Faculty of Resource Science and Technology

Morphology and Distribution of Starch Granules from Trunking and Non-Trunking Sago Palm (*Metroxylon sagu*)

Mohd Izzuddin bin Alias

Master of Science
2016

Morphology and Distribution of Starch Granules from Trunking and Non-Trunking Sago Palm (*Metroxylon sagu*)

Mohd Izzuddin bin Alias

A thesis submitted
In fulfillment of the requirement for the degree of
Master of Science (Molecular Biology)

Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2016

DECLARATION

I hereby declare that all the work presented in the thesis is entirely my own work and has not been submitted in support of an application for another degree or qualification to this or any other university or institute of higher learning.

.....

MOHD IZZUDDIN BIN ALIAS

ACKNOWLEDGEMENTS

Alhamdullilah, I thank Allah S.W.T for His blessing and guidance throughout this time, as that enables me to live, and finally reached the accomplishment this project. I would like to express my deepest gratitude to my supervisor, Assoc. Prof. Dr. Mohd Hasnain bin Md. Hussain, for providing me the opportunity to pursue my master studies under his supervision in Universiti Malaysia Sarawak (UNIMAS), guidance, and endless support. I was pleased to work with him, thanks also for his advice, kind and constant support, encouragement and help in all my difficulties in accomplishment of this project. Not to forget, with love and many thanks I give to my parents for having faith on me. Their sincere prays have helped me to go through all difficulties during completion of this project. Their hopes give me a greater strength and motivation to give out my best to accomplish the project.

My colleagues in the Proteomics' lab, Siti Izyan Liyana Bt Kamarol, Nikson Chong Fatt Ming, Yan Wei Jie and Mohd.Noorzaifiqrudin Bin Khairol also deserve warm thanks for providing a pleasant working atmosphere during my work in the lab, helping me in many different ways throughout my research, and being good friends who will live in my heart forever. Not to forget the laboratory assisstants, Saifulrizzan providing the needs in the laboratory. I would like to thanks to my fellow friends, Norizawati Bt Muhamad Nur, Lai Mei Kin, Norsyafikah Asyilla Bt Nordin, Norhafizah Bt Sidek, Latifah Suali, Nurul Haniza Bt Zaini and Fazidah Bt Junaidi for their sincere assistant and constructive guidance in term of knowledge and through ups and downs till my project done.

My thanks also extends to all lecturers in Faculty of Resource Science & Technology especially from Department of Molecular Biology for their invaluable knowledge and guidance through out my study period. I would to like to thank Kementerian Pengajian Tinggi Malaysia for financial support throughout my study (MyBrain15). This work has

been partially funded through grant FRGS/961/2013(02) and Tun Openg Sago Chair grant ORC/2011/06(02).

Last but not least, thanks to all who help me directly or indirectly throughout the project. Because of them and their support, I was able to successfully complete this journey. Thanks again and I love you all so much.

ABSTRACT

Sago palm (*Metroxylon sagu*) naturally grows in Malaysia and this species covers a large area in the state of Sarawak. The starch of sago palm is in its trunk. Sago palm is one of the oldest tropical plants exploited by man for its stem starch and one of the main starch-producing crops in the world. The starch granule is mainly composed of amylose and amylopectin. Trunking sago palm is a normal sago palm with the trunk full with starch at the maturity stage. Meanwhile, the non-trunking sago palm is abnormal sago palm where the growth and development is stunted. The purpose of this research is to profile the morphology and distribution of starch from trunking and non-trunking sago palm from selected areas and selected time. The morphology and distribution of starch was viewed under Scanning Electron Microscope (SEM) and also Transmission Electron Microscope (TEM). The shape of the starch granules for trunking sago palm trunk was the same as non-trunking sago palm trunk, where the shape was oval and truncated. The average size of starch granules from trunk of both trunking and non-trunking sago palm are around 20-40 μm. The starch granules extracted from trunking and non-trunking sago palm leaves have disc shaped and oval, where the size of granules range between 1-5 μm. In TEM, the growth rings formation in the trunk of trunking and non-trunking sago palm were the same; they were made up of alternating amorphous and semi-crystalline layers. Fourier Transform Infrared (FTIR) analysis showed there were no differences between trunking and non-trunking sago palm functional group. The starch granule distribution of trunking sago palm were not significantly difference from non-trunking sago palm.

Keywords: sago palm, starch granules, trunking, non-trunking, distribution

Morfologi dan Pengagihan Granul Kanji Pokok Sagu Berbatang dan Tidak Berbatang (Metroxylon sagu)

ABSTRAK

Pokok sagu (Metroxylon sagu) secara semula jadi tumbuh di Malaysia dan spesies ini meliputi kawasan yang besar di negeri Sarawak. Kanji pokok sagu berada di batangnya. Pokok sagu juga merupakan salah satu tumbuhan tropika tertua yang dieksploitasi oleh manusia untuk kanji stem dan salah satu tanaman pengeluar kanji utama di dunia. Granul kanji terutamanya (98-99%) terdiri daripada amilosa dan amilopektin. Pokok sagu berbatang ialah pokok sagu normal dengan batang dipenuhi dengan kanji pada peringkat matang. Sementara itu, pokok sagu tidak berbatang adalah pokok sagu yang tidak normal dimana pertumbuhan dan perkembangannya terbantut. Tujuan kajian ini adalah untuk mendapatkan profil kanji daripada pokok sagu berbatang dan tidak berbatang dari kawasan yang terpilih dan masa yang terpilih. Morfologi dan pengagihan kanji telah dilihat di bawah Mikroskop Imbasan Elektron (SEM) dan juga Mikroskop Transmisi Elektron (TEM). Bentuk granul kanji untuk batang pokok sagu berbatang adalah sama seperti pokok sagu tidak berbatang, dimana bentuk bujur dan dipenggal, manakala saiz purata granul kanjinya adalah di antara 20-40 mikron. Granul kanji yang diekstrak daripada daun pokok sagu berbatang dan tidak berbatang mempunyai bentuk cakera dan bujur, di mana saiz granul di antara 1-5 mikron. Pemerhatian menggunakan TEM menunjukkan pembentukan lingkaran pertumbuhan dalam kedua-dua pokok sagu adalah sama; iaitu masing-masing terdiri daripada susunan lapisan amorfus dan semi-kristal. Analisis spektroskopi Fourier transformasi infra-merah (FTIR) menunjukkan tiada perbezaan kumpulan berfungsi antara kedua-dua pokok sagu. Kesimpulannya, tiada perbezaan yang ketara untuk pengagihan granul kanji pokok sagu berbatang dan tidak berbatang.

Kata kunci: *pokok sagu, granul kanji, berbatang, tidak berbatang, pengagihan*

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

LIST OF ABBREVIATION

°C	degree Celcius
µm	micrometer / micron
µm	millimolar
ADPase	adenosine 5’-diphosphate glucose pyrophosphorylase
ADPG	adenosine 5’-diphosphate glucose
AFM	atomic force microscopy
ATP	adenosine triphosphate
C	carbon
cm^{-1}	reciprocal centimeter
DBE	starch debranching enzyme
DP	degree of polymerization
FTIR	Fourier transform infrared spectroscopy
g	gravity
GBSS	granule bound starch synthase
KBr	potassium bromide
kV	kilovolt
M	Molar

mg	milligram
ml	milliliter
mm	millimeter
NADPH	nicotinamide adenine dinucleotide phosphate
NaOH	Sodium hydroxide
nm	Nanometer
SBE	starch branching enzyme
SEM	Scanning electron microscope
SS	starch synthase
TEM	Transmission electron microscope
α	alpha
β	beta

CHAPTER 1

INTRODUCTION

1.1 General introduction

Sago palm (*Metroxylon* spp.) is one of the main starch-producing crops in the world. It is one of the few tropical crops which can tolerate wet growing conditions including peat swamps (Jong, 1995). Sago palm is also one of the oldest tropical plants exploited by man for its stem starch (Mathur *et al.*, 1998). There are several different species of Metroxylon such as *M. rumphii*, M. warburgii, *M. amicarum*, and *M. salomonense* (Flach, 1997). The true sago palm or scientifically called *Metroxylon sagu* naturally grow in Malaysia and this species covers a large area in the state of Sarawak (Karim *et al*., 2008). Since the 1900s, much study has been carried out on sago palm cultivation. The Sarawak state government was aware with the potential of sago palm. Therefore, the Land Custody and Development Authority was involved in rehabilitation of small farms holdings in Mukah division and developed three sago plantations namely Mukah Sago Plantation, Dalat Sago Plantation and Sebakong Sago Plantation (Karim *et al*., 2008). According to Karim *et al*. (2008), sago palm contains a large amount of starch in its trunk and its productivity was calculated to be four times that of paddy rice. In Southeast Asia especially, sago starch has been used in the cooking of various types of dishes such as jellies, puddings, soups, noodle, biscuit, sago pearls, and many more. The state of Sarawak is so far the main sago starch exporter and in 2011 with 51,000 metric tonne of sago starch was produced. It earned Sarawak USD24.5 million (RM91 million) (Agricultural Statistic of Sarawak, 2011). Most of the sago starch is exported to Peninsular Malaysia, Japan, Singapore,

Thailand, Taiwan, Vietnam, China and United States of America. In 2012, 56,000 hectares of land were planted with sago palms and about 47,000 hectares of smallholdings are in Mukah Division (Agricultural Statistic of Sarawak, 2011).

