ENZYMATIC PRODUCTION OF SAGO STARCH OLIGOSACCHARIDES AND THEIR FERMENTATION BY *LACTOBACILLUS* SPP.

by

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RINANI SHIMA BINTI ABD RASHID

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Abbreviations	Caption
AOS	Alginate-oligosaccharides
CFU/ml	Cell forming unit per ml
DE	Dextrose equivalents
Dp	Degree of polymerization
FOS	Fructo-oligosaccharides
GC	Gas chromatography
HPLC	High performance liquid chromatography
NAOS	Neoagarose-oligosaccharides
NDO's	Non digestible oligosaccharides
RI	Refractive Index
SCFA	Short chain fatty acids
SCO	Short chain oligosaccharides
XOS	Xylo-oligosaccharides

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PENGHASILAN BERENZIM OLIGOSAKARIDA KANJI SAGU DAN PEMFERMENTASIANNYA OLEH *LACTOBACILLUS* SPP.

ABSTRAK

Potensi oligosakarida rantaian pendek (ORP) kanji sagu dalam meningkatkan pertumbuhan *Lactobacillus* spp. adalah dikaji. Kanji sagu separa tergelatinisasi dihidrolisiskan pada pH 6.2 selama 48 jam pada pelbagai suhu (45 °C - 75 °C) dan kepekatan (1% - 6% (v/w)) enzim α-amilase (Termamyl 120L). Hidrolisis kanji sagu dengan 2% (v/w) α-amilase pada suhu 65 °C menghasilkan jumlah keseluruhan ORP sebanyak 14.6 g/kg, sementara hidrolisis berenzim pada suhu 75 °C dengan 1% (v/w) α-amilase menghasilkan ORP tertinggi sebanyak 15.0 g/kg selepas 48 jam hidrolisis. ORP kanji sagu yang diperolehi daripada hidrolisis berenzim pada 45 °C - 75 °C dan 1% - 6% (v/w) α-amilase pada 6 jam, 24 jam dan 48 jam hidrolisis digunakan sebagai pengganti glukosa dalam medium MRS (deMann Rogosa dan Sharpe) untuk pertumbuhan *Lactobacillus acidophilus* FTCC 0291, *Lactobacillus bulgaricus* FTCC 0411 dan *Lactobacillus casei* FTCC 0442. ORP kanji sagu didapati meningkatkan pertumbuhan *Lactobacillus* spp.. Pertumbuhan terbaik *L. acidophilus*, *L. bulgaricus* dan *L. casei* diperolehi daripada medium yang ditambah ORP hasil hidrolisis kanji sagu pada 65 °C, iaitu masing-masing sebanyak 7.93, 8.16 and 8.99 $\log_{10}$ CFU/ml. Pertumbuhan yang terbaik juga diperolehi daripada medium yang mengandungi ORP hasil hidrolisis kanji sagu dengan 1% (v/w) α-amilase dengan hitungan jumlah plat masing-masing sebanyak 7.93, 8.14 and 9.76 $\log_{10}$ CFU/ml. Penambahan 4% (w/v) ORP di dalam medium MRS tanpa glukosa adalah kepekatan terbaik untuk menyokong pertumbuhan *Lactobacillus* spp.. Medium mengandungi ORP hasil hidrolisis kanji sagu dengan 1% (v/w) α-amilase pada 65 °C selama 24 jam meningkatkan pertumbuhan dengan signifikan ke atas semua strain

dibandingkan dengan medium kawalan; iaitu masing-masing sebanyak 7.24, 7.39 dan 8.90 $\log_{10}$ CFU/ml untuk *L. acidophilus*, *L. bulgaricus* dan *L. casei* dibandingkan 5.49, 5.56 dan 6.25 $\log_{10}$ CFU/ml untuk medium kawalan. Keputusan juga menunjukkan bahawa pertumbuhan semua *Lactobacillus* spp. di dalam ORP kanji sagu adalah setanding dengan FOS dan inulin. Penurunan pH medium pertumbuhan yang mengandungi ORP kanji sagu mencadangkan berlakunya penghasilan asid-asid organik di dalam medium. Peratus penggunaan ORP kanji sagu adalah yang tertinggi dibandingkan dengan glukosa, FOS dan inulin oleh *L. acidophilus*, *L. bulgaricus* dan *L. casei*, iaitu masing-masing sebanyak 20.06%, 22.47% dan 27.88%. Metabolisme ORP kanji sagu oleh *L. acidophilus*, *L. bulgaricus* dan *L. casei* menghasilkan jumlah asid lemak rantaian pendek yang signifikan, iaitu masing-masing sebanyak 398.07 mM, 413.57 mM dan 427.33 mM. Keputusan kajian menunjukkan pengaruh positif ORP kanji sagu dengan meningkatkan pertumbuhan *Lactobacillus* spp dan kajian ORP kanji sagu sebagai prebiotik harus diteruskan.

ENZYMATIC PRODUCTION OF SAGO STARCH OLIGOSACCHARIDES AND THEIR FERMENTATION BY *LACTOBACILLUS* SPP.

ABSTRACT

The potential of sago starch short chain oligosaccharides (SCO) to enhance the growth of *Lactobacillus* spp. was studied. Partially gelatinized sago starch was hydrolyzed at pH 6.2 for 48 hours at various temperatures (45 °C - 75 °C) and concentrations (1% - 6% (v/w)) of α-amylase (Termamyl 120L). Sago starch hydrolysis using 2% (v/w) α-amylase, at 65 °C yielded a total SCO of 14.6 g/kg, while enzymatic hydrolysis at 75 °C with 1% (v/w) α-amylase produced the highest concentration of SCO reaching 15.0 g/kg after 48 hours hydrolysis. Sago starch SCO obtained from enzymatic hydrolysis at 45 °C - 75 °C with 1% - 6% (v/w) α-amylase at 6 h, 24 h and 48 h hydrolysis were used as glucose replacer in the MRS (deMann Rogosa and Sharpe) medium for the growth of *Lactobacillus acidophilus* FTCC 0291, *Lactobacillus bulgaricus* FTCC 0411 and *Lactobacillus casei* FTCC 0442. The sago starch SCO was found to enhance the growth of *Lactobacillus* spp. The best growths of *L. acidophilus*, *L. bulgaricus* and *L. casei* were from the media with SCO hydrolyzed at 65 °C, with 7.93, 8.16 and 8.99 $\log_{10}$ CFU/ml respectively. The best growths were also obtained from media containing SCO hydrolyzed with 1% (v/w) of α-amylase with total plate counts of 7.93, 8.14 and 9.76 $\log_{10}$ CFU/ml respectively. Inclusion of 4% (w/v) SCO in the media was the best concentration to support the growth of *Lactobacillus* spp.. Media containing SCO from sago starch hydrolysis with 1% (v/w) α-amylase at 65 °C for 24 h increased significant growth of all strains compared with the control media; reaching 7.24, 7.39 and 8.90 $\log_{10}$ CFU/ml for *L. acidophilus*, *L. bulgaricus* and *L. casei* respectively as compared to 5.49, 5.56 dan 6.25 $\log_{10}$ CFU/ml for the control media. Results also showed that the

growth of all *Lactobacillus* spp. in sago starch SCO were comparable to FOS and inulin. The pH reduction in the growth media containing sago starch SCO suggested the production of organic acids in the media. Percent utilizations of sago starch SCO by *L. acidophilus, L. bulgaricus* and *L. casei* were the highest compared to glucose, FOS and inulin, which were 20.06%, 22.47% and 27.88%, respectively. Metabolism of sago starch SCO by *L. acidophilus, L. bulgaricus* and *L. casei* produced significant amount of short chain fatty acids at 398.07 mM, 413.57 mM and 427.33 mM, respectively. Results of this study indicated that sago starch SCO had a positive effect by increasing the number of *Lactobacillus* spp. in the MRS media and study of its use as a prebiotic should be furthered.

CHAPTER 1

INTRODUCTION

1.1 Background

It is now well established that the colonic microflora has a profound influence on health. Consequently, there is a great deal of interest in the use of prebiotic oligosaccharides (Gibson and Roberfroid, 1995; Roberfroid, 2000; Gibson and Fuller, 2000; Losada and Olleros, 2002; Hammes and Hertel, 2002) as functional food ingredients to manipulate the composition of colonic microflora in order to improve health (Rastall and Maitin, 2002). From a standpoint of general wellness, both prebiotic and probiotic supplements have been shown to improve digestion and absorption of some nutrients; enhance detoxification by intestinal mucosal cells; reduce the concentrations of large bowel mutagens and carcinogens; aid elimination processes; and favorably affect the immune system of the gut and the systemic immune system. These benefits are related to favorable changes in the concentrations of gut bacteria resulting from prebiotics and probiotics consumed (Meschino, 2003).

Prebiotics as a functional food helps growth of certain bacteria especially Bifidobacteria and Lactic Acid bacteria (LAB) in our gastrointestinal tract. Prebiotic is also used as food substance which promotes the growth of beneficial bacteria in the intestines. Prebiotics are defined as non digestible but fermentable food ingredients that confers a health benefit on the host associated with modulation of microbiota in the colon (FAO, 2007).

Prebiotics mainly consisting of non-digestible oligosaccharides (NDOs) are defined as short-chain carbohydrates that are not digested in the small intestine and

enter the large intestine unaltered. They are mostly sugar-like compounds comprising between two and ten carbohydrate units that are soluble in 80% ethanol and largely resistant against digestion by pancreatic and brush boarder enzymes. They are substrates for fermentation but their effects on bowel habit are negligible (Cummings *et al*., 2004).

One of the main sources of oligosaccharides is starch. In Malaysia, the main source of starch is sago (Pei-Lang *et al*., 2006; Karim *et al*., 2008). However, other potential sources of starch include tapioca, rice, sweet potato, yam, breadfruit and banana. Sago starch has been chosen as the source of short chain oligosaccharides in this study due to high yield and low cost of production. As reported by Abd-Aziz (2002), the functions of sago starch as an alternative cheap carbon source to be used in the fermentation process make it attractive for both economic and geographical considerations. The crop requires no fertilizer and demands very little husbandry (Cecil *et al*., 1982). At the moment, only sago and tapioca starch are being processed at industrial scale in Malaysia.

Sago (*Metroxylon sagu*) has been an economically important crop of Sarawak. The focus of the development is in Mukah division. To date, a total of 15,006 hectares of peat land in Mukah had been designated and progressively developed (PELITA, 2008). In Malaysia, sago palm is inexpensive and not nearly as agriculturally intensive as rice. Although rice is generally preferred as the main staple food crop in Malaysia, sago is also a reliable source of carbohydrates. Presently, commercial production of sago flour in Malaysia occurs mainly in Sarawak and small parts of Johor. In Sarawak, the planting area of sago is estimated

over 60, 000 hectares (Sarawak Department of Agriculture, 2008). Sarawak is also one of the biggest exporters of sago. Sago brings above RM30 million in exports earning in latest year for Sarawak (Sarawak Department of Agriculture, 2008) and this value is expected to rise in coming years. Sago palm has a high starch yield; one palm may yield between 150 to 300 kg of starch. Sarawak exports up to 40,000 tons sago a-year and the effluent resulting from sago debarking and processing are often discharged to nearby rivers (Mona, 2008). Sago palm, which grows in swamp areas inhabitable for most other crops is also the world's highest starch producer; however, the utilization of this material is still limited as compared to that in other countries due to the limited data of basic knowledge on its physico-chemical and functional properties.

The prebiotics that are available in the market are inulin, derived from natural chichory root, fructo-oligosaccharide (FOS), galacto-oligosaccharides, soy-oligosaccharides and xylo-oligosaccharide. The study on sago starch oligosaccharides as an alternative prebiotic oligosaccharides source may be a good start and show promising development as a new food ingredient, thus promoting economic growth of Sarawak.

This research also focused on the production of sago short chain oligosaccharides by partial enzymatic hydrolysis. The enzyme, α-amylase which has a shape that allows it has to wrap around starch (substrate) polymers and cut it up into smaller oligomers and individual glucose units. The enzyme specifically hydrolyses α–1,4- glycosidic bond in amylose, amylopectin and glycogen in endo-fashion, but the α–1,6-glycosidic linkages in branched point are not attacked

(Forgarty, 1983). As a result, the hydrolysis by α-amylase on amylose will yield maltose and maltotriose while hydrolysis of amylopectin causes production of glucose and maltose, in addition to a series of branched limit α-dextrins which contains four or more glucose residues of all the α–1,6- glycosidic linkages of the original structure (Palmer, 1970; Forgarty and Kelly, 1990).

Pretreatment of starch by heating it to a temperature below gelatinization temperature at lower pHs resulted in an increase in the ability of enzyme to digest the sago starch as compared to untreated starch (Haska and Ohta, 1991).

The present investigation was done to explore the potential of utilizing sago starch oligosaccharides as a new source of nutrient supplement specifically to promote the *Lactobacillus* spp. growth. It was also to generate new information on the influence of sago starch oligosaccharides on the growth of *Lactobacillus* spp. compared to that of commercial prebiotics (fructo-oligosaccharide and inulin). The work was also done to obtain the oligomers in a manner that requires minimal energy input through pretreatment and partial enzymatic hydrolysis. Through enzymatic hydrolysis, the effect of enzyme concentration and temperature were taken into important consideration in order to observe the pattern of sago oligosaccharides profiles obtained and their effect on the bacteria growth. All the data obtained should provide a better understanding of the process and mechanism of sago starch hydrolysis and the bacterial growth.

1.2 Objectives

The aim of this research was to study the potential of short chain (Dp < 5) oligosaccharides (SCO) of sago starch obtained via partial enzymatic hydrolysis to promote the growth of beneficial *Lactobacillus* spp. compared to that of commercial prebiotics FOS and inulin. Therefore, the specific objectives in this research were:

1. To study the effect of temperature and time on the formation of short chain oligosaccharides (SCO) from sago starch by α-amylase.
2. To analyze the growth patterns of *L. acidophilus, L. bulgaricus* and *L. casei* in the media supplemented with sago starch SCO.
3. To evaluate the suitability of sago starch SCO as glucose replacement for *L. acidophilus, L. bulgaricus* and *L. casei* growth in comparison with commercial prebiotics, namely fructo-oligosaccharide and inulin.

In objectives number 3, total viable count of probiotics, percentage of substrate consumption, total short chain fatty acids (SCFA) and pH changes in growth medium will be determined.

CHAPTER 2

LITERATURE REVIEW

2.1 Starch

Starch is the most important, abundant, digestible food polysaccharide. It occurs as the reserve polysaccharide in the leaf, stem (pith), root (tuber), seed, fruit and pollen of many higher plants and are therefore the major source of carbohydrate in human diet, traditionally supplying about 70 – 80 % of the calories consumed by humans with approximately two – third of this calories coming from starch (Knill and Kennedy, 2005) . It occurs as discrete, partially-crystalline granules whose size, shape and gelatinization temperature depend on the botanical source of the starch (FAO, 1998).

Starch can be used in a variety of different ways other than a foodstuff such as in adhesives, coatings sizing, paper, pharmaceuticals, building materials and textiles industries (Swinkels, 1985, Thomas and Atwell, 1997). The industry of starch is based on its unique chemical and physical characteristics since it can be used directly as intact granules, in the swollen granular state, in the dispersed form, as an extrudate powder, after controlled partial hydrolysis to a mixture of oligosaccharides, after hydrolysis and isomerization to glucose, or after chemical modification (Knill and Kennedy, 2005).

2.1.1 Occurance and appearance

The origins of starch as an industrial material date back to Egyptian times in the manufacturing of starch based adhesive and papyrus parchment. In Roman times starches were used as a whitener and stiffener for textiles, while in Europe the starch

industry was mainly came from potato starch for the textiles industry where it was served as a sizing agent (Dziedzic and Kearsley, 1995).

Starch basically is composed of tiny, white granules, ranging from 2 – 100 μm in diameter and the size and shape of the granules are peculiar to each variety of starch (Swinkels, 1985). The shapes usually are range from round to polygonal and truncated. Table 2.1 shows the granule properties of various starches.

Table 2.1: Granule properties of various starches

Starch	Type	Size (diameter) range (μm)	Size (diameter) average (μm)	Shape
Corn	Cereal	3 – 26	15	Round, polygonal
Potato	Tuber	5 – 100	33	Oval, spherical
Wheat	Cereal	2 – 35	15	Round, lenticular
Tapioca	Root	4 – 35	20	Oval, truncated
Waxy maize	Cereal	3 – 26	15	Round, polygonal
Sorghum	Cereal	3 – 26	15	Round, polygonal
Rice	Cereal	3 – 8	5	Polygonal, angular
Sago	Pith	5 – 65	30	Oval, truncated
Arrowroot	Root	5 – 70	30	Oval, truncated
Sweet potato	Root	5 - 25	15	Polygonal

(Swinkels, 1985).

Generally, there are many sources of starch available in the world; however the commercial sources of starch can be divided into three groups. The first group is seeds of cereal grain (corn, wheat, sorghum, and rice). The second group comprises of tubers (potato), roots (tapioca, sweet potato, and arrow root), and the pith of sago palm. The third group includes the waxy starches (waxy maize, waxy sorghum and waxy rice) (Dziedzic and Kearsley, 1995). These starches are originated from cereals but the physical properties of the waxy starches are similar to root starches. The properties of starch vary depending from which source it's derived.

2.1.2 Starch composition

Starch composes of two types of glucose polymers, amylose and amylopectin (Bank and Greenwood, 1975; Dziedzic and Kearsley, 1995; Biliaderis, 1998). These two polymers are built up of six-carbon sugar D-glucose, often referred to as "building block of starch". However the structural differences between these two polymers contribute to large differences in starch properties. Amylose is a linear polymer form of α-D-glucose units bonded by α–1,4 linkage (Figure 2.1), while amylopectin is branched types of polymer. The backbone of amylopectin is still α-D-glucose units bonded by α–1,4 linkage but the branches are connected by α–1,6 glycosidic bonds every 10 – 12 glucose units (Figure 2.2). There is also occasional branching at carbon – 3. The side chains off the main backbone are 20 – 30 glucose units in length (Dziedzic and Kearsley, 1995). Both Tables 2.2 and 2.3 show some important characteristics of amylose and amylopectin and properties of the starch components respectively.

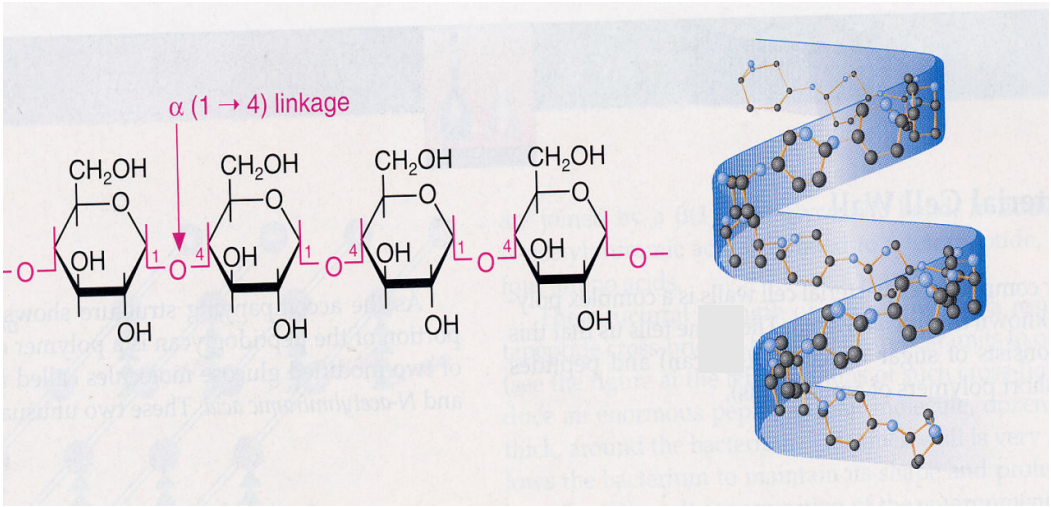


Figure 2.1: Amylose structure (Danniston, 2004)

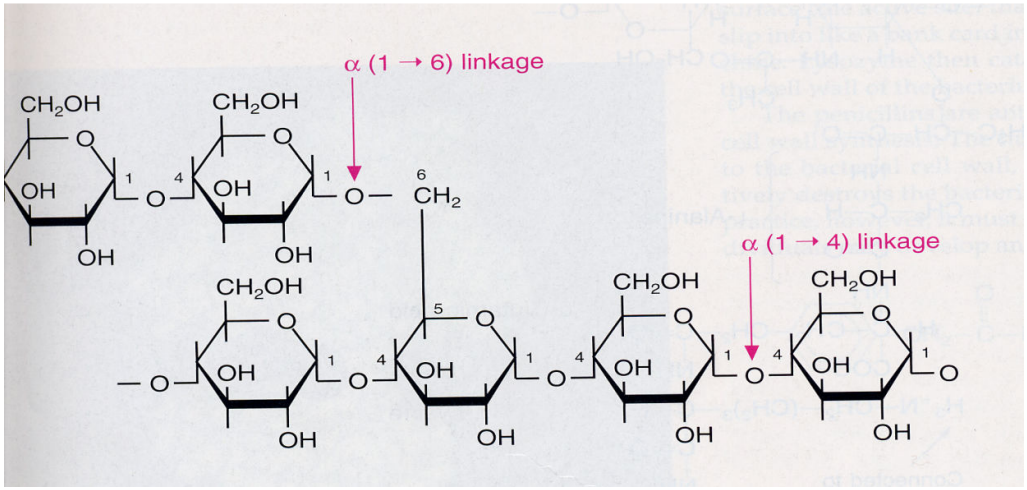


Figure 2.2: Amylopectin structure (Danniston, 2004)

Table 2.2: General characteristics of amylose and amylopectin

Characteristic	Amylose	Amylopectin
Shape	Essentially linear	Branched
Linkage	α–1,4 [some α–1,6]	α–1,4 and α–1,6
Molecular weight	Typically <0.5 million g/mol	50-500 million g/mol
Films	Strong	Weak
Gel formation	Firm	Non gelling to soft
Color with iodine	Blue	Reddish brown

(Thomas and Atwell, 1997)

Table 2.3: General properties of the starch components

Property	Amylose	Amylopectin
Average chain length (CL)	~ 10^3	20-25
Degree of polymerization (DP)	~ 10^3	10^4 - 10^5
Solubility of aqueous solution	Unstable	Stable
Conversion into maltose (%)		
with α-amylase	~ 110	~ 90
with β-amylase	~ 70	~55
with debranching enzyme, then β-amylase	~ 100	~75

(Manners, 1979)

2.1.2.1 Amylose

Amylose covers a range of degrees of polymerization (Dp), depending upon the source of the starch. The degree of polymerization of amylose is approximately from 200 – 20000, with molecular weight between 30 – 3200 kDa (Knill and Kennedy, 2005, Kennedy *et al*., 1983). Although amylose was considered to be linear, there were numbers of studies suggested a small number of branches or a limited amount of branching involving α–1,6 glygosidic linkages exist in the molecule (Kennedy *et al*., 1983; FAO, 1998). There are 9-20 branch points per molecule and the side chains range in chain length from 4 to over 100 D-glucose units (Hizukuri *et al.*, 1981). Amylose chain contain reducing end which is the highly reactive aldehyde group (Thomas and Atwell, 1997)

Dziedzic and Kearsley (1995) have reported based on various studies that amylose is a straight chain glucose polymer with a conformational helical structure. Amylose has the ability to form inclusion complexes with iodine. The complex of amylose with iodine gives a characteristic blue colour and is essentially insoluble in water, which can be used to establish the presence of amylose in starch. Apart from that, solutions of amylose have high viscosity and are unstable, with the tendency to precipitate spontaneously or to retrograde and release some of the bound water (syneresis) (Manners, 1979). Increasing amylose concentration decreases gel stickness but increases gel firmness. Knill and Kennedy (2005) have reported that the abundance of hydroxyl groups makes amylose hydrophilic and its uniform linear chain nature permits crystallization from solution as well as in a semi solid state in films and coating. On cooling, amylose readily crystallizes and precipitates.

The many different starches range in amylose, on the average, from 16 to 25% and maybe as high as 30%. There are some high amylose rice starches which may have as much as 80 to 90% amylose. These starches are unusual and are occasionally used in edible casings. At the other end of the scale, there are the waxy corn starches, waxy rice starches, waxy sorghum, which contains 100% amylopectin and no amylose (Swinkels, 1985; Anonymous, 2005). Table 2.4 shows amylose content of various starches.

Table 2.4: Amylose contents of various starches

Starch Source	% Amylose
Waxy Rice	0
High Amylose Corn	70
Corn	28
Cassava	17
Waxy Sorghum	0
Wheat	26
Sweet Potato	18
Arrowroot	21
Sago	26
Potato	20

Satin (2005)

2.1.2.2 Amylopectin

Amylopectin is much branched with an average of 17–26 D-glucosyl units separating α–1,6 branch points (Kennedy *et al*., 1983). The degree of branching and chain length between branch points in amylopectin varies within the source of starch. Hydrolysis of starch with a debranching enzyme and measurement of the molecular weight of resulting linear chains can give excellent profile of branch lengths. Amylopectin molecules are thus very large, consisting of molecular weight between 10 000 to over 500 000 kDa (Knill and Kennedy, 2005). Amylopectin is unable to form stable complexes with iodine because of the short side chains, so only small

amounts of iodine are bound, thus a red brown complex is formed with iodine (Banks and Greenwood, 1975).

According to Manners (1979); Knill and Kennedy (2005), amylopectin chains can be classified into three types namely A, B, and C chains. A-chains are are linked to the molecule by only a single linkage through their potential reducing end, while B-chains which carry one or more A-chains and are themselves linked to an adjacent chain by the potential reducing group and C-chains carry the single reducing end-group (Figure 2.3).

Various amylopectin structures have been proposed since seventy years ago. Five of the models are shown in Figure 2.4. In the Haworth structure (Figure 2.4A), the chains consist a single branching while in other models, many chains contain several interchain linkages or multiple branching. In the Staudinger comb-type structure there are no B-chains occur (Figure 2.4B) and in the other Meyer 'bush' structures, there are approximately equal numbers of A- and B-chains (Figure 2.4C, 2.4D and 2.4E). However in Whelan structure, indicates the ratio of A-chains to B-chains is about 2:1 (Figure 2.4F). Later on 'cluster model' was proposed by several researchers.

Robin *et al.* (1974) have led to a new model of amylopectin containing clusters of highly ordered side chains with Dp of 15 (Figure 2.5). They indicated that crystalline region of amylopectin consisting of closely packed chains and appeared to be acid resistant. The cluster model was then further developed by Manners and

Matheson (1981), which claimed the branch points are arrange in tiers of some kind and not distributed randomly throughout the macromolecule (Figure 2.6).

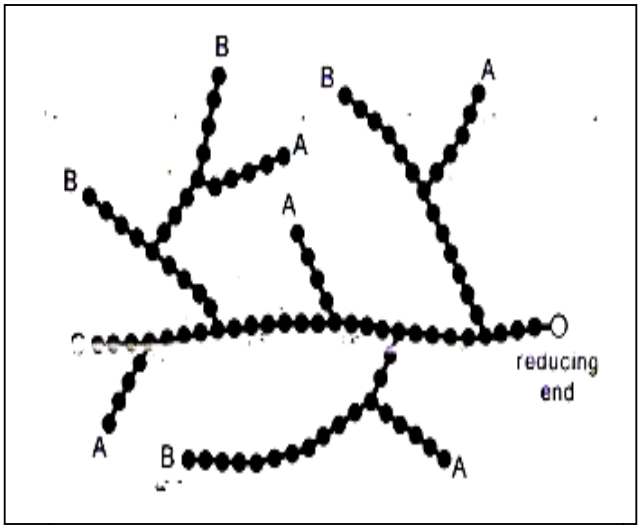
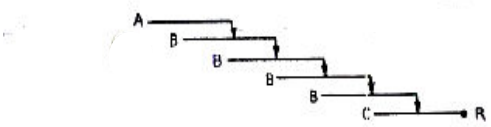


Figure 2.3: Representation of A, B, and C chains in amylopectin (Knill and Kennedy, 2005).

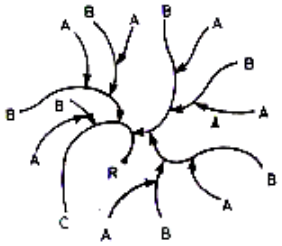


Haworth structure

A

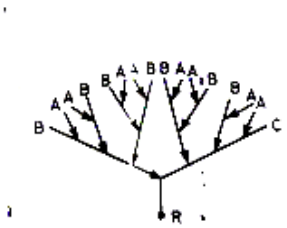
Staudinger structure

B



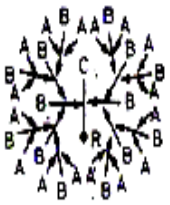
Meyer structure

C



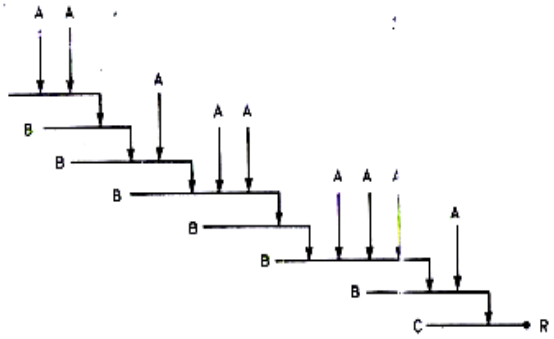
Redrawn Meyer structure

D



Revised Meyer structure

E



Whelan structure

F

Figure 2.4: Diagrammatic representation of the molecular structure of amylopectin as proposed by various authors : (A) laminated structure by Haworth; (B) comb-like structure by Staudinger; (C) bush structure by Meyer; (D) and (E) redrawn and revision of Meyer structure; (F) Whelan structure. R: reducing group. (Manners, 1979).

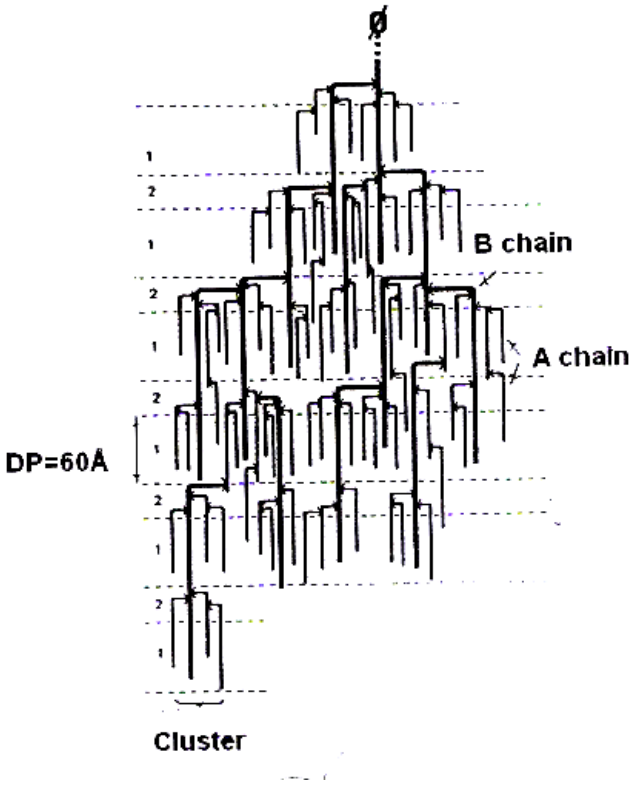


Figure 2.5: Proposed structure of amylopectin by Robin *et al.* (1974).
1: compact area; 2: less compact area; arrow indicate branching points; 0: reducing end point.

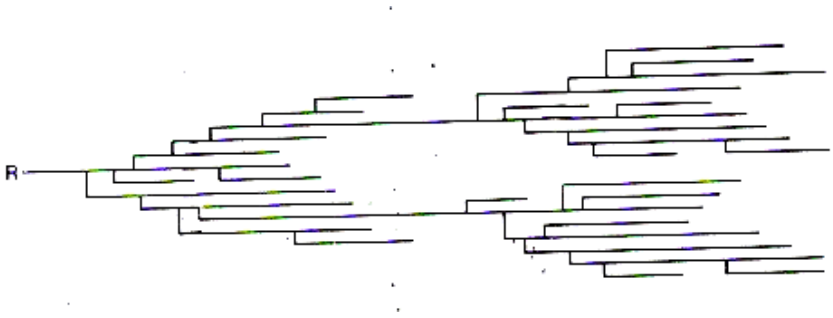


Figure 2.6: Cluster model proposed by Manners and Matheson (1981).
R: reducing group.

2.1.3 Solubility of starch in water

Native starches are insoluble in water below 50 °C (Swinkels, 1985). When a suspension of starch is heated in water above the critical temperature, it will start absorbing water and swelling, make the granules size bigger many time than its original size. The critical temperature at which this phenomenon occurs is known as gelatinizing temperature usually at 55 – 85 °C, depending on types of starch. When heating is continued, the swollen starch granules begin to disintegrate into swollen starch aggregates. This will lead to the increasing of starch viscosity, and turning the starch into thick paste forms. The process which involves the transformation of starch solution into starch paste is known as gelatinization.

Starch granules are insoluble in cold water due to the granules are densely packed in a polycrystalline state that are held together by inter- and intra-molecular hydrogen bonding within and between the amylose and amylopectin and are hence insoluble in coldwater and often resistant to chemicals and enzymes (Mitsuiki *et al*., 2005, Hamilton *et al*., 1999). Starch granules swell slightly in cold water (10 – 15% increase in diameter), but this process is reversible. Their granules shrink back to their original size on drying (Swinkels, 1985).

2.2 Sago starch

2.2.1 Introduction

Since prehistoric times, starch extracted from the stem of the sago palm, *Metroxylon sagu*, has been used in trade amongst the islands of the Sumatera-New Guinea archipelago and become the main source of sustenance for the inhabitants before the emergence of rice (Cecil *et al*., 1982, Abd-Aziz, 2002). According to

Ahmad *et al*. (1999), the name "sago" originated from Malaya and East Indies where the starch was obtained from the pith of palms, especially the sago palm and fern.

The sago palm *(Metroxylon* spp.) (Figure 2.7) is 6-14 m tall and hapaxantic - that is, it flowers once and dies shortly thereafter and commonly distributed in tropical swampy areas such as Southeast Asia (Cui and Oates, 1997). Just before flowering, the plant converts its stored nutrients into starch, which fills the trunk (Abd-Aziz, 2002). Since sago palm thrives well in swampy areas and on peat soils, which would require extensive engineering and costly maintenance to exploit for other plantations and annual crops, it has been a staple food in these areas (Tan, 1980). Sago starch is isolated from sago palm (*Metroxylon spp*.) which is better known as 'rumbia' and distributed throughout South East Asia. Some of the important species widely used in sago starch production includes *M. longispinum, M. sylvestre, M. microcanthum, M. sagu, and M. rumphii.* (Ahmad *et al*., 1999).

Sago palm is an important resource especially to the people in rural areas because it has various uses especially in the production of starch either as sago flour or sago pearl. Although sago starch was used for a long time especially in South East Asia in the food industry for the production of vermicelli, bread, crackers, biscuits, and many other traditional foods only a few limited studies on the physicochemical characteristics and properties were reported. Sago starch represents an alternative cheap carbon source for fermentation processes that is attractive out of both economic and geographical considerations. Besides the fact that sago starch is cheap some other important properties were reported such as its ease to gelatinize, its high

viscosity if properly extracted and the ease with which it can be moulded. It has also been reported to undergo little syneresis (Takahashi, 1986).

Figure 2.7: Sago palm in Sarawak (Singhal *et al.*, 2008)

The sago palm also requires no fertilization and has few natural pest or disease. It is an ideal crop to plant in peat because of its easy and less intensive management compared with other field crops. On average, the modern factories with their improved processing machinery require about 5-6 mature sago palms to produce one tonne of dry sago flour. Sago flour is a valuable source of carbohydrate (Zulpilip *et al.*, 1991). It may also be utilized for the production of fermentable sugars. Utilization of sago as a part of a sustainable agrotechnological system will provide a source of starch and lead to conservation of marshy areas of land (Wang *et al*., 1996).

Besides multitude uses of sago starch, there are a number of characteristics of sago palm that makes it quite a unique and unusual plant. Sago is an extremely hardy plant, thriving in swampy, acidic peat soils, where few other crops survive. The palm

is immune to floods, drought, fire and strong winds. It's large fibrous root system traps silt loads and removes pollutants, faecal contaminants and heavy metals. Sago forest also acts as an excellent carbon sink for carbon sequestration, thereby mitigating the greenhouse effect and global warming arising from the release of carbon dioxide into the atmosphere due to industrialization and increases in motorized vehicles (Stanton, 1991).

2.2.2 Properties and chemical composition of sago starch

Sago starch accumulates in the pith core of the stem of the sago palm (Cecil *et al.*, 1982). Sago starch granules are rather larger than those from most other plants. The size of the sago starch granules vary between 15 – 65 microns but mostly between 20 – 60 microns and the granules are oval or egg-shaped but with a number of truncated oval granules (Radley, 1976; Ahmad *et al*., 1999). Figure 2.8 shows a typical scanning electron micrographs (SEM) for sago starch. Takahashi (1986) has listed some sago starch properties including the ease of gelatinization, as its gelatization temperature is low, it has a high viscosity, it is easily moulded and the gel syneresis is low. Sago starch has a higher pasting temperature, of 69°C, than most starches, a higher ratio (27:73) of amylose to amylopectin and, at 97%, a much greater swelling power (Cecil *et al*., 1982). Sago starch shows a similar moisture content and granular size to those of potato starch and similar swelling power, solubility, gelatinization temperature and temperature at maximum viscosity to those cassava and sweet potato starches but the retrogradation and amylose content are like those of corn starch (Maaruf *et al*., 2001). Table 2.5 compares some properties of sago and other starches.

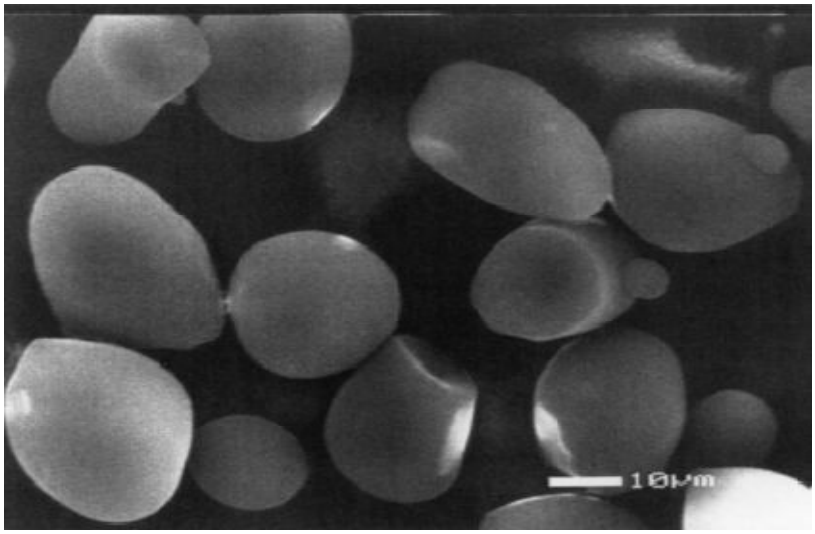


Figure 2.8: Scanning Electron Micrographs of sago starches (Ahmad *et al*., 1999)

Table 2.5: Properties of sago and other starches

	Origin of starch				
	Sago	Maize	Rice	Tapioca	Wheat
Granule shape	Oval	Round, polygonal	Polygonal	Oval – with indentation	Round
Granule size (microns)	20-60	15	3-8	5-35	2-10 and 20-35
Amylose %	27	26	17	17	25
Swelling power %	97	24	19	71	21
Initial pasting temperature °C	69	62	66	58	65

(Cecil *et al*., 1982)

Sago starch is nearly pure carbohydrate and has very little protein, fat, or minerals. Several analysis on proximate compositions of sago starch shows that sago starch contains about 10–20% moisture, 0.1-1.0% protein, 0.1-0.3% fat, 0.1-0.5% fibre and 0.1-0.8% ash (Radley, 1976; Swinkels, 1985; Ahmad *et al.*, 1999; Wong *et al*., 2005).

Apart from sago granules, the pith of sago trunk also contained other associated components. Cecil *et al.* (1982) reported that the chemical analysis of pith showed about 6-12 % of soluble solids (dry substance) and 1-3 % of ash, apart from 79-88 % of apparent starch plus sugar content. The sago pith also contained most of the constituents in other plant materials such as fibre, hemicelluloses, other cell structural materials, soluble solids and traces of other unidentified substances.

Sago starch exhibits a C-type (a mixture of A and B crystalline types) diffraction pattern consisting of about 65% A-type and 35% B-type as some faba bean starches. Newly accumulated starch in the top of the sago palm revealed the C-type structure is dominantly affected by the A-type structure. On the other hand, developed starch granules accumulated in the bottom of the sago palm also showed the C-type structure, but the relative percentage of A-type structure in the starch gradually decreased compared to that of the B-type structure (Karim *et al.*, 2008).

2.2.3 Processing and production of sago starch

The sago palms are felled in about 10 to 15 years after planting, depending on the fertility of the terrain. Starch accumulates in the pith core of the stem of sago palm, which has to be separated from the cellulosic cell wall of the trunk. After removing cortex, rachis and leaflets from the pith, which is the most labour-intensive operation in sago palm processing, starch will be extracted from the pith (Abd-Aziz, 2002).

Azudin and Eng-Tian, (1991) and Chew *et al.* (1998) have explained the modern method used in sago starch processing. The sago trunks are cut into sections,

each 75 cm to 90 cm long. Each sago trunk yields from 10 to 12 sections. These sago sections are tied together in the form of rafts and tugged by boats to sago factories for processing. The sago factories are invariably situated along river banks, for ease of transportation. Independent agents collect the sago sections and arrange for the transportation of the sections to the factories, for a fee. Floating the sago sections along the canals and rivers is the most common form of transportation to the factories. Lorries are also used in cases where the canals are too dry for movement by water. Logs of sago palm on arrival at the mills are immediately processed. Logs are first debarked, followed by maceration using a rasper. The rasper which consists of nails disintegrates the pith to produce a fine mass of sago pith. New designs of raspers have eliminated the necessity of debarking. These new raspers remove the sago pith by the scraping action of a series of wires. This process is extremely effective leaving behind 1 cm of bark. The chips are then further disintegrated using a hammer mill. The starch slurry is then passed through a series of centrifugal sieves to separate the coarse fibres. Further purification is achieved by separation in a nozzle separator. A series of cyclone separators have also been used to obtain very pure starch. Dewatering of starch is carried out using a rotary vacuum drum drier, followed by hot air drying.

The crude starch is usually referred to by importers as ‘sago flour’, similar to the term ‘tapioca flour’. It is essentially a raw material which is refined, purified or slightly bleached by oxidation with small quantities of hypochlorites (0.25-6.0% chlorine on dry starch basis) (Radley, 1976).

2.2.4 Sago production in Malaysia

It is estimated that there are about 2 million ha of natural sago palm forests and about 0.14 million ha of planted sago palm at present, out of a total swamp area of about 20 million ha in Asia and the Pacific Region, most of which are under- or non-utilized (Karim *et al.*, 2008). Sarawak has been the principal exporter of sago flour to the world market. The sago industry in Sarawak has made sago flour one of the most important export commodities, with a current output of 45 000 metric tons per year, with revenue expected to increase from RM 38 million/year to 2.7 billion/year in 2015 (Jackson, 2007). Sago palm has a high starch yield; one palm may yield between 150 to 300 kg of starch. Sarawak exports up to 40,000 tons sago a-year and the effluent resulting from sago debarking and processing are often discharged to nearby rivers (Mona, 2008). Sago can compete economically on yield and price compared to other crops for example the yield of sago starch is 2000 – 3000 kg/ha-yr compared to cassava 2000 kg/ha-yr and maize 1000 kg/ha-yr (Stantan, 1992).

Sago starch has great potential for starch production in Malaysia. Starch is well-known and abundant renewable raw material, and sago is one of its sources. In Malaysia, sago starch ranks fifth highest in terms of agricultural revenue after pepper, palm oil, cocoa, and rubber (Abd-Aziz, 2002). Most of the sago was exported to Peninsular Malaysia, Japan, Taiwan, Singapore and other countries. It is widely used in Indonesia, and in India it is boiled with sugar to make a jelly, while starch from several genera of palms in the form of small whitish, pinkish or brownish grains is exported to Europe and America, where it is mainly used for thickening the soups and making puddings (Wang *et al.*, 1996).

Sago starch is a very versatile multiple-use product. In Sarawak, sago is widely used to produce sago pearls and "tabaloi", a local biscuit delicacy. Sago pearls can be boiled, either alone or mixed with other foods, and consumed directly as a carbohydrate source. Whether in its original, purified or modified forms, sago starch is quite widely used by the local food industries. Together with rice, corn and potato starch, sago starch is used by the 'Bee Hoon' (or vermicelli), noodles and 'Koay-Teow' manufactures (Zulpilip *et al*., 1991). It is primarily because it can supplement both corn and tapioca starch for manufacturing monosodium glutamate and glucose. Ajinomoto (M) Sdn. Bhd. is the sole company manufacturing monosodium glutamate in Malaysia (Zulpilip *et al*., 1991). New uses for sago include its use in the manufacture of biodegradable plastics, alcohol, ethanol and citric acid. Besides its use as a foodstuff, sago starch can also be utilized to produce adhesives for paper, textiles, and plywood; as a stabilizer in pharmaceuticals; or converted to other types of food (Abd-Aziz, 2002).

2.3 Enzymatic hydrolysis of starch

Acid hydrolysis of starch has had widespread use in the past. It is now largely replaced by enzymatic processes, as acid hydrolysis required the use of corrosion resistant materials, gave rise to high color and saltash content (after neutralization), needed more energy for heating and was relatively difficult to control. A shortcoming of these methods is also the necessity for the purification of the product from salts and non-desired contaminants derived from the thermal treatment (Yankov *et al.*, 1986). Further hydrolysis using acid is not satisfactory because of undesirably colored and flavored breakdown products. Acid hydrolysis appears to be a totally random process which is not influenced by the presence of α-1,6-glucosidic

linkages. Enzymatic hydrolysis exhibits some substantial advantages over acid hydrolysis, since there is no need for high temperature and pressure.

Marchal (1999) had reported that back to the history, Payen and Persoz (1833) were the first to become aware of enzymatic starch hydrolysis; they found that malt extract converted starch to sugar. Much later, a fungal amylase was employed for commercial syrup manufacture in 1938 (Dale and Langlois, 1940). The enzyme preparation contained a mixture of glucoamylase and a-amylase and was used to further hydrolyse an acid hydrolysate (Schenck, 1992).

Starch can be hydrolyzed enzymatically to yield several commercially relevant hydrolysates (Marchal, 1999). There are three stages in the conversion of starch (Figure 2.9). (1) gelatinization, involving the dissolution of the nanogram-sized starch granules to form a viscous suspension and required to increase the accessibility of the substrate and to enhance the hydrolysis rate; (2) liquefaction, involving the partial hydrolysis of the starch, with concomitant loss in viscosity and to form a product with a dextrose equivalent that varies between 15 and 30; and (3) saccharification, these partially hydrolysed starch chains are broken down into glucose, maltose, maltotriose, and some higher oligomers. The dextrose equivalent varies between 40 and 98 depending on the enzyme that was used (Baks *et al.,* 2006; Chaplin, 2004).