PHYSICOCHEMICAL PROPERTIES OF STARCH FROM SAGO (*Metroxylon sagu*) PALM GROWN IN MINERAL SOIL AT DIFFERENT GROWTH STAGES

by

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LIST OF SYMBOLS & ABBREVIATIONS

Symbols	**Caption**
AM	Angau muda
AT	Angau tua
B	Bubul
P	Plawei
T_c	Conclusion temperature
T_g	Glass temperature
T_o	Onset temperature
T_p	Peak temperature
ΔH	Gelatinization enthalpy
%T	Percentage of transmittance
cP	Centipoist

Abbreviations	**Caption**
DMSO	Dimethyl sulphoxide
DP	Degree of polymerization
DSC	Differential scanning calorimetry
GOPOD	Glucose Determination Reagent
PELITA	Land Custody and Development Authority
RVA	Rapid Visco Analyzer
SEM	Scanning Electron Microscopy
PT	Pasting Temperature
PV	Peak Viscosity
TV	Through Viscosity
FV	Final Viscosity

BD	Breakdown
SB	Setback

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CIRI – CIRI FIZIKOKIMIA KANJI DARIPADA PALMA SAGU (*Metroxylon* sagu) DITANAM DALAM TANAH MINERAL PADA TAHAP PERTUMBUHAN YANG BERBEZA.

ABSTRAK

Malaysia pada masa kini adalah pengeksport terbesar kanji daripada palma sagu (*Metroxylon sagu*) iaitu 47,000 metrik tan/tahun yang mana 96% kanji tersebut dihasilkan di Sarawak. Masalah besar yang dihadapi oleh industri atau pengilang makanan adalah variasi kualiti dalam kanji sagu daripada satu set kepada set yang lain. Data yang sedia ada tidak mencukupi untuk menampung permintaan tinggi kepada kanji sagu berkualiti tinggi daripada industri. Pemahaman kepada ciri-ciri asas diperlukan untuk mengeksplotasikan kanji dengan lebih efisyen yang mana data masih tidak mencukupi bagi palma sagu yang tumbuh dalam tanah mineral. Kajian ini dijalankan untuk menentukan kesan-kesan perbezaan tahap pertumbuhan dan perbezaan ketinggian terhadap ciri-ciri fizikokimia granul kanji yang diekstrak daripada palma sagu. Empat tahap komersil pertumbuhan palma sagu dikaji iaitu Plawei (palma sagu pada pertumbuhan vegetatif yang maksimum), Bubul (pertumbuhan struktur pendebungaan), Angau Muda (peringkat berbunga) dan Angau Tua (peringkat berbuah). Sampel kanji telah diambil daripada dua ketinggian yang berbeza bagi setiap tahap pertumbuhan iaitu: bawah (1 meter dari aras tanah) dan pertengahan (5 meter dari aras tanah) pada batang sagu yang sama. Keputusan daripada kajian ini menunjukkan kandungan kanji, amilosa, lemak, protein dan abu di dalam kanji sagu berkumpul dengan banyak pada ketinggian bawah pokok sagu seterusnya berkurangan pada ketinggian tengah palma sagu pada

semua peringkat pertumbuhan kecuali peringkat Bubul. Kandungan kanji tertinggi didapati pada peringkat Plawei (94.2%) dan peringkat Angau Muda (97.9%) pada ketinggian bawah dan pertengahan pokok sagu, bagi setiap satu. Penyebaran saiz granul didapati sama apabila palma tumbuh ke peringkat yang seterusnya. Diameter min tertinggi bagi kanji sagu ditemui pada peringkat Angau Muda (33.3μm) pada ketinggian bawah. Peringkat Angau Muda mempunyai sifat kerintangan yang paling tinggi iaitu tiada sineresis berlaku selepas lima pusingan analisis nyahbeku. Ciri-ciri fizikokimia kanji sagu daripada kedua-dua perbezaan tahap pertumbuhan dan perbezaan ketinggian; tidak berbeza secara signifikan.

PHYSICOCHEMICAL PROPERTIES OF STARCH FROM SAGO (*Metroxylon sagu*) PALM GROWN IN MINERAL SOIL AT DIFFERENT GROWTH STAGES

ABSTRACT

Malaysia is currently the largest world exporter of starch from sago (*Metroxylon sagu*) palm i.e. 47,000 metric ton/year where 96% from the starch was produced in Sarawak. The major problem faces by the industry or food manufacturer is the variation in quality of sago starch from batch to batch. The existing data are not sufficient to cater the increasing demand for high quality sago starch from the industry. An understanding of basic properties is required to effectively utilize the starch which data is still lacking for sago palm grown in mineral soil. This study was carried out to determine the effects of different growth stages and different heights on the physicochemical properties of starch granule extracted from sago palm. Four commercial growth stages of sago palm i.e. Plawei (palms at maximum vegetative growth), Bubul (appearance of flowering structure), Angau Muda (flowering) and Angau Tua (fruiting) were studied. The sampling point was taken at two different heights for each growth stage: base (1 meter above the ground) and middle (5 meter above the ground) at the same sago palm trunk. Results from this study indicated that starch, amylose, fat, protein and ash content of the starches accumulated plentifully at the base of the palm then lessen towards the middle height for all growth stages except for Bubul stages. The highest starch content was found at Plawei stage (94.2%) and Angau Muda stage (97.9%) at base and middle height, respectively. Granule size distributions were similar as the palm grows to the

later growth stages. The highest mean diameter of sago starches granules was found at Angau Muda stage (33.3μm) at base height. Angau Muda stage has the highest resistance where syneresis does not happen after five cycles of freeze-thaw analysis. The physicochemical properties characteristics of sago starch from both different growth and height did not differ significantly.

1 INTRODUCTION

1.1 Background

Starch is a mixture of two polysaccharides, the linear molecule of amylose which consists of polymers of glucose, and amylopectin, a highly branched molecule. Starch can be sub-divided into cereal, legumes, palm and tuber or root starches (Lideboom *et al*., 2004). However, starches of different botanical origins have different characteristics, shapes, sizes and morphology (Oates, 1997). Sago starch; is an edible starch extracted from pith-like center of several Asian palms (including *Metroxylon sagu*) or sometimes cycads (Cabalerro *et al.*, 2003) and it has been distributed throughout South East Asia (Ahmad *et al.*, 1999). The word 'sago' is originally Javanese, meaning starch-containing palm pith. The scientific name is derived from 'metra', meaning pith or parenchyma and 'xylon' meaning xylem (Rekha *et al.*, 2008).

Sago palm is characterized by a crown of compound leaves and terminating in a tall, woody and unbranch stem with non-branching roots go straight down to the soil (Caballero *et al*., 2003). It grows well in humid tropical lowlands, up to an altitude of 700 meters. Temperature above 25 ^{o}C and relative air humidity of 70 % are favorable. It is extremely hardy plant, thriving in swampy, acidic peat soils, submerged and saline soils where few other crops survive but growing slowly in peat soil than mineral soil (Rekha *et al.*, 2008). The sago palm is hapaxantic, means it flowers once and dies shortly thereafter. The palm is mostly propagated vegetatively through its suckers in the wild as well as cultivation. Individual sucker firstly grows into a rosette of leaves, then produce a stout trunk (Caballero *et al*., 2003). During the vegetative stage, just before flowering, the plant converts its stored nutrients into starch, which accumulates in the trunk (Rekha *et al.*, 2008).

The matured trunk will be harvested, leaving the immature and suckers, thereby sustaining sago production (Caballero *et al*., 2003). The trunk consists of a central core of soft pale-pink pith (Figure 1) that contains most of the starch stored by growing palm, protected by 2 cm thick of fibrous bark. The bark is progressively denser towards the outside where the surface is covered with a thin shiny reddish-brown skin. This shell provides most of the structural strength of the palm and protects against predatory organisms (Rekha *et al.*, 2008). The sago starch accumulates in the pith core of the stem of the sago palm. Trunk formation starts in the third and forth year of growth of the palm. The vegetative phase of the sago palm takes about 7 to 15 years, during which time the pith is saturated with starch from the base of the stem upwards (Tie *et al.*, 2008). The classification of sago palm stages are tabulated in Table 1.

Figure 1: Cross section of sago palm trunk showing the pale-pink pith

Table 1: Four different growth stages of sago starches (Jong, 1995)

Local name	Estimated age from planting (years)	Duration of trunk growth (years)	Growth description
Pelawai	10	4.5	75 % trunk growth; trunks are 6 to 8 in length (Figure 2a).
Bubul	12	6.5	Bolting; appearance of torpedo-shaped flowering structures at the palm terminal. It is characterized by the elongation of the trunk at the top of the crown and frond reduction to bract-like structures (Figure 2b).
Angau Muda	12.5	7	Flowering; well-developed flowering structure with primary, secondary and tertiary flowering axes spreading out at the terminal. Flowers are in the pre- or post anthesis stage (Figure 2c).
Angau Tua	14	8.5	Mature fruiting; fruits are mature, of diameter 30 to 40 mm. Seeds (if any) are well developed with dark brown seed coat and bony endosperms. Most fronds are in senescent stage (Figure 2d).

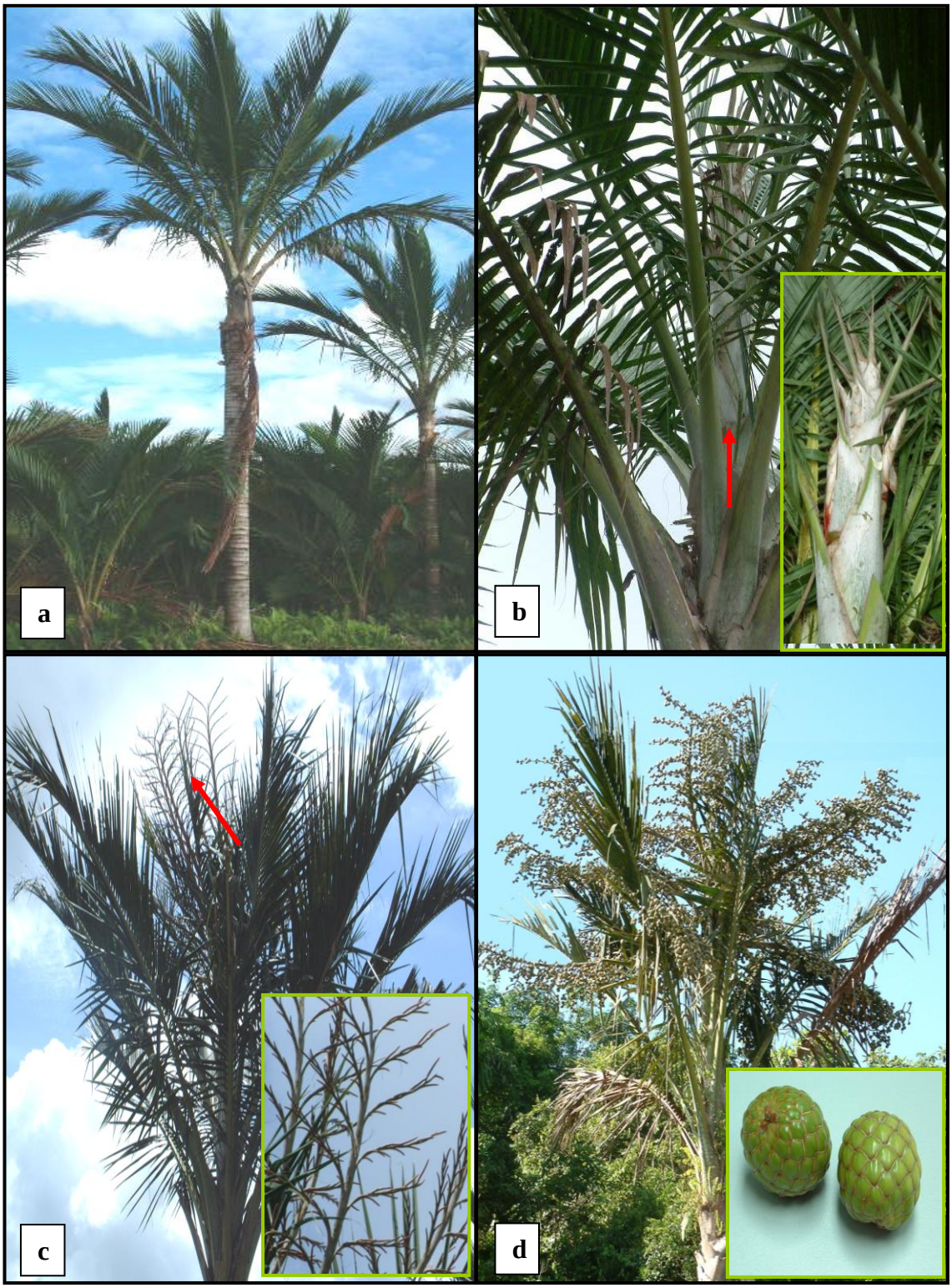

Figure 2: Sago (*Metroxylon sagu*) palm at different growth stages (a) Plawei, (b) Bubul, (c) Angau Muda and (d) Angau Tua (CRAUN Research Sdn Bhd)

Sago starch is becoming an important carbohydrate source owing to its lower production cost and higher yield compared to other crops such as cassava and maize. Caballero *et al.* (2003) reported that the productivity of sago palm is four times of paddy (*Oryza sativa*) where a single palm yields up to 300 kg of starch. Three leading world sago starch producers are Malaysia, Indonesia and Papua New Guinea, where sago palm is grown commercially for the production of sago starch and conversion to animal food or ethanol. A 25 ton per hectare of sago starch was produced every year from sago plantation under development of Sarawak state of Malaysia, the highest in productivity among the starchy crops of the world (Rekha *et al*., 2008).

Sago starch is essential diet for people of South East Asia where sago is used in various food items and also to stiffen cloth material in the textile industry. Sago is widely used to produce sago pearls. It can be boiled, either alone or mixed with other foods, and consumed directly as a carbohydrate source (Caballero *et al*., 2003). In Malaysia, sago starch was utilized in the making of noodles, crackers, adhesive and glucose syrup (Anthonysamy *et al*., 2004).

Quality of sago starches is important when starch is designated for export or when it is sold to large-scale food processors (Oates and Hicks, 2002). For example, good quality sago starches give high viscosity during gelatinization (Azudin and Lim, 1991). The poor quality of sago starch has been attributed to a number of factors such as poor processing conditions, presence of metal ions during processing, freshness of the raw pith, presence of polyphenol compounds and the consequent activity of polyphenol (Sim *et al.*, 1991). Quality problems associated with sago starch are inconsistent viscosity (or variable pasting properties), variable moisture content, distinct odor, low profile viscosity, high level of fiber and dull color. Mature palms are essential for the

production of high-quality starch. Immature pith contains more impurities per unit weight of starch and has a greater tendency for browning (Karim *et al.*, 2008).

Ruddle *et al.* (1978) reported that the starch reserve inside sago palm trunk apparently at their maximum just before flowering and fruiting but scientifically little information available about the timing of starch build up. Jong (1995) found that the starch content is low in the early stages of trunk development and is mainly confined to the lower portion of the trunk. In Indonesia and Sarawak, the general belief is that the felling of the sago palm is best carried out after flowering but before the fruiting stage (Karim *et al.*, 2008). Therefore sago starch properties at different growth stages has to be established as the maturity, the location of the starch at different parts of sago palm trunks and the types of soil have been found to influence the physicochemical and functional properties of sago starch (Karim *et al.*, 2008). Jong (1995) reported that high content and density of starches are constant throughout the whole length of the trunk until the flowering stage. Thereafter, the level of starch decreases sharply towards the topmost and bottommost position of the trunk. Thus, it is important to study sago starch properties at different heights of sago palm.

Previous study of sago starch in peat soil by Tie *et al.* (2008) found that there was a variation in the morphology of starch, amylose content, particle size and distribution profile, pasting, thermal and retrogradation profile of sago starch from different growth stages obtained at different heights of the palm. Nozaki *et al.* (2004) also reported that sago palm grows quickly in mineral soil with most starch accumulated from base towards the middle heights of the palm; while no morphological differences in the starch granules among the soil conditions and position for sago palm grown in peat soil (Rekha *et al.*, 2008). However these data are not sufficient to cater the increasing

demand for high quality sago starch from the industry. The presence of quality variations for sago starch from batch to batch such as viscosity and color; will effects the processing condition, mechanization and end product. Thus the effective utilization of this starch requires an understanding of its basic properties for which data is still lacking for sago palm grown in mineral soil at different growth stages obtained from different heights.

It is hope that the output of this study will contribute to the improvement of sago starch quality; hence a reliable large volume supply of good quality sago starch will be available. A competitive price and sufficient supply of high quality sago starch would provide an alternative of starch source for the starch processors.

1.2 Objectives

The objectives in this study are as follows:

i) To study the effect of growth stages of sago palm i.e. Plawei (palms at maximum vegetative growth), Bubul (appearance of flowering structure), Angau Muda (flowering) and Angau Tua (fruiting) on the physicochemical properties of sago starch granule;

ii) To investigate the variation (chemical content, amylose content, swelling factor, pasting & thermal profiles, granule morphology and granule size distribution) of sago starch derived from sago palm at two different heights i.e. Base (from 1 meter above the ground) and Middle (from 5 meter above the ground) part of the trunk.

2 LITERATURE REVIEW

2.1 Starch

2.1.1 Amylose and Amylopectin

Starch consists of two polysaccharides which are amylose and amylopectin. The approximate weight amounts of amylose and amylopectin is in the range of 15-30% and 85-70%, respectively (Jane *et al*., 1994). Tester and Karkalas (2002) reported that both amylose and amylopectin represent approximately 98 – 99 % of the dry weight. Most starches contain 20 – 30 % amylose and 70 – 80 % amylopectin where the ratio varies with the botanical source of the starch. It is known that the amylose – amylopectin ratio of starch greatly affects the starch functional properties (Jane *et al*., 1999). In starch granules, the amylose and amylopectin molecules are radially oriented with their single reducing end-groups towards the centre or hilum, and synthesis is by apposition at the outer non-reducing ends (French, 1984).

Amylose has long linear chains of (α-1-4) – linked D-glucopyranose residues (Figure 3), some with a few (more than 10) branches (Jane *et al*., 1999). Amylose can be made of several thousand glucose units and the number of repeated glucose subunits (n) can be many thousands. Amylose is found with molecular weights ranging from 10^5-10^6 and with the number of glucose residues per molecule, (DP – degree of polymerization) ranging from 500 to 5000 (Galliard and Bowler, 1987). DP is the total number of anhydroglucose residues present divided by the number of reducing ends. Amylose is present in the amorphous structure. In general, cereal starches have smaller amylose molecules than tuber starches, and large amylose molecules contain more branch linkages than small amylose molecules.

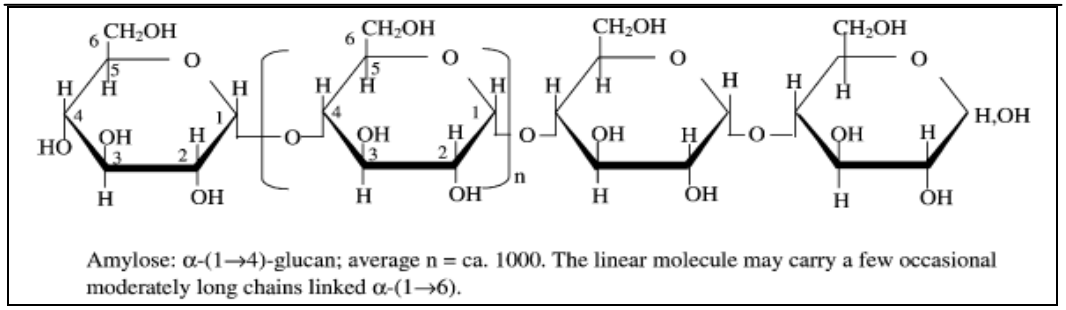

Figure 3: Structure of amylose (Tester *et al*., 2004).

A single amylose helix has a relatively hydrophobic inner surface that can hold guest molecules. Each amylose helical turn has been shown to hold polyiodide ions and the iodine-amylose complex gives an intense blue color. The color formation of the iodine-amylose complex is used in the analysis of amylose. Amylose content can be quantitatively determined by measuring the absorbance of the blue color from iodine reaction at 680 nm under specified conditions, and this absorbance is called "Blue Value". Potentiometric iodine titration is also another technique to determine the quantity of amylose in starch (Kuakpetoon, 2006).

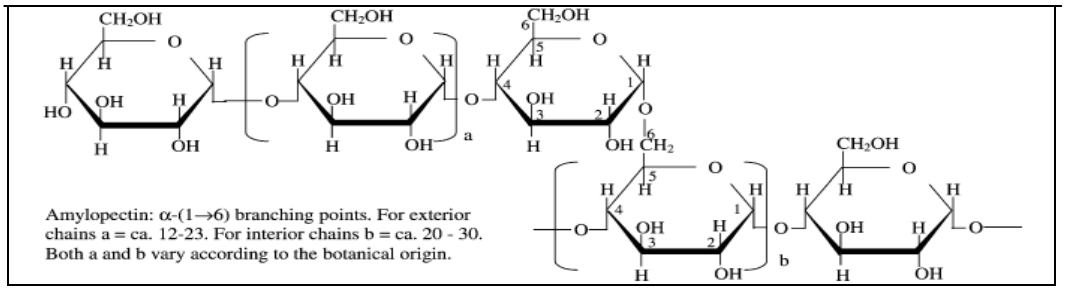

Figure 4: Structure of amylopectin (Tester *et al*., 2004).

Amylopectin has large molecular weight and highly branched structures consisting of much shorter chains of (1-4) α-D-glucose residues. The branch-chains are connected by (α-1-6) –D glucosidic linkages (Figure 4). Amylopectin has molecular weights ranging from 10^7-10^9, depending upon the source. The amylopectin branches may be classified according to their pattern of substitution: A-chains are defined as unsubstituted, B-chains are substituted by other chains and there is a single C-chain that carries the reducing glucose. Chen (2003) suggested that amylopectin structure consists of three type chains (Figure 5) i.e., A-chain, B-chain and C-chain. The C-chain carries the sole reducing group in the molecule to which the B-chains are attached, while the terminal A-chain is attached to B-chain (Manners, 1989).

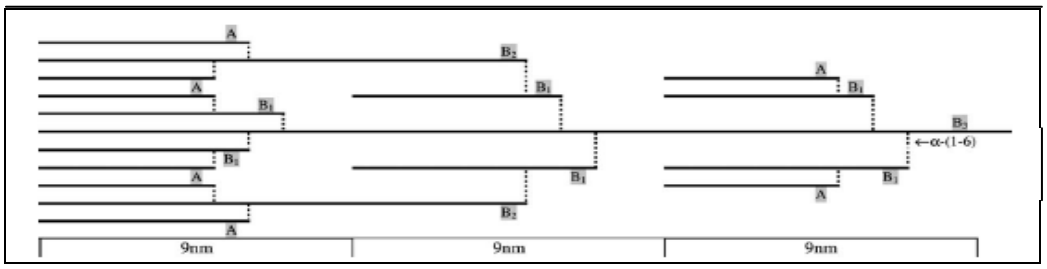

Figure 5: Chain distribution of amylopectin (Tester *et al*., 2004).

Amylopectin is the major component of starch. The molecules are very large, ranging from 50 million to over a 100 million in molecular weight (Whistler *et al*., 1984). Being a major component in starch, its crystalline structure nature and its swelling power, amylopectin plays an important and dominating role in the starch properties such as gelatinization and pasting properties. It is highly crystalline and it is responsible for the crystallinity of starch granules and results in insolubility of starch granules in cold water.

The packing of the amylopectin clusters form two alternating crystalline and amorphous lamellae regions (Figure 6). The amylopectin double helices fall within the crystalline lamellae regions, while the amylopectin branch points line in the amorphous lamellae. The crystalline double helices are more compact and less susceptible to acid compared with the amorphous lamellae. The width of one crystalline and one amorphous lamellae is 9 nm and the width of one growth ring is around 120 – 400 nm (Kuakpetoon, 2006). Table 2 shows some important physicochemical properties of amylose and amylopectin.

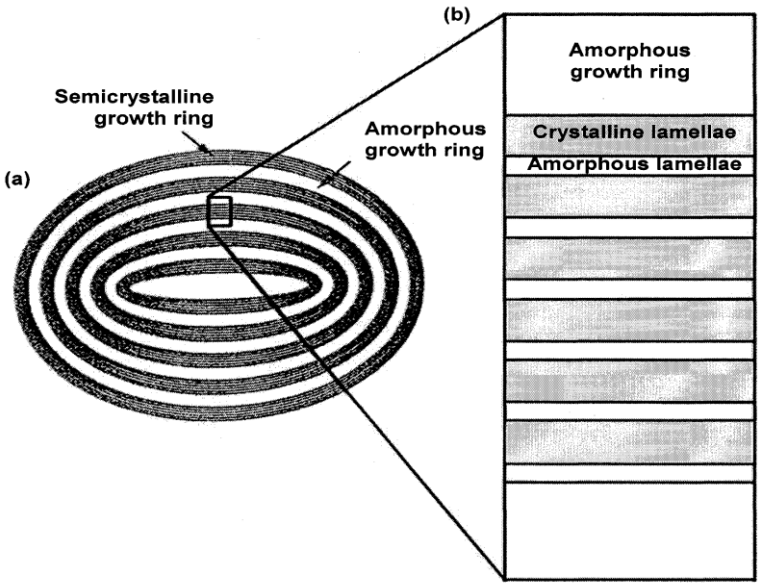

Figure 6: Schematic diagram of starch structure (a) A single granule comprising concentric rings of alternating amorphous and semi-crystalline composition (b) Expanded view of the internal structure. The semi-crystalline growth ring contains stacks of amorphous and crystalline lamellae (Kuakpetoon, 2006).

Table 2: Some important physicochemical properties of amylose and amylopectin

Property	Amylose	Amylopectin
Molecular structure[a]	Linear (α-1,4)	Branched (α-1,4; α-1,6)
Molecular weight[b]	~10^6 Daltons	~10^8 Daltons
Degree of polymerization[a]	1500-6000	$3x10^5$-$3x10^6$
Helical complex[b]	Strong	Weak
Iodine colour[a]	Blue	Red-purple
Dilute solutions[a]	Unstable	Stable
Retrogradation[b]	Rapidly	Slowly
Gel property[a]	Stiff, irreversible	Soft, reversible
Film property[a]	Strong	Weak and brittle

a: from Jane (2000); b: from Zobel (1988).

2.1.2 Starch Granules

Starch granules are semi-crystalline, comprised of crystalline and amorphous regions, and may have some transitional regions. Under polarized light, starch granule shows birefringence and a 'Maltese Cross' pattern where the hilum, the growing point of the starch granule, is at the geometric center of the granule. Birefringence implies a high degree of molecular orientation within a granule. Fresh start granules exhibit growth rings, which may originate from the concentric deposition of starch molecules. Each ring represents shells of high and low starch contents, presumably from variation in rate or mode of starch deposition during growth (Buleon *et al*., 1998). Starch varies greatly in form and functionality between and within botanical species, and even from the same plant cultivar grown under different conditions.

Starches from different botanical sources, from different cultivars and from the same cultivars grown under different conditions are characterized by differing physicochemical properties. Starch granules from different botanical sources have different characteristic shapes, sizes and morphology. The sizes of starch granules vary from submicron to more than 100 microns in diameter. Starch granules varies in shape include spherical, disk, oval, polygonal, dome-shape, elongated rod shape and compound starch (Jane, 2006). Huber and BeMiller (2001) reported that starches from various sources can be differ in terms of the granule morphology (size, shape, presence or lack of pores, channels and cavities), molecular structure (amylose and amylopectin fine structures) and composition (amylose-to-amylopectin ratio, content of non-starch components) lead to variations in starch properties (X-ray diffraction pattern, gelatinization temperature range, gel properties, retrogradation tendency, granule swelling power and pattern, etc.) which are indications of structural divergence.

Starch occurs in granular form, with the shape of the granules being characteristic of the source of the starch. Analysis of granules with polarized light shows evidence of a layered structure in the granule, particularly for wheat, although starch from many plants only exhibits the rings or lamellae after pretreatment with acid or hydrolytic enzymes. It was reported that starch granules have a symmetrical arrangement thought to be crystalline structure which can be seen as birefringence patterns when the granule is viewed between crossed polarizer (Kennedy *et al.*, 1987). Isolation of starch granule from plant tissues can be achieved without degradation because they are insoluble in cold water, whereas many of the contaminants are soluble. The granules swell reversibly in cold water and this process is used for the extraction on the industrial scale to loosen the granules in the matrix. As temperature is raised, the

swelling process becomes irreversible and the ordered structure is lost. Above this gelatinization temperature the granule bursts to form a starch paste. Starch granules will burst at different temperature where the temperature of gelatinization is characteristic of a particular starch (Kennedy *et al.*, 1987).

2.1.3 Thermal Analysis of Starch

2.1.3.1 Starch Gelatinization and Retrogradation

The physical form of the starch components undergoes several transformations during the processing and storage of starch-based food. Heat and shear treatment during food processing implies swelling, loss of crystallinity and disruption of the starch granules (Vesterinen *et al.*, 2002). Gelatinization of starch in water is the collapse of the crystalline structure in the granules accompanied by the increase in volume, due to swelling and leaching of soluble amylose and amylopectin into the surrounding aqueous media as a result of heating. Gelatinization temperature is reflection of the crystallite perfection (Abera and Rakshit, 2003).

At molecular level, gelatinization involves the uncoiling of external chains of amylopectin that are packed together as double helices in clusters (which creates crystalline regions in the native starch). Crystalline and double helices melting during starch gelatinization are assisted by hydration and swelling of the amorphous regions of the starch granules by imparting a stress on the crystalline regions and thereby stripping the polymer chains from the surface of the starch crystallites (crystal melting) (Tie *et al.*, 2008). According to Karim *et al.* (2000), when a thermal transition occurs, the energy observed by the sample is replenished by increased energy input to the sample to maintain the temperature balance. This energy input is equivalent in magnitude to the

energy absorbed in the transition and yield a direct colorimetric measurement of the energy transition which is then recorded as a peak.

Starch gelatinization within the starch granule manifested in reversible changes in properties such as granular swelling, native crystallite melting, loss of birefringence and starch solubilization. The point of initial gelatinization and the range over which it occurs is governed by the starch concentration, method of observation, granule type and heterogeneities within the granule population under observation (Maaruf *et al.*, 2001). Differential scanning calorimetry (DSC) is particularly well suited to investigate the phase transitions of starch/water system because it allows: (1) a study of starch gelatinization over a wide range of starch/water ratio; (2) determination of gelatinization temperature above 100 ^{o}C and (3) estimation of transition enthalpies.

Enthalpy values have been used as an indicator of degree of molecular order for comparative purposes. The chain lengths associated with the molecular order in sago starch are shorter than for the wheat (9.7 J/g), potato (16.2 J/g) or tapioca (16.97 J/g) leads to lower value of enthalpy and temperature of melting of the starches (Maaruf *et al.*, 2001). The gelatinization temperature for sago starches are high compared to corn, pea and potato but lower compared to starch from sweet potato, tania and yam. The gelatinization temperature and enthalpy of the starches depends on the microstructure and degree of crystallinity within the granule and also on granule size and the amylose to amylopection ratio. Normally the smaller the granule the higher will be the gelatinization temperature (Ahmad *et al.*, 1999).

Retrogradation is a result of re-association of starch molecules in an ordered structure. It is a situation where the dissolved amylose chains associate to form helices and insoluble double helices when starch paste cools (Abera and Rakshit, 2003). During

retrogradation, amylose forms double helical associations of 40 – 70 glucose units whereas amylopectin crystallization occurs by re-association of the outermost short branches. Extent of retrogradation and the nature of crystallite formed may be affected by the starch source, concentration and storage temperature (Narpinder *et al.*, 2005).

The variation in the thermal properties of starches after gelatinization and during refrigerated storage may be attributed to the variation in amylose to amylopectin ratio, size and shape of the granules and presence/absence of lipids; although both amylose and amylopectin component appears to be more responsible for long term quality changes in foods. Recrystallization of amylopectin branch chains has been reported to occur in less ordered manner in stored starch gels as it is present in native form. This explains the observation of endotherms at temperature range below that for gelatinization (Narpinder *et al.*, 2005).

Retrograded starches are problematic as food components. Retrograded starchy foods cause an unacceptable texture, because parts of starch molecules associate to form a more rigid or toughened structure. Retrogradation occurred within the swollen granule during aging the paste, which stabilizes the granule against swelling when heated to a higher temperature. Retrograded starch is more soluble in cold water than raw starch and it produces viscous solutions even at room temperature (Hibi and Hikone, 2000).

2.1.3.2 Pasting, Swelling and Solubility

When starch molecules are heated in excess water, the crystalline structure is disrupted and water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of amylose and amylopectin, which cause an increase in granule swelling and solubility (Narpinder *et al.*, 2005; Lee *et al.*, 2005). After gelatinization, a

starch paste consists of solubilized carbohydrate molecules and swollen starch granules or their fragments. Granule swelling occurs after the melting of the starch crystallites. After melting of the crystallites, the granules hydrate and swell irreversibly to give a paste (Hibi and Hikone, 2000).

Lin *et al.* (2003) reported that swelling factor of potato starches was greatly influenced by growth time and experimental temperature. Swelling factor of starch is also influenced by molecular structure including crystalline structure and chemical composition. Another factor influencing starch swelling at different temperatures could be the leaching of amylose from starch granules. Different in swelling factors indicate that different interactions between amylose and amylopectin may exist in these potato starches (Lin *et al.*, 2003).

Factors such as properties and intensity of the three-dimensional network of micelles in starch granule, bonding degrees at the molecular level, branching of outer parts in the amylopectin molecules and properties, and amount of non-starch components such as lipid had great influence on swelling power. The different in the swelling and solubility pattern appears to be the basis for differences in their functional properties, thus making them useful for the preparation of various products (Odeku and Picker-Freyer, 2007).

Sandhu *et al.* (2005) mentioned that swelling power and solubility provide evidence of the magnitude of interaction between starch chains within the amorphous and crystallite domains. Factors like amylose-amylopectin ratio, chain length and molecular weight distribution, degree/length of branching and confirmation determine the degree of swelling and solubility. High amylose content and presence of higher number of intermolecular bonds can reduce swelling. Swelling volumes also affected by

the formation of lipid-starch complex that presence with naturally occurring carbohydrate and non-carbohydrate. Solubility of starch depends on a number of factors such as source, inter-associative forces, swelling power and presence of other components (Moorthy *et al*., 2002).

2.1.4 Particle Size Distribution

The Malvern laser diffraction technique generates a volume distribution for the analyzed light energy data. During the laser diffraction measurement, particles are passed through a focused laser beam. These particles scatter light at an angle that is inversely proportional to their size. The angular intensity of the scattered light is then measured by a series of photosensitive detectors. These volumes distribution can be converted to any number or length diameter. Laser diffraction can generate the D [4, 3] or equivalent volume mean. This is identical to the weight equivalent mean if the density is constant. The volume mean diameter is the mean of the diameters of the spheres having the same volume as the real particles (Pei-Lang, 2004). D [v, 0.5] is the volume median diameter where 50 % of the distance is above and 50 % is below this value. It divides the distribution exactly in half. D [v, 0.1] is the 10 % cut-off point as 10 % of the distribution is below this point. D [v, 0.9] is the 90 % cut-off as 90 % of the distribution is below this point (Pei-Lang, 2004).

From previous study, Tie *et al.* (2008) reported that starch granules from Angau Muda stage showed the largest mean diameter (25.7 μm) at base height while smallest mean diameter was observed in the Plawei stage (16.8 μm) at middle height. The most widely distribution in granule size was observed in the base height of the Angau Muda stage (15.0 to 28.1 μm) while the narrowest distribution was observed in the middle

height of the plawei stage, i.e., from 9.7 to 18.2 μm (Tie *et al.*, 2008). Physicochemical properties such as percent light transmittance, amylose content, swelling power and water binding capacity are significantly correlated with the average granule size of the starches separated from different plants (Shujun *et al*., 2006).

2.1.5 Intrinsic Viscosity

Intrinsic viscosity is a measure of hydrodynamic volume of macromolecules in dilute solution. It is generally accepted that macromolecule conformation and molecular weight play a fundamental role, through their relationships with the molecular dimensions and shapes, in determining the value of intrinsic viscosity (Xu *et al.*, 2008). The conformation of a polymer molecule also influence by its components and solution concentration. In extremely dilute solutions, the polymer coils are separated and isolated from each other. The coils begin to overlap when the concentration increases and finally the coils are packed in more concentrated solution. Consequently, polymer shows different solution behavior in different concentration regimes due to interactions between polymer chains (Xu *et al.*, 2008). The intrinsic viscosity is a characteristic of macromolecules that is directly related to their ability to disturb flow and indirectly to the size and shape of the molecules. This value is obtain by measuring specific viscosities at different concentrations at the same shear rate and extrapolating the course of specific viscosity to infinite dilution (Nurul Islam *et al.*, 2001).

2.1.6 Microscopic Observation

2.1.6.1 Scanning Electron Microscopy (SEM)

Basic SEM principle application involve fine probe of electrons with energies focused on a specimen, and scanned along a pattern of parallel lines. Various signals are generated as a result of the impact of the incident electrons, which are collected to form an image or to analyse the sample surface (Bogner *et al*., 2007). Eliasson (2004) reported that SEM can either be used to examine the gross morphology, or by the use of etch on fractured samples to study variations in internal packing. The resolution possible with SEM also provides a more detailed perspective on granule surface characteristics and granule morphology (Chmelik, 2010). Ahmad *et al.* (1999) reported that SEM results for sago starches samples from South East Asia showed that the sago starch consists of oval granules with diameters in the range of 20 – 40 μm. In contrast, Tie *et al.* (2008) reported that sago starch granules are oval in shape, with some granules showing a truncated shape. From the micrograph, it is estimated that the granule size ranges from approximately 10 – 30 μm.

2.1.6.2 X-Ray Diffraction

The melting thermodynamic properties of starches were directly correlated to their amylose content. X-ray diffraction patterns have been used to reveal the characteristics of the crystalline structure of starch granules (Huang *et al*., 2006). Tian *et al.* (1991) reported that the crystalline nature of a starch granule can be defined by the position of the x-ray diffraction peak. Starch granules possess different types of crystallinity, displaying A-, B- and C-type X-ray patterns, depending on their amylopectin branch chain length (Hizukuri, 1985). Most cereal starches such as wheat, corn and rice are A-type and B-type pattern is shown by tuber, fruit and high-amylose

corn starch and by retrograded starch (Tester and Karkalas, 2002). C-type pattern is an intermediate between A- and B-type starches and its typical of legume seed starches, such as pea and bean. The V-type structure has not been found in native starches, but it may form if starch recrystallizes in the presence of a fatty acid or long-chain alcohol (Hoseney, 1994). Figure 7 shows the X-ray diffraction patterns of A, B and C type.

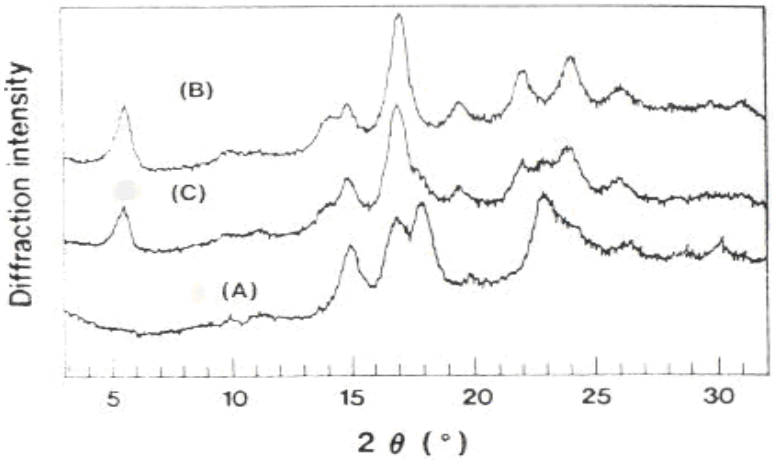

Figure 7: X-ray diffraction patterns of A, B and C type.
(Adapted from Hizukuri et al., 1996).

The A-type polymorphic starch has a monoclinic unit cell and the B-type polymorphic starch has a hexagonal unit cell (Imberty *et al*., 1991; Zobel, 1988). The C-type polymorphic starch consists of a combination of the A-type and the B-type unit cells. This classification, based on diffractometric spectra, does not follow the morphological classification but is able to group most starches conveniently according to their physical properties (Gallant *et al*., 1992). In addition, Jane (2006) had also reported that the A type starch consists of shorter branch chains whereas B-type starch consists of longer B chains. The short crystalline structure in A-type polymorphs are less stable and more susceptible for rearrangement and therefore generate more loosely packed areas of voids. On the other hand, B type polymorphs and some C type

polymorphs have long chains, which extended through two or more clusters and stabilize the internal structures of granules.

2.1.7 Freeze-thaw Study

Starch gel or paste undergoes structural changes upon freezing. Numerous publications have reported physical changes of starch gels induced by freezing or freeze-thawing treatment. The changes include synerisis and formation of a sponge structure, which are attributed mainly to starch retrogradation. When a starch gel is frozen, the gel system becomes heterogeneous and separates into starch-rich and starch-deficient ice phases (Jeong and Lim, 2003). Gels of amylose become very firm on standing due to the formation of crystallites, whereas amylopectin gels are softer, more stable and less crystalline because of extensive branching. The addition of amylopectin will improve the stability of starch products because it prevents association of amylose molecules which follows the release of water molecules, as shown in Figure 8.

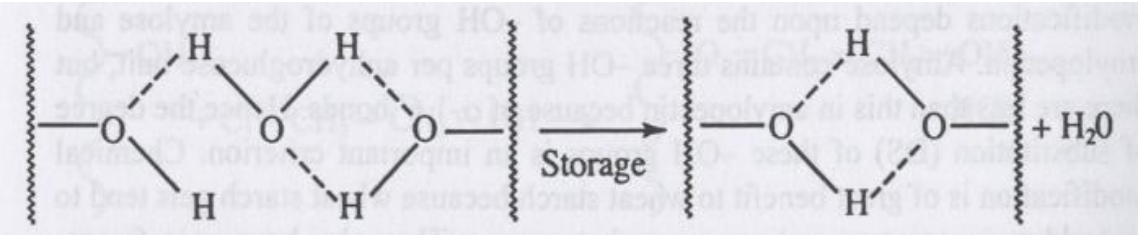

Figure 8: Synerisis of starch gel, exemplified by release of water from hydrogen bonded amylose gel (Eliasson, 2004).

This process is termed ‘retrogradation’, which means the return from salvated, dispersed state to an insoluble, aggregated state. The change is accompanied by an increase in cloudiness as well as formation of free water, the latter being termed ‘synerisis’. Amylopectin undergoes retrogradation more slowly and to a much lesser

extent than amylose, because of its highly branched structure. Unlike linear amylose, amylopectin molecules are not able to align so readily, this only happens over limited regions. (Eliasson, 2004).

Synerisis occurs due to increased molecular association between starch chains, at reduced temperature, thus excluding water from the gel structure. Waxy rice starch gel was reported to be more resistant to synerisis after a freeze-thaw cycle due to the formation of fewer inter-molecular associations (Eliason, 2004). Freezing modified the quality attributes of the starch pastes by increasing exudates production, structure deterioration and rheological changes. Starch retrogradation and ice recrystallization both contributed to the deterioration of the frozen paste during storage. Freezing rate was also an important determinant of structure and texture, with faster freezing rates showing improved quality (Eliasson, 2004).

2.2 The Sago Palm

2.2.1 Taxonomy and Botany

The term sago means an edible starch extracted from the pith-like center of several Asian palms (including *Metroxylon sagu*) or sometimes cycads. Palm is the common name for members of the plant family Palmae. Palmae is a large family and includes tropical trees, shrubs and vines. Most members of this family are tree-like, characterized by a crown of compound leaves and terminating in a tall, woody, unbranched stem (Caballero *et al.*, 2003). Sago palm belongs to the order Arecales Nakai, family Palmae Jussieu, subfamily Calamoideae Griffith, tribe Calameae Drude, subtribe *Metroxylinae* Blume and genus *Metroxylon* Rottboell (Caballero *et al.*, 2003; Rekha *et al.*, 2008; Karim *et al.*, 2008). The two most important starch-producing

species in Malaysia and Indonesia regions are *Metroxylon sagu* Rottb. and *Metroxylon rumphii* Mart., of which the latter has spines on the petioles, spathes and even on the leaflets. Rauwerdink (1986) has grouped the 2 species into *Metroxylon sagu*, and this taxonomy is now widely accepted (Karim *et al.*, 2008).

Sago palm is commonly grown in wild swampy areas of Malaysia, Indonesia and New Guinea. This palm grows between 10 ^{o}N and 10 ^{o}S latitudes and up to an elevation ranging from 700 to 1000 m. It is also found growing in dry lands (Caballero *et al.*, 2003). The sago palm is soboliferous (suckering) and has a massive rhizome that produces suckers freely. Sago may be propagated from suckers or seedlings. The plant forms a rosette of leaves in the early stage. Trunk formation starts during the 3rd to 4th year growth of the palm. Sago trunks may reach 7 to 15 m in length and attain an average girth of 120 cm at the base of the palm. The vegetative phase in the sago palm lasts 7 to 15 year, during which excess photosynthate from the leaves is transported to the trunk and stored as starch (Karim *et al.*, 2008).

The pith is saturated with starch from the base of the stem upwards, and at maturity the trunk is fully saturated with starch almost to the crown. After the mature fruits fall off, the palm will soon die. The development of the inflorescence to the production of ripe fruits lasts about 2 year, during which the remaining leaves fall and the carbohydrate supply in the stem is exhausted (Karim *et al.*, 2008). Starch accumulation in palms on a massive scale as found in *Metroxylon* is almost always associated with the hapaxanthic flowering method, where starch is accumulated in the pith of the stem and is mobilized at the onset of the production of a mass of inflorescence state. As flowering proceeded, the stem apex aborts and flowering and

fruiting are followed by the death of the stem. Due to the massive size and lengthy vegetative phase, vast quantities of sago are stored in the stems (Karim *et al.*, 2008).

2.2.2 Soil of the Sago Palm Areas

Soil is the central organizer of the terrestrial ecosystem. Minerals, organic components and microorganisms are among major solid components of soils. These components are not separate entities but rather a unified system constantly in association with each other in the environment (Huanga *et al.*, 2005). Major inorganic solid compounds are quartz, clay minerals, (hydrous) oxides of Ferum, Mangan, and Aluminium, carbonates as well as anthropogenic compounds. Soil organic matter (SOM) represents a complex mixture of partially recalcitrant substances composed of humified and nonhumified materials that derive from plant litter, faunal, and microbial biomass (Totschel *et al.*, 2010). Murtedza (2002) reported that the parameters commonly used to describe the physical properties of organic soil are those related to texture, loss on ignition, bulk density, porosity, wetting and drying process, moisture relationships and hydrology. The physical properties of organic soils are dependent on the four major components which make up the organic soil system; the organic material, the mineral material, water and air.

Metroxylon sagu species can grow on a wide variety of soils, preferring medium and heavy soil texture. They can persist on well drained, poor quality materials including sand, clay, or lava. The palms will grow in soil that is periodically inundated by salt water as long as fresh water flow is more prevalent. It grows best soils with impede drainage, or with seasonal water-logging because water-logging for long periods impedes growth and productivity of sago palm (McClatchey *et al.*, 2004). Table 3