The two primary uses of sago palm are for the production of edible starch and durable leaf thatch. Several secondary uses have also been recorded, but these are not comparable in economic importance to the primary uses. *M. sagu* is a staple food crop in the Sepik and Gulf provinces of lowland Papua New Guinea, where most of the sago grows in wild, uncultivated stands. Among the Asmat of Papua New Guinea, felling of the palm and harvesting of the sago starch is accompanied by ritual. In house construction, the leaves of sago are used for roof thatch and wall siding, and the wood is also used for floorboards and rafters. In the Solomon Islands, the thatch is known to last five years or longer (Karim *et al*., 2008).

In Asia, rice has long been the staple food for most populations and it is the crop that is most cultivated and consumed. Similarly, cassava or tapioca is a staple food for nearly half of the poor people of the tropics, particularly in Africa and parts of Asia (Karim *et al*., 2008). As a result of their importance, the cultivation and processing of rice and cassava have been extensively studied, and many advances have been made. In contrast, sago starch has been somewhat neglected and relatively less attention has been devoted to the sago palm and its starch. Nevertheless, a number of studies have been published covering various aspects of sago starch such as molecular structure, physicochemical and functional properties, and chemical/physical modifications (Karim *et al*., 2008). In addition, a regular international symposium on sago starch has been organized to discuss the development and progress on sago palm and sago starch studies.

The decaying trunks of the sago palm are a source of sago palm beetle grubs, an excellent source of protein. According to McClatchey *et al*. (2006) in the other parts of the Pacific, *M. warburgii* and *M. amicarum*are are viewed as emergency food and are rarely or no longer eaten by people, although they are used for thatch and animal feed. Various parts of the plant are used for traditional medicine, toys, and other miscellaneous items. *M. sagu* is considerably more productive for starch production compared to other *Metroxylon* species (McClatchey *et al*., 2006).

Starch is the main stored polysaccharide of green plants. Starch is composed of two major polysaccharides which are the amylose and amylopectin. Starch is a remarkable substance in that it consists of simple polymers of glucose organized to form semi-crystalline, insoluble granules with an internal lamellar structure (Buléon *et al*., 1998b). This granular structure is relevant when considering the mechanism of starch degradation, as many glucan-metabolizing enzymes appear to be unable to act upon intact granules as a substrate. Most information on the structure of starch comes from studies of the starch-storing organs of crop species rather than leaves, but recent work has shown that leaf starch is similar in many respects to storage starches (Matheson, 1996; Zeeman *et al*., 2002). Amyloplast is the place where the starch is produced and then the starch is directed to the main storage areas which are the seeds, tuber and root. The starch that produced is very stable and acts as a supply of energy and carbon for the developing plant.

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 trunk consists of a central core of soft pale-pink pith that contains most of the starch stored by growing palm, protected by 2 cm thick fibrous bark. The sago starch accumulates in the pith core of the stem of the sago

palm. Trunk formation starts in the third and fourth 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 (Karim *et al*., 2008).

1.2 Problem statement

Sago palm (*M. sagu*) is one of the typical underutilized indigenous food crops with very little attention and research in the past. It can be grown in underutilized wetlands and peat swamps where other food crops do not fit to grow economically, and produce high yield of starch (150-400 kg of dry starch per plant). Thus, sago palm has a high potential to contribute to food security as an additional source of staple foods without (or less) competition on the use of arable lands with other food crops, as well as for other industrial use including bio-plastic and bioethanol production. The possibility to use sago starch in the production of food, polymer, pharmaceutical and textile industry will lead to the high demand of sago starch in the market (Awg-Adeni *et al*., 2010).

The major problem faced by the food manufacturing industry is the variation in quality of sago starch from batch to batch. The existing data are not sufficient to cater to the increasing demand for high quality sago starch from the industry. An understanding of starch granules properties of sago palm is required to effectively utilize the starch where data is still lacking for sago palm.

This study was done to characterize starch granules distribution from various tissues of the trunking sago palm and non-trunking sago palm using several methods. The tissues compared were leaves and trunk. By comparing trunking sago palm and non-trunking sago

palm, the differences in starch morphology and distribution from leaves and trunk of the sago palm could be determined. The starch granules from trunking and non-trunking sago palm from different areas and different time were also investigated. This was done to compare the starch content from different areas and time. The hypothesis for this study is the morphology of starch granule of trunking sago palm is different with non-trunking sago palm.

1.3 Objectives

This study on morphology and distribution of starch granules from trunking and non-trunking sago palm comprises of two main objectives, which are:

- To characterize the morphology and distribution of starch granule from trunk and leaves in trunking and non-trunking sago palm.
- To profile starch from trunking and non-trunking sago palm from selected areas and selected time.

CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomy and uses of sago palm

The word sago is originally Javanese, and means starch containing palm pith. In quite a number of languages, however, the word has become a common name for all sources of starch. In the region in which the Indonesian-Malay language is spoken, the word sago is used to mean the starch of any palm (Schuiling & Jong, 1996; Chong & James, 2015).

Sago palm belongs to the order *Arecales* Nakai, family *Palmae* Jussieu, subfamily *Calamoideae* Griffith, tribe *Calameae* Drude, subtribe *Metroxylinae* Blume and genus *Metroxylon* Rottboell (Uhl & Dransfield, 1987). *Metroxylon* has previously been classified in the subfamily *Lepidocaroideae* (Moore, 1973) but this name has been changed back to *Calamoideae* by Uhl and Dransfield (1987), in agreement with the original classification of Griffith (1844). Further to this, Rauwerdink (1986) also attempted to classify and distinguished the species of *Metroxylon* based on the fruit morphology and size among other considerations in species and subspecific classifications (Jong, 1995).

Starch is considered to be one of the most abundant plant products and a major source of energy in the human diet. The world production of starch is estimated to be around 27.5 million tons (Bujang, 2006). The world consumption of sago starch lies between 200,000 to 300,000 tons per annum and accounts for about 3% of the total world market which is dominated by corn, potato and tapioca starches.

In Malaysia, more than 90% of all sago-planting areas are found in the state of Sarawak in East Malaysia. The largest (75%) sago planting area is Mukah where over 50% of the sago starch is produced. The annual export of sago starch from Sarawak fluctuates between 30,000 to 50,000 tons between the years 1988-1990's, procuring incomes of between US$3.4 million to US$10.8 million. The downtrend of sago starch prices saw a decline to US$9.15 million in spite of increased production at 61,000 tons for the year 2000. About 100,000 tons of sago starch is used annually in Malaysia for various applications, mainly in the production of glucose (15,600t), MSG (15,000t) and noodle (13,200t) while other household uses account for 36,000t (Bujang, 2006).

2.2 Starch granule from the trunk and leaves

In plants, plentiful of carbohydrate storage are found in the form of starch. These are two types of starch that can be found in the plant. These two types starch are classified based on their functions which are the storage starch and transitory starch (Zeeman *et al*., 2002). Storage starch is placed in the amyloplast and amasses in the perennation and dispersal organs. The transitory starch is produced in the chloroplast during the photosynthesis process which occurs during the day and is degraded during the night. These storage starch granules differ to a great extend between species in terms of size and shape but are alike in terms of the structure and composition (Zeeman *et al*., 2002).

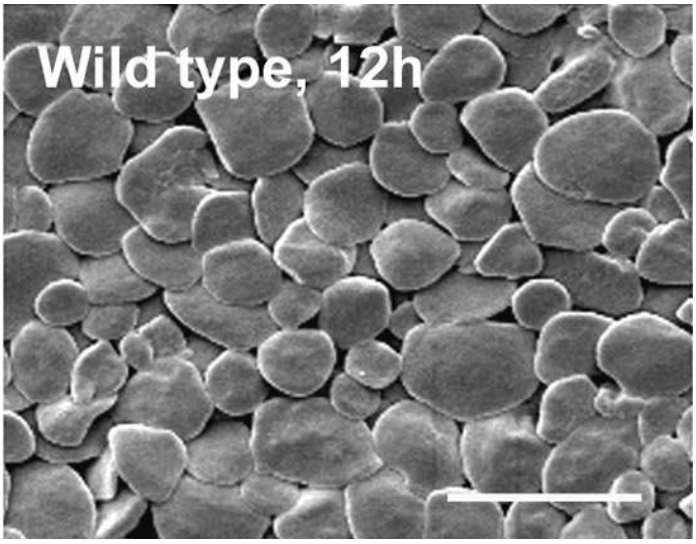


Figure 2.1: Scanning electron micrographs of starch granules isolated from Arabidopsis (Zeeman *et al.*, 2002)

The storage starch granules are composed by two different types of glucose polymers which are amylose and amylopectin. The starch storage granules are eventually semi-crystalline which consist of concentric ring of irregular crystalline and amorphous lamellae (Trethwey & Smith, 2000). In the transitory starch granule the proportion of amylose to amylopectin is lower than storage starch granule. The transitory starch amylopectin has an extremely robust polymodal distribution of chain length in extend of 5-40 glucose residues. This configuration was viewed in spite of the age and growth state of the plant (Trethwey & Smith, 2000). According to Grennan (2006), the transitory starch accumulates during the day and is degraded during the night into glucose and maltose. The degraded starch is then transported to the storage organs through the phloem. The storage starch is produced and stored in the storage organs.

2.3 Environmental preferences and tolerances of trunking and non-trunking sago palm

The suitable climate for *Metroxylon* species is the humid tropical rainforest of South East Asia and the equatorial Pacific. The relative humidity should be at least 70%, but the plant can tolerate lower humidity for short periods without damage, and incidental light should be above 800 k/cm^2 /day (Flach, 1997). *Metroxylon* species do not tolerate water shortage well. In rainfall-dependent sago palm growing localities, rainfall should be uniform and ample. Flooding for prolonged periods and stagnant water are detrimental to growth. As long as sufficient water is present, there does not seem to be an upper temperature limit for growth of sago (McClatchey *et al*., 2006).

Trunking sago palm is a normal sago palm with the trunk full with starch at the maturity stage. The formation of trunk is mainly associated with the depth of the peat. Normal and good conditions of trunking palms were all on shallow peat. Soil of trunking sago palms area were more humidified compare to those in the non-trunking sago palms area (Wan Sulaiman *et al*., 2004). The soil need to be extra moistened for the trunking sago palms compared to the non-trunking sago palms founded on the elevated mass compactness and better exhaustion of total nutrient contents (Wan Sulaiman *et al*., 2004).

The extra moistened soil around the trunking sago palms with elevated mass compactness enhances nutrient assimilation by roots owing to the improved soil-root bond. Most of the trunking sago palms were situated closer to the drain on moderately deep peat (Wan Sulaiman *et al*., 2004).

Figure 2.2: Seven year old trunking sago palm in Mukah plantation.

The non-trunking sago palm is abnormal sago palm where the growth and development is stunted. The non-trunking sago palms were all on deep peat (Wan Sulaiman *et al*., 2004). According to Wan Sulaiman *et al*., (2004), soils of non-trunking sago palms are were less humidified compare to those in the trunking sago palms area. The non-trunking sago palm has poor growth development, high mortality rate and poor ability to develop trunk (Wan Sulaiman *et al*., 2004).

Figure 2.3: Seventeen year old non-trunking sago palm in Mukah plantation.

Metroxylon species can grow on a wide variety of soils. They can persist on well drained, poor quality materials including sand or clay. The palms will grow in soil that is periodically inundated by salt water as long as fresh water flow is more prevalent (McClatchey *et al*., 2006). *Metroxylon* species grow best in soils with impeded drainage or with seasonal waterlogging. Waterlogging for long periods impedes growth and productivity (McClatchey *et al*., 2006).

2.4 Life cycle of sago palm

In Sarawak, *Metroxylon sagu* is the preferred sago palm to be planted by the local farmers as the smooth sheathed and thornless nature of the palm makes it easier to manage. The criteria by which sago palms are selected for harvesting are poorly documented. The starch reserves are apparently at their maximum just before flowering and fruiting deplete these reserves, but scientifically little more is known of the timing of starch build-up (Ruddle *et al*., 1978). 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 (Lim, 1991; Nabeya *et al.*, 2016).

For the model, a palm cultivar with relatively short growth duration, obtained through propagation, by means of suckers, has been chosen. Planting is rather uniform. In this model, the ideas of Corner (1966) concerning palm growth have been applied. Palm growth is strictly regular. There are as many unfolded leaves visible in the crown as there is developing, still folded, within the growing point. The newest leaf, the spear leaf, appears midway between the developing leaves in the growing point and those visible in the crown. Tomlinson (1990) doubts that this growth model is valid for all palms. It does apply to the sago palm in the bole-forming stage, although possibly not as strictly as Corner assumes. Only if the number of leaves increases or decreases is the number of developing leaves within the growing point larger or smaller than in the visible crown.

In plants with a terminal inflorescence, flowering starts after a fixed number of leaves has been formed. In the case of rice, banana and sisal, for example, this naturally only holds within certain limits. The sago palm is assumed to follow this flowering pattern, and the duration of the bole growth stage can thus be estimated by counting the number of leaf scars on the bole

at flower initiation. In our palm, grown under optimum ecological conditions, and with uniform planting material, there were 54 leaf scars. At a production rate of one leaf per month, this would correspond to a physiological age of 54 months. Deviations from this number in other palms might form an interesting research topic.

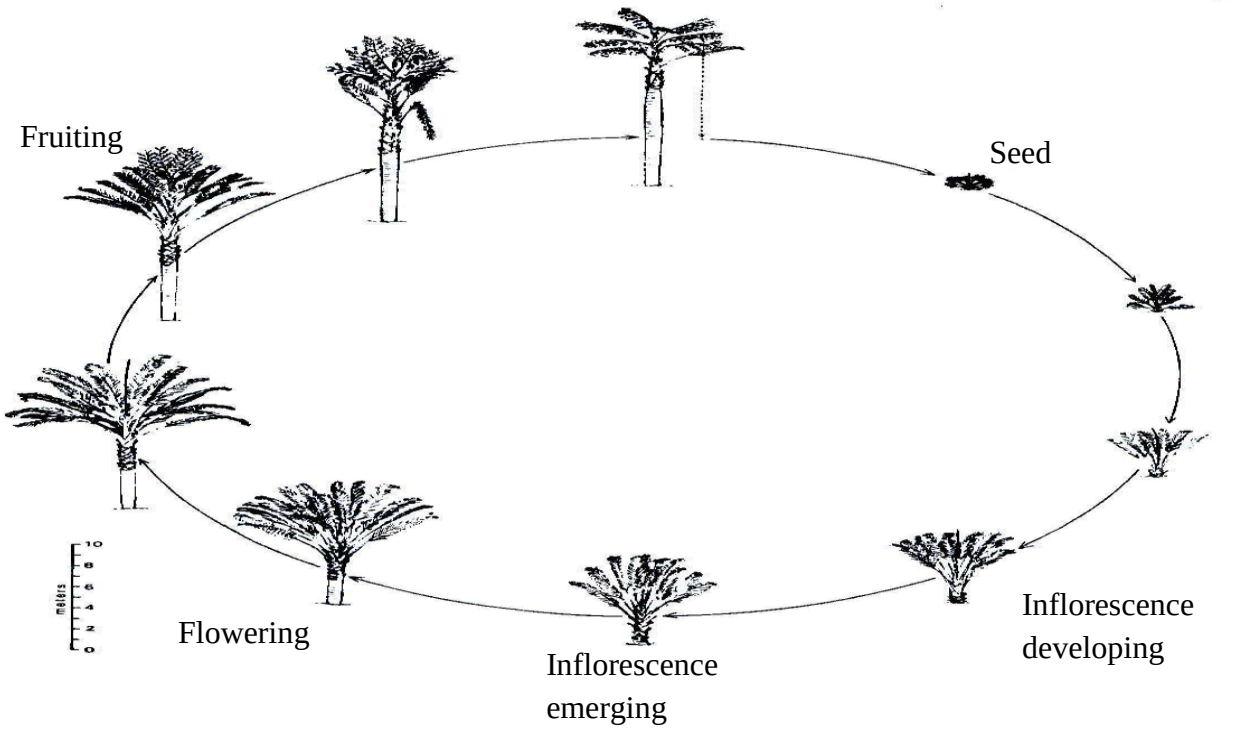


Figure 2.4: Life cycle of a sago palm (Flach & Schuiling, 1989).

In Sarawak, the local farmers have classified the mature sago palms into the following five stages (Table 2.1).

Table 2.1: Different physiological growth stages of sago palm (Lim, 1991).

Growth stage	**Palm description**
Plawei	Palms that have reached maximum vegetative growth
Plawei Manit	Inflorescence emerging
Bubul	Inflorescence developing
Angau Muda	Flowering
Angau Tua	Fruiting

Lim (1991) reported that the maximum starch yield per trunk is at the 'Angau Muda' stage (flowering stage) and declining at the 'Angau Tua' stage. No significant difference in starch yield among 'Plawei Manit', 'Bubul', and 'Angau Muda' stages was observed. Hence, Lim (1991) concluded that the earliest stage at which a palm can be felled for maximum yield would be at 'Plawei Manit' stage.

2.5 Starch in general

2.5.1 Production and utilization

Starch is abundant in all major agricultural crops. It is the major energy reserve for a large variety of higher plants such as cereals, legumes and roots and tubers (Buléon *et al*., 2007), over 90% of world starch production comes from cereals (Tetlow, 2006, Smith, 2015). Maize is by far the most important source of starch in the world contributing to over 80% of world starch production, while wheat (>8%), potato (>5%), and cassava (>5%) mainly share the rest of the production. Starches such as rice, barley, oats, sweet potato, sago, etc., also contribute to the world starch production in small quantities.

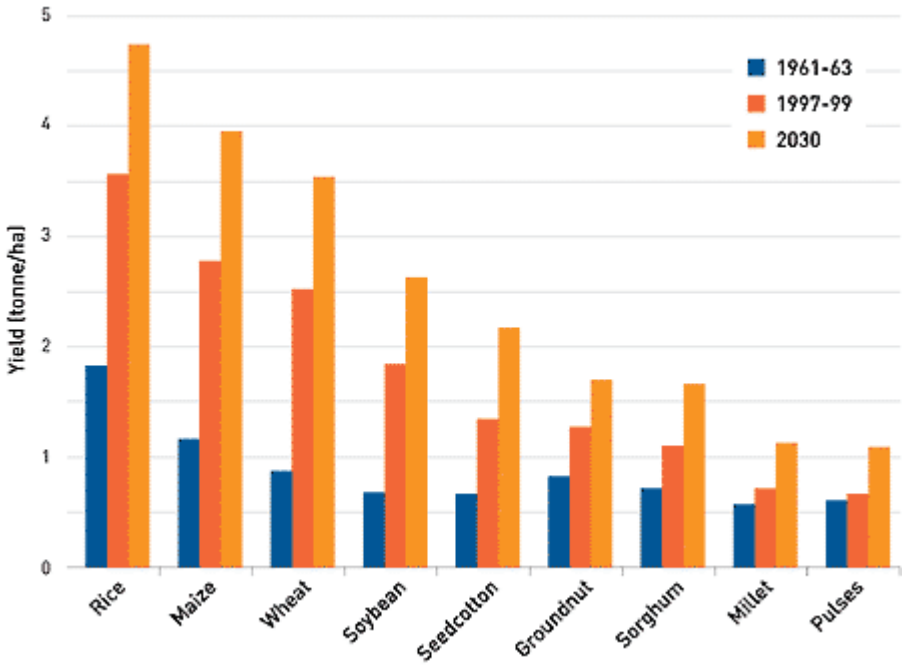


Figure 2.5: Crop yield in developing countries, 1961 to 2030 (FAO, 2002).

2.5.2 Starch granule biosynthesis

Storage starch is deposited in the endosperm (in amyloplasts) of the seed, while transient starch is deposited in leaves (in chloroplasts) (Tetlow *et al*., 2004). Starch is deposited as semi-crystalline granules and consists of two main glucan polymers named amylose and amylopectin. Amylose is essentially a linear polymer with α-1,4 branches between glucose units, amylopectin is highly branched polyglucan. In amylopectin molecules, linear chains connecting glucose monomers via α-1,4 branches, are interconnected via α-1,6 branches. Although the macromolecular components of starch granules contain only one type of sugar residue and two types of chemical branches, the biosynthesis of a starch granule is more complex than it appears (Bulèon *et al*., 1998a). The biosynthesis is coordinated through interactions of a group of biosynthetic enzymes as demonstrated in Figure 2.6 (Tetlow, 2011).

The first step in the biosynthesis of both transient and storage starch in plant tissues is the production of adenosine 5'-diphosphate glucose (ADPGlc), which is the soluble precursor and the substrate for the starch synthesizing enzymes called starch synthases (SSs), by the enzyme adenosine 5'-diphosphate glucose pyrophosphorylase (AGPase) (Jeon *et al*., 2010; Tetlow, 2006; Tetlow *et al*., 2004). AGPase catalyzes the rate limiting step in starch biosynthesis and exerts a high degree of control on the flux of carbon into this pathway (Buléon *et al*., 1998a; Smith *et al*., 1997; Tetlow, 2006). SSs generally function in the elongation of linear glucan chains by catalyzing the transfer of the glucosyl moiety of the soluble precursor ADPGlc to the non-reducing end of a pre-existing α-(1, 4)-linked glucan chain (Jeon *et al*., 2010; Tetlow, 2006, 2011). SSs are divided into two major classes, the first is granule bound starch synthases (GBSSs), which are involved in amylose biosynthesis (Leloir *et al*., 1961), and the second is

SSs that are involved in amylopectin biosynthesis (Tetlow, 2006, 2011). These SSs are also called soluble starch synthases (Cao *et al*., 1999).

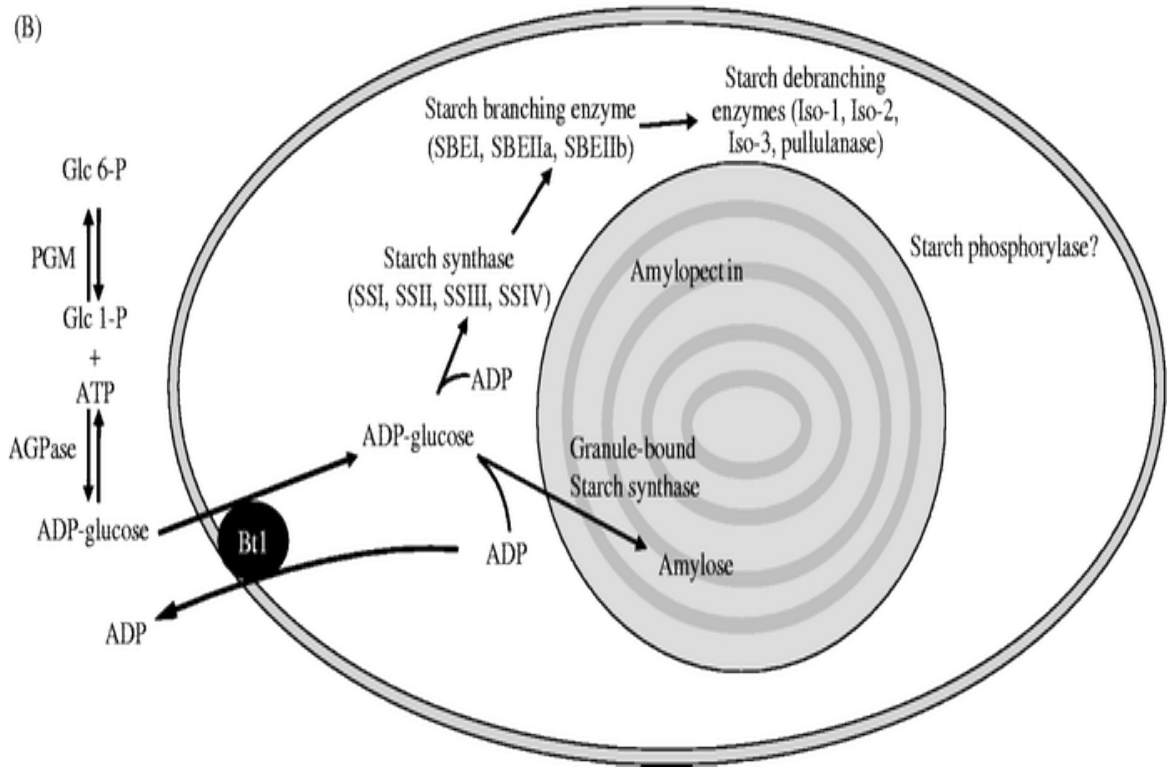


Figure 2.6: Starch granule biosynthesis pathway in the cereal endosperm (Tetlow, 2011).

There are two types of GBSS isoforms known as GBSSI and GBSSII. GBSSI catalyzes the elongation of amylose in storage starch (De Fekete *et al*., 1960; Nelson & Rines, 1962), while GBSSII is thought to be involved with the starch synthesis in leaves and other non-storage tissues (Nakamura, 2002). Mutants of rice (Sano, 1984), maize (Tsai, 1974), barley (Patron *et al*., 2002), and wheat (Fujita *et al*., 2001; Nakamura *et al*., 1995) produced by lacking GBSSI have demonstrated either low or zero amylose. GBSSI is found within the granule matrix and

therefore one of the so-called granule associated proteins. In addition to its role as a limiting enzyme in amylose production, GBSSI is involved with the extension of long amylopectin chains (Delrue *et al*., 1992; Maddelein *et al*., 1994).

The second group of SSs, which is the group of enzymes involved with amylopectin biosynthesis, is divided into four isoforms named SSI, SSII, SSIII, and SSIV (Tetlow *et al*., 2004). Studies with mutants lacking specific SS isoforms have shown that each of these isoforms as its own role in amylopectin biosynthesis. SSI is primarily responsible for the synthesis of the shortest glucan chains (degree of polymerization (DP) ≤ 10) (Commuri & Keeling, 2001) and their elongation is achieved by SSII and SSIII. Studies with mutants lacking SSI have shown that SSI elongate short (DP 4-7) glucan chains.

Short A and B1 chains are extended by SSI up to a critical length (Jeon *et al*., 2010). SSI is tightly bound to longer amylopectin chains, whereupon it becomes entrapped within longer glucans as a relatively inactive protein in the starch granule. Rice mutant lacking SSI has shown a deficiency in shorter glucan chains of DP 6-12 (Fujita *et al*., 2006). These authors further reported that the lack of SSI in cereal endosperms has no effect on either the size and shape of developing seeds and starch granules or the crystallinity of rice starches suggesting that other SS isoforms compensate at least partly the starch biosynthesis. In wheat endosperm, SSI is expressed early in development producing glucan chains with DP 5-10 (Peng *et al*., 2001).

There are two classes of SSII isoform designated SSIIa and SSIIb. Although the role of SSIIb is not known yet, SSIIa plays a specific role in the elongation of shorter glucan chains (DP≤10) to intermediate size with DP of 12-24 in cereals (Morell *et al*., 2003; Tetlow, 2006, 2011; Tetlow *et al*., 2004). SSIIa mostly predominates in cereal endosperm while SSIIb is

mostly restricted to photosynthetic tissues (Tetlow, 2011). The absence of SSIIa in wheat and barley results in elevated amylose, more short chains and less intermediate size chains in amylopectin chain length distribution, and reduced crystallinity and altered granule morphology (Morell *et al*., 2003; Zhang *et al*., 2004; Kosar-Hashemi *et al*., 2007). Both SSI and SSII are trapped within the starch granules in many cereals (Peng *et al*., 2001; Morell *et al*., 2003; Umemoto & Aoki, 2005).

The primary role of SSIII is amylopectin synthesis, although it possesses regulatory properties with respect to control over the starch biosynthetic pathway (Tetlow, 2011). The impact of loss of SSIII is also genetically controlled. The maize and rice mutants lack of SSIII produced granules with altered morphology, crystallinity and amylopectin with reduced long glucan chains of DP≥30 (Inouchi *et al*., 1983; Fujita *et al*., 2007) and approximately 15% starch as intermediate size highly-branched polyglucans (Gao *et al*., 1998; Jane *et al*., 1999). These observations suggest that SSIIIa functions in the provision of long chains, which extends between clusters of amylopectin (James *et al*., 2003; Jeon *et al*., 2010). Furthermore, an enhanced activity of SSI was found in SSIIIa mutants (Cao *et al*., 1999; Fujita *et al*., 2007) and GBSS was found in SSIII antisense potato tubers (Fulton *et al*., 2002) suggesting interactions among SS isoforms, which would partially compensate for non-functional or insufficient SS isoforms (Jeon *et al*., 2010). SSIV was found recently (Dian *et al*., 2005) and is active only in the presence of ADPGlc (Tetlow, 2011). The lack of SSIV isoform produced only one large granule in *Arabidopsis* chloroplast suggesting that it controls the number of granules within the plastid (Roldán *et al*., 2007). In addition, a pattern in the expression of SSs and GBSSs during the growth of the wheat grain is also found (Zhang *et al*., 2010). For instance, SSI was found to be generally expressed over the grain filling period, while SSII and SSIII were expressed over

the early and mid-grain filling stages and GBSSI was expressed during the mid to late-grain filling periods. Besides AGPase and SSs, starch branching enzymes (SBE) and starch debranching enzymes (DBE) are involved with the starch synthesis process. SBEs catalyze the formation of branch points by cleaving internal α-(1,4)-bonds in a glucan polymer and transferring the released segment to a C6-hydroxyl to form the branched structure of the amylopectin molecule (Jeon *et al*., 2010; Tetlow *et al*., 2004). There are two isoforms of SBE, namely SBEI and SBEII. These two classes of SBEs are different from each other in terms of the length of the glucan oligomer transferred and their substrate specificities; SBEIIs transfer shorter chains and show a higher affinity towards amylopectin, whereas SBEIs show higher rate of branching with amylose (Guan *et al*., 1997; Guan & Preiss, 1993; Takeda *et al*., 1993). Cereals contain two distinct SBEII isoforms called SBEIIa and SBEIIb (Han *et al*., 2007; Nakamura, 2002). While SBEIIa is ubiquitously present in cereal plant tissues, SBEIIb is specifically expressed in the endosperm (Ohdan et al., 2005; Yamanouchi & Nakamura, 1992). While SBEIIb is responsible for producing high-amylose starches with altered amylopectin structures (Nishi *et al*., 2001; Sawada *et al*., 2009), SBEIIa is assumed to play a role supporting other SBE isoforms (Blauth *et al*., 2001; Nakamura, 2002). Besides, a preferential association of some starch branching enzymes in large granules of wheat (Peng *et al*., 2000; Yin *et al*., 2012) and GBSSI in large granules of maize (Utrilla-Coello *et al*., 2010) is also reported.

Isoamylase and pullulanase are the two types of DBEs that are involved with starch biosynthesis. While isoamylase debranches phytoglycogen and amylopectin, pullulanase acts upon pullulan and amylopectin, but not phytoglycogen (Nakamura *et al*., 1996). Mutants produced with lacking isoamylase at different extents have given amylopectin structures with a wide variation, from amylopectin with more short chains to phytoglycogen with highly and randomly branched glucan polymers (Burton *et al*., 2002; Cao *et al*., 1999; James *et al*., 1995;

Kubo *et al*., 1999). Isoamylase is assumed to function in the editing of excessively branched chains or in removing improper branches of amylopectin formed by branching enzymes in order to maintain the cluster structure of amylopectin during starch biosynthesis (Jeon *et al*., 2010). Compared with isoamylase, the functionality of pullulanase is less established. Although, it is believed to function in starch degradation within the germinating grain, some activity has been detected during starch biosynthesis by compensating partially for the defect in isoamylase (Beatty *et al*., 1999; Dinges *et al*., 2003; Fujita *et al*., 2009; Li *et al*., 2009; Nakamura *et al*., 1996). In the endosperm, all these enzymes are present as heteromeric protein complexes and are involved with the starch granule biosynthesis (Tetlow, 2011).

Two models have been proposed to explain the role of debranching enzymes in starch biosynthesis. The glucan trimming model suggests that debranching enzymes work on irregularly branched pre-amylopectin in the plastid stroma and at the edges of the starch granule (Ball *et al*., 1996; Myers *et al*., 2000). As the pre-amylopectin increases in size, debranching enzymes cleave the widely spaced branches and generate a regularly branched glucan structure that is competent to crystallize. Debranching enzymes hydrolyze some of these α-1,6 branches, especially those that are widely spaced, to produce more regions of crystallization-competent glucan. This model proposes that the extensive branching of pre-amylopectin followed by trimming of the outer glucan chains to produce regions of glucan with the competence to crystallize may explain the regular distribution of α-1,6 branch clusters (the 9-nm repeat) in amylopectin.

An alternative model, the water-soluble polysaccharide-clearing model, was suggested by Zeeman *et al*. (1998b). This model proposes that the principal substrate for starch debranching enzymes during starch synthesis is branched, water-soluble glucan.

The principal difference between these two models is the nature of the glucan that is the primary target of debranching enzyme activity during starch synthesis, whether or not it goes on to form amylopectin and consequently whether debranching enzymes play a direct or an indirect role in amylopectin synthesis.

Starch-debranching enzymes in plants are of two types. The pullulanases which also have been referred to as R-enzymes or limit dextrinases hydrolyze α-1,6 branches in amylopectin and β-limit dextrin (glucans that are produced as a result of β-amylase activity during starch breakdown) but do not hydrolyze glycogen (Hussain *et al*., 2003). These enzymes show greatest activity on the yeast glucan pullulan, which has regularly spaced α-1,6 branches after every three α-1,4–linked glucose units. Isoamylase-type debranching enzymes are distinct from pullulanases in their substrate preferences. They are most active on amylopectin, but they also are active on glycogen and β-limit dextrin substrates. Isoamylases are inactive on pullulan. Although the two types of debranching enzyme have related primary amino acid sequences, pullulanases can be distinguished structurally from isoamylases by a number of distinct motifs (James *et al*., 1995; Beatty *et al*., 1999). Biochemical analysis of debranching enzymes in potato tubers shows that Stisa1 and Stisa2 interact in a multimeric enzyme (probably a hexamer), whereas Stisa3 is not associated with this complex (Hussain *et al*., 2003).

2.5.3 Starch degradation

Transitory starch in leaves is degraded primarily by hydrolysis of the constituent glucans to maltose and glucose, both of which can be exported from the chloroplast and metabolized in the cytosol (Figure 2.6) (Niittyla *et al*., 2006; Weber *et al*., 2000; Weise *et al*., 2004). Mutations affecting key degradative enzymes decrease starch breakdown, resulting in the accumulation of starch over repeated diurnal cycles (Caspar *et al*., 1991). There is good evidence that starch degradation is dependent on the reversible phosphorylation of glucans at the surface of the starch granule, which serves to solubilize the granule surface, thus allowing hydrolases access to the glucan chains.

Hydrolysis of the linear chains is catalyzed primarily by β-amylases (α-1,4-glucan maltohydrolase;EC: 3.2.1.2), exo-acting enzymes that release maltose from exposed non reducing ends of chains. β-amylases cannot hydrolyze α-1,6 branch points or act immediately adjacent to them. Thus, the complete degradation of amylopectin also requires hydrolysis of branch points by DBE activity. Higher plant genomes encode large numbers of β-amylase like proteins. *Arabidopsis*, for example, has nine β-amylase genes (*BAM1–9*). The β-amylase family can be divided into four distinct classes and higher plants contain one or more genes of each class (Fulton *et al*., 2008). The importance of chloroplastic β-amylase activity for transitory starch degradation was established by experiments with transgenic potatoes, in which repression of a chloroplastic isoform led to a *sex* phenotype (Scheidig *et al*., 2002). This finding has been confirmed and extended in *Arabidopsis* (Fulton *et al*., 2008; Kaplan & Guy, 2005). Four of the nine *Arabidopsis* BAM proteins (BAMs 1–4) have been shown to be chloroplastic (Fulton *et al*., 2008). BAM1 and BAM3 are active enzymes and partially redundant: *bam1* and *bam3* mutants have a wildtype and a mild *sex* phenotype, respectively, but the *bam1bam3* double

mutant has a strong *sex* phenotype (Fulton *et al*., 2008; Kaplan & Guy, 2005). No function has yet been ascribed to BAM2. The recombinant BAM2 protein has a very low specific activity compared with BAM1 and BAM3, and *bam2* mutants are indistinguishable from wild-type plants (Fulton *et al*., 2008).

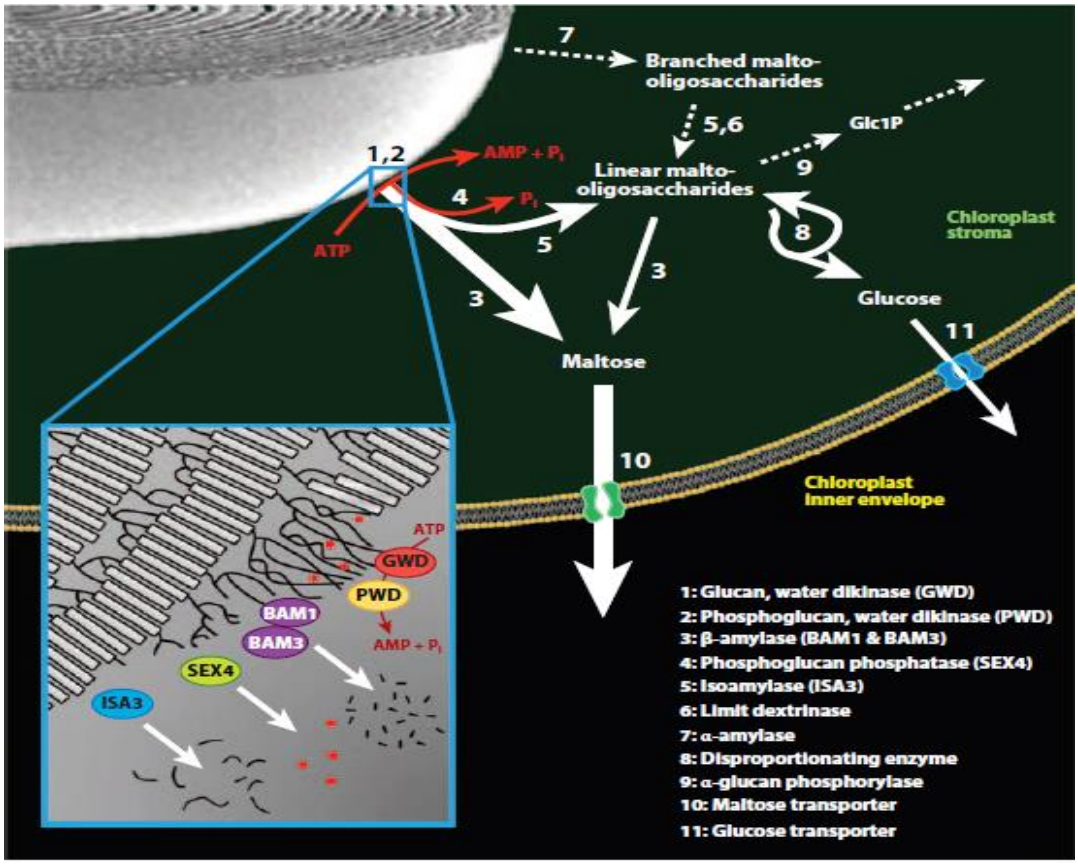


Figure 2.7: The pathway of starch degradation in chloroplasts and the role of transient glucan phosphorylation (Zeeman *et al.*, 2010).

BAM4 is unusual because its putative active site has multiple substitutions of amino acid residues that are strictly conserved in other, active β-amylases, and the recombinant protein does not have β-amylase activity (Fulton *et al*., 2008). Other members of this class of BAMs, including *Arabidopsis* BAM9 and proteins from other species have similar substitutions in their

putative active sites (Fulton *et al*., 2008), and recombinant BAM9 has no enzymatic activity. Although BAM4 is apparently non-catalytic, the *bam4* mutant has a *sex* phenotype, indicating that it is required for starch degradation. Genetic analysis shows that BAM4 does not act simply by modulating the activity of the active β-amylases, as its loss in the *bam1bam3* double mutant background enhances the *sex* phenotype (Fulton *et al*., 2008). One possible explanation for the requirement for BAM4 is that it senses maltose levels and transduces this information to modulate the starch degradation rate by interaction with other starch-degrading enzymes.

Several isoforms of BAM lack discernable plastid transit peptides. If they are indeed outside the plastid, they are unlikely to be involved in starch degradation. A high proportion of the β-amylase activity in *Arabidopsis* leaves is contributed by one such isoform, BAM5 (also called RAM1), which has been shown to be present within phloem sieve elements (Wang *et al*., 1995). Studies of the *bam5* mutant failed to discern a starch related phenotype (Laby *et al*., 2001) and its function remains unclear.

DBEs release short, linear malto-oligosaccharides into the plastid stroma (Delatte *et al*., 2005; Zeeman *et al*., 2007). Two DBEs (ISA3 and LDA) function in starch degradation in *Arabidopsis* leaves. Both enzymes preferentially remove short branches and have their highest activity on β-limit dextrins. They differ in the range of branch point configurations that they are able to degrade, as illustrated by the ability of LDA-type, but not ISA-type, to degrade the fungal glucan pullulan (Delatte *et al*., 2005; Hussain *et al*., 2003; Takashima *et al*., 2007; Wu *et al*., 2002). There is functional overlap between ISA3 and LDA. Loss of LDA alone has no effect on starch metabolism in *Arabidopsis* leaves, whereas loss of ISA3 leads to a *sex* phenotype (Delatte *et al*., 2005; Wattebled *et al*., 2005). Loss of both enzymes together (the *isa3lda* double mutant), however, results in a phenotype more severe than that of *isa3* (Delatte *et al*., 2005). The relative

importance of ISA3 and LDA in leaf starch degradation may differ between species. Unlike *Arabidopsis*, maize mutants lacking LDA alone (*Zmpu1* mutants) have reduced rates of starch degradation in leaves at night (Dinges *et al*., 2003).

The shortest malto-oligosaccharide on which β-amylase can act is maltotetraose. Maltotriose formed during the degradation of starch is metabolized by disproportionating enzyme (D-enzyme; α-1,4-glucan 4-α- glucanotransferase; EC: 2.4.1.25), which transfers a maltosyl group from maltotriose to another glucan, generating glucose and a longer glucan that can be further degraded by β-amylase. This activity is necessary for normal starch degradation in *Arabidopsis* leaves: In mutants lacking D-enzyme (*dpe1* mutants: 22), the rate of starch degradation is reduced and maltotriose accumulates in leaves at night. Two additional enzymes, plastidial α-amylase and plastidial α-glucan phosphorylase, are widely conserved in plants and may participate in starch degradation. In addition to β-amylase, research has also shown that in vitro degradation of starch particles in enhanced when acting together with glucan water dikinase (GWD) (Edner *et al*., 2007).

In *Arabidopsis*, mutations resulting in loss of activity (the *amy3* and *phs1* mutants, respectively) do not result in *sex* phenotypes under normal laboratory conditions (Yu *et al*., 2005; Zeeman *et al*., 2004). However, AMY3 does participate in starch degradation in *Arabidopsis* plants in which other starch-degrading enzymes are missing. For example, in mutants lacking the two DBEs that participate in starch degradation (the *isa3lda* double mutant), AMY3 is induced and is responsible for releasing branched malto-oligosaccharides from the starch granule surface (Delatte *et al*., 2005). The importance of chloroplastic α-amylase in leaf starch degradation appears to differ between species. Rice plants with reduced activity of chloroplastic α-amylase have a *sex* phenotype (Asatsuma *et al*., 2005).

2.5.4 Starch granule morphology

Depending on the biological origin, starch granules demonstrate different shapes, sizes, and morphology increasing its specificity in food applications (Jane *et al*., 1994; Stark & Lynn, 1992). Variously shaped granules such as lenticular/oval, round/spherical, polygonal, disk, truncated ellipsoid, and irregular are found in nature with size ranging from 1 to 100 μm in diameter (Bulèon *et al*., 1998a; Cisneros *et al*., 2009; Waduge *et al*., 2006; Watcharatewinkul *et al*., 2009). Granules from roots and tuber starches are relatively larger in size (2-100 μm) and have oval shape although some varieties with round, polygonal, and irregularly shape granules are found (Cisneros *et al*., 2009; Singh & Kaur, 2004; Velde *et al*., 2002; Watcharatewinkul *et al*., 2009).

The outer surface of starch granules plays an important role in many applications (Huber & BeMiller, 2001). Generally, root and tuber starches have a smooth surface (Jane *et al*. 1994). Jane *et al*. (1994) further observed indentations/cuts in many legume starches (lima bean, chick pea, lentil, mung bean, green pea), sharp edges in rice (wild type and waxy) and maize (normal) starches, pocks in wheat, oat, millet, and triticale starches, and rough surfaces in waxy maize starches. Equatorial grooves or furrows are present in large granules of barley and maize (Li *et al*., 2001, 2003; Waduge *et al*., 2006) and wheat (Thomas & Atwell, 1999; Kim & Huber, 2008) starches. In addition, surface pores in starches of maize (Li *et al*., 2001; Jayakody & Hoover, 2002; Velde *et al*., 2002; Sandu *et al*., 2004; Sujka & Jamroz, 2009), rice (Jayakody & Hoover, 2002), sorghum (Fannon *et al*., 1992; BeMiller, 1997), barley (Fannon *et al*., 1992; Li *et al*., 2001, 2004), triticale (Juszczak, 2003), millet (Fannon *et al*., 1992), large granules of wheat (Fannon *et al*., 1992; Juszczak *et al*., 2003b ; Glaring *et al*., 2006; Kim & Huber, 2008), and rye (Fannon *et al*., 1992) have been observed. More recent studies with atomic force microscope

(AFM) has allowed researchers to go into the nanometer scale and have a more closer look at the granule surface. Observations in these studies have demonstrated surface pores in potato (Juszczak *et al*., 2003a; Sujka & Jamroz, 2009) and tapioca starches (Juszczak *et al*., 2003a) in contrast to earlier reports (Fannon *et al*., 1992; Jane *et al*., 1994). Furthermore, studies with AFM have demonstrated that the shapes and sizes of these depressions depend on the starch origin (Juszczak *et al*., 2003a, 2003b).

The concept of the "hairy billiard ball structure" for the surface of starch granules was brought by Lineback in 1986. The surface of starch granules consists of ends of amylose and amylopectin chains which are protruded through the surface as hairs with different lengths (Lineback, 1986). This was proved by recent work with potato and corn (Park *et al*., 2011) and immature wheat (Waduge *et al*., 2010) starches.

2.5.5 Granule composition

The starch granule is mainly (98-99%) composed of amylose and amylopectin, as discussed above. Although, the ratio of amylose to amylopectin in a normal starch is about 1:3, the exact value depends on the botanical origin (Buléon *et al*., 1998a; Jane, 2009; Singh *et al*., 2003; Tester *et al*., 2004). However, breeding technologies have produced starches with low amylose (waxy; <5% amylose) and high amylose (>35% amylose) (Tester *et al*., 2004). There are two types of amylose in a starch granule distinguished as lipid-free amylose and lipid-complexed amylose (Morrison, 1988, 1995).

Apart from amylose and amylopectin, starch contains small quantities of surface and integrated proteins and lipids, as well as trace amount of minerals (Tester, 1997; Tester *et al*., 2004). Starch lipids are found both on the surface and inside granules (Morrison, 1988, 1995). It is likely that both surface and internal lipids may be present in the free state as well as bound to starch components, or linked via ionic or hydrogen bonding to hydroxyl groups of the starch components (Vasanthan & Hoover, 1992). Lipids in cereal starches are proportional to the amylose content and are fully complexed with a portion of amylose, but the relationship between starch lipids and amylose is quite different among starches (Morrison, 1988, 1995).

Nitrogen in starch is generally present as protein, but it may also be a part of starch lipids. Protein content in purified starch, less than 0.6% generally, is a good indicator for starch purity. In common with starch lipids, proteins occur both on the surface and interior of the granule (Li *et al*., 2003; Tester *et al*., 2004). Collectively, the proteins are referred to as starch granule associated proteins (Baldwin, 2001) and are mainly located in the granule interior as integral proteins (Li *et al*., 2003). The integral proteins may represent residual material from synthesis of the starch granule (Stark & Lynn, 1992; Tetlow, 2011). Both lipids and proteins

may influence starch physicochemical properties such as digestibility, swelling, solubilization, retrogradation, and granule integrity (Appelqvist & Debet, 1997; Han & Hamaker, 2002).

Starches also contain relatively small quantities (<0.4%) of minerals such as calcium, sodium, potassium, and phosphorous, which are, with the exception of phosphorous, of little functional significance (Tester *et al*., 2004). The degree of phosphorylation depends on the cultivar, growth conditions, temperature, fertilizer, and storage (Blennow *et al*., 2002; Hizukuri *et al*., 1970; Nielsen *et al*., 1994). Amylopectin from most plant sources contain small amounts of glucose moieties (0.1-1%) with phosphate groups (Blennow *et al*., 1998). Generally amylopectin from tuber and root starches have the highest degree of phosphorylation (Lim *et al*., 1994).

Some starches contain a third polysaccharide fraction, usually referred to as an intermediate fraction, which has more or less branched materials (Banks & Greenwood, 1975). In this fraction, the average chain length and the number of chains per molecule differ from those of amylose and amylopectin. Therefore, this intermediate fraction cannot be categorized either as amylose or amylopectin (Hizukuri, 1996). The presence of an intermediate fraction is reported in both normal (barley, oat, rye, and wheat) and high-amylose (maize, barley, rice, and pea) starches (Bertoft *et al*., 1993; Yamamori *et al*., 2000; Zhou *et al*., 1998).

CHAPTER 3

MATERIALS AND METHODS

3.1 Plant materials preparations

3.11 Sampling

The leaves and trunk of plant samples were collected from Dalat Sago Plantation and Sebakong Sago Plantation, Sarawak. Leaves from 7 year old trunking sago palm and 17 year old non-trunking sago palm were used for this study. The samples were selected from the third frond of the sago palm. The leaves were wiped with 70% ethanol and packed in a zip lock bag covered with ice. The samples for leaves were collected around 8.00 a.m. in the morning and 4.00 p.m. in the evening at different time. The samples were stored in a -80 °C freezer until used.

Figure 3.1: Sampling of leaves and trunk of sago palm.

3.1.2 Starch granule extraction from leaves and trunk

The method used by Vasanthan and Bhatty (1995) to extract starch was used and starch was extracted from the trunks and leaves from trunking and non-trunking sago palm with some modification. The leaves and trunks were homogenized with distilled water (500 ml) in a blender for 5 minutes. The slurry was passed through double muslin cloth (additional water was used to was the residue) and then through double nylon cloth with mesh size 20 μm. The filtrate was adjusted with 0.05 M NaOH, stirred for 30 minutes, and centrifuged at 5500 g for 15 minutes. The starch residue was washed twice and neutralized with 0.05 M NaOH and recovered by filtration. The filter cake was washed with distilled water, then washed with 80% ethanol and air-dried. The starch granule that extracted from the trunks and leaves from trunking and non-trunking sago palm were kept in powder stage.

3.1.3 Cracking of starch granules and digestion of starch granules

To crack starch granules, 1 g of dry starch was suspended in 1ml of water, frozen in liquid nitrogen and then ground in a mortar until the slurry began to thaw. The slurry was frozen and ground three additional times. Cracked starch granules were suspended in 100 μm MES-NaOH, pH 6.0, added with 100 to 200 units of α-amylase and incubated at 37 °C for 16 hours. The samples were then centrifuged at 10,000 *g* and the pellet was washed three times with acetone at -20 °C, dried, and stored at -20 °C.

3.2 Starch granules analysis

3.2.1 Scanning electron microscopy of starch granules

The starch pellet from starch granule extraction was sprinkled on the double-sided adhesive tapes mounted on circular aluminium stubs, coated with titanium using auto-finer coater JEOL 1600. The sample was coated with titanium until it was fully dried by observing the reading to be 0, which took about 10 minutes to coat the sample. The sample was examined in a JEOL 6390 Scanning Electron Microscope (SEM) at an accelerating voltage of 10 kV using a secondary electron detector.

3.2.2 Determination of growth ring distribution and ImageJ

Granules from each SEM sample, all cracked along their major axis, were analyzed from digital images obtained from the SEM. Growth ring distribution was measured at the proximal and distal ends of the granule. For both ends, the distance from the innermost visible ring to the periphery was measured perpendicular to the curve of the growth ring and was divided into three equal segments: center, intermediate, and periphery. The number of rings in each segment were counted and the average ring width (in micrometers) was calculated. ImageJ was used to measure the starch granule distribution. A line was drew over scale bar and the scale was set. The image was converted into grayscale and the threshold was adjusted. The analyze particles button was selected and a summary of the particle count was also shown in another data window.

3.2.3 Preparation of granules for transmission electron microscope (TEM)

The sample was fixed by immersing the sample in Karnovsky's solution for 30 minutes, and fixation was continued for an additional 2-5 hours. The sample was washed thrice with cold 0.1 M cacodylate buffer for 30 minutes each time, followed by with at least one overnight submersion. The sample was then post-fixed for 2 hours in 1.33% osmium tetroxide buffered with 0.1M cacodylate buffer. The sample was again immersed in buffer solution for three 15 minute washes with a final rinse lasting 12 hours. Dehydration of the sample followed a series of alcohol in 50% ethanol for 15 minutes, 70% ethanol for 15 minutes, 90% ethanol for 15 minutes, and 100% ethanol thrice for 20 minutes at room temperature.

In the infiltration stage, the general procedure was to expose the sample to one or more mixtures of propylene oxide and embedding medium. The first part involved preparation of the appropriate number of labeled molds dried in the oven. The second part involved preparation of the infiltration resin, which was a complete mix of LX-112 embedding medium. The third part was after the second 30 minutes rinse in propylene oxide, the following mixtures were prepared and used to infiltrate the sample. After preparing embedding mixture, molds were retrieved from the oven. Sample was placed in a small drop of resin mixed into the bottom of each mold. The molds were then filled to the top with resin. After all samples were embedded, the molds were cured in a 48 hours in a 60 °C oven.

After the molds were cured, the samples were rough trimmed. The resin block was placed in a vice and trimmed by shaving the resin blocks into a trapezoid having the dimensions not exceeding 0.5 mm across the base, 0.4 mm across the top, and 0.3 mm along the sides. Sectioning was accomplished with the use of a Nova Ultratome. Sections of the

trapezoids coming off the knife and were floated onto water in the boat of a glass knife. Once trapezoids of gold or silver were available, a chloroformed Q-tip was wafted above them to expand the sample, the sample was then collected on 300 mesh copper grids.

Grids were stained in two steps. After covering a petri-dish containing a few NaOH pellets and a small sheet of dental wax, several drops of uranyl acetate stain were placed on the wax. The grids, with the specimen side down, remained in uranyl acetate for 10 minutes and then were rinsed in a series of four beakers of pure water. After rinsing, the grids were then stained with lead citrate for 15 minutes, rinsed again in pure water and stored in a grid box. After drying, the grids were ready to be viewed.

3.2.4 Sample preparation for Fourier transform infrared spectroscopy (FTIR)

The starch powder sample and KBr were ground to reduce the particle size to less than 5 mm in diameter. Otherwise, large particles scatter the infrared beam and cause a slope baseline of spectrum. A spatula full of KBr was added into an agate mortar and was ground to fine powder until crystallites could no longer be seen and it became pasty and sticky. A small amount of powder sample (about 0.1-2% of the KBr amount) was taken and mixed with the KBr powder. The mixture was subsequently ground for 3-5 minutes. The die-set was assembled. When assembling the die, the powder was added to the 7mm collar. The die was put together with the powder into the Qwik Handi-Press. The powder was pressed for 2 minutes to form a pellet. A good KBr pellet was thin and transparent. The die set was disassembled and taken out of the 7 mm collar. The collar was put together with the pellet onto the sample holder. FTIR spectra

were recorded as KBr pellets on Perkin Elmer 1605 FTIR Spectrophotometer provided by Universiti Malaysia Sarawak (UNIMAS).

3.3 Statistical analysis using ANOVA

A single factor or one-way ANOVA was used to test the null hypothesis that the means of several populations are all equal. Statistical analysis was carried out by using one-way ANOVA analysis using the Microsoft Excel 2013 to compare means. Triplicate analysis were done for each sample while the significant difference was determined at $\alpha = 0.05$ (95 % confidence level). To perform a single factor ANOVA (analysis of variance) in Excel, the hypotheses were set.

$H_0: \mu_1 = \mu_2 = \mu_3$

H_1: at least one of the means is different

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Scanning electron microscope (SEM) analysis

The method that used from Vasanthan and Bhatty (1995) to extract starch was done and starch was extracted from trunking and non-trunking sago palm leaves and trunks. The starch granule was examined in a JEOL 6390 scanning electron microscope at an accelerating voltage of 10 kV using secondary electron detector. The SEM analysis was done to view the structure of starch granule of trunk and leaves. The starch granule was compared between trunk of trunking and non-trunking sago palm at Dalat. Also the starch granule for leaves of trunking and non-trunking sago palm at Dalat were also compared.

For the Dalat samples, the starch granules from leaves of trunking and non-trunking sago palm were compared morphologically between morning and evening period, while for Sebakong samples, only the starch granules from trunking sago palm were compared between morning and evening period.

The two kinds of sago starch granules from trunk of trunking and non-trunking sago palm showed bimodal size distributions (Figures 4.1 and 4.2 respectively). The large starch granules displayed an oval shaped and truncated with diameter of 20-30 μm while the isolated small starch granules displayed a spherical shape with diameter of about 1-10 μm. The scanning electron micrographs showed that sago starch had a greater proportion of small granules. The size of the large granules from the two sago starches was nearly the same.

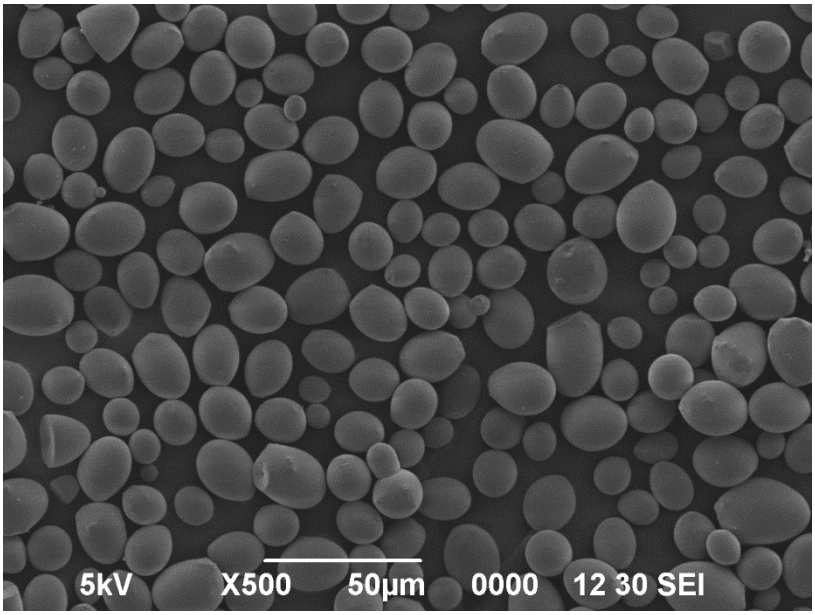


Figure 4.1: Scanning electron microscope image of starch from trunk of trunking sago palm from Dalat. The shape of granules were oval and truncated.

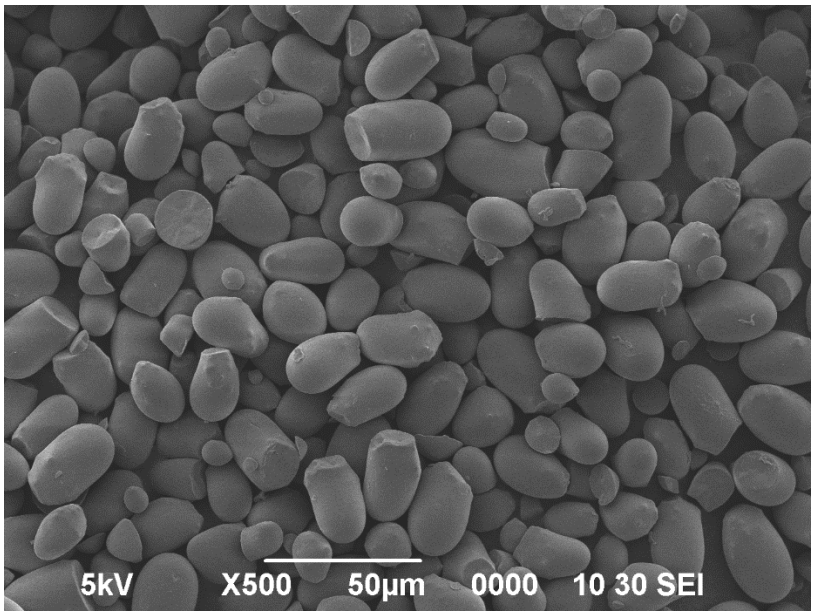


Figure 4.2: Scanning electron microscope image of starch from trunk of non-trunking sago palm from Dalat. The shape of granules were oval and truncated.

According to (Sim *et al*., 1991), the native sago starch granules are found to be oval to round shaped or polygonal-shaped with well-defined edges with a number of truncated oval granules. The granules surface is usually smooth but with pitting of granules on some sago starch granules. The shape of the sago starch granules were almost similar at all stages but differed in range of particle size distribution.

A starch granule from the trunk of trunking sago palm was observed in Figure 4.3. The shape of the extracted starch granule was oval and the size of the granule was 35 μm. The shape of the starch granule for trunking sago palm trunk was the same as non-trunking sago palm trunk in Figure 4.4, where the shape was oval and truncated. The round shape of the starch granule represents the shape of the starch granule when it was positioned horizontally.

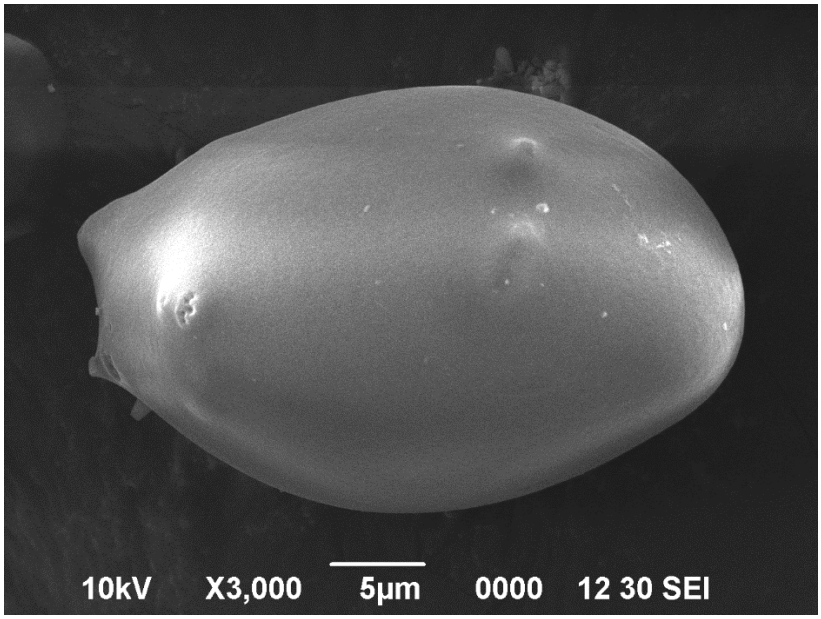


Figure 4.3: Starch granule from trunk of trunking sago palm. The size of starch granule was 35 μm. The shape of granules were oval and truncated.

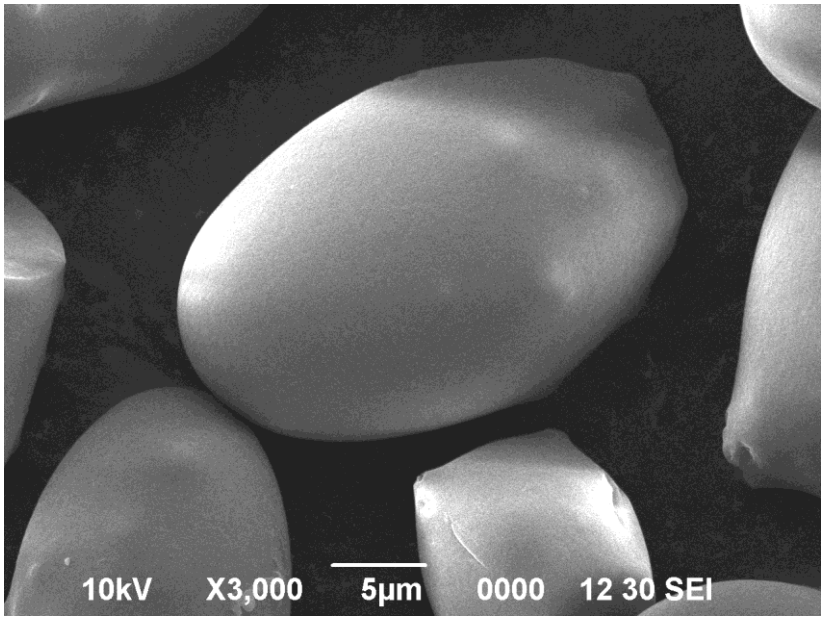


Figure 4.4: Starch granule from trunk of non-trunking sago palm. The size of starch granule was 35 µm. The shape of granules were oval and truncated.

There are no observable differences between the shape of the starch granules from the trunk of both trunking and non-trunking sago palm, which were oval and truncated. It might be due to the proteins involved in the starch synthesