and quantify the RS content. From data acquired, insignificantly, the debranched at 8 hour sago starch sample (T5) is the best to compete with the commercialized resistant starch and it is the most potential RS3 to be further research as prebiotic. With the advanced of enzyme technology, it is possible that a complete debranching process of sago starch can be conducted at sub-gelatinization temperature and a more economical process can be developed to produce product with high yield of resistant starch. Different methods of RS determination should be conducted in future study to obtain and compare for the best result.

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APPENDIX A

Original Data Table

TREATMENT	UV-ABSORBANCE (mM)			CONCENTRATION OF GLUCOSE (RESISTANT STARCH) (%)		
	Replications			Replications		
	R1	R2	R3	R1	R2	R3
Native Sago Starch (T1)	0.054	0.054	0.054	1.22	1.22	1.22
FIBERSYM (T2)	0.066	0.062	0.064	1.82	1.62	1.72
Retrograded Starch (T3)	0.049	0.050	0.053	0.97	1.02	1.17
Retrograded by Pullulanase at 4 Hour (T4)	0.047	0.053	0.053	0.87	1.17	1.17
Retrograded by Pullulanase at 8 Hour (T5)	0.049	0.053	0.055	0.97	1.17	1.27
Retrograded by Pullulanase at 12 Hour (T6)	0.054	0.050	0.049	1.22	1.02	0.97

APPENDIX B

Statistical Analysis of Data

The SAS System

The ANOVA Procedure

Class Level Information		
Class	**Levels**	**Values**
Treatment	6	T1 T2 T3 T4 T5 T6

Number of Observations Read	18
Number of Observations Used	18

The ANOVA Procedure

Dependent Variable: RS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.98791667	0.19758333	12.93	0.0002
Error	12	0.18333333	0.01527778		
Corrected Total	17	1.17125000			

R-Square	Coeff Var	Root MSE	RS Mean
0.843472	10.20110	0.123603	1.211667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	5	0.98791667	0.19758333	12.93	0.0002

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for RS

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	0.015278
Critical Value of Studentized Range	4.75020
Minimum Significant Difference	0.339

Means with the same letter are not significantly different.			
Tukey Grouping	**Mean**	**N**	**Treatment**
A	1.7200	3	T2
B	1.2200	3	T1
B			
B	1.1367	3	T5
B			
B	1.0700	3	T4
B			
B	1.0700	3	T6
B			
B	1.0533	3	T3

The MEANS Procedure

Treatment=T1

Analysis Variable : RS	
Std Dev	**Std Error**
0	0

Treatment=T2

Analysis Variable : RS	
Std Dev	**Std Error**
0.1000000	0.0577350

Treatment=T3

Analysis Variable : RS	
Std Dev	**Std Error**
0.1040833	0.0600925

Treatment=T4

Analysis Variable : RS	
Std Dev	**Std Error**
0.1732051	0.1000000

Treatment=T5

Analysis Variable : RS	
Std Dev	**Std Error**
0.1527525	0.0881917

Treatment=T6

Analysis Variable : RS	
Std Dev	**Std Error**
0.1322876	0.0763763

PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "Production of Resistant Starch Type III from Native Sago Starch as Potential Prebiotic" by the supervisor in a join authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus and published in the form approved by the faculty.

Abdul Muhaimin Bin Ahmad

Date: 8/7/2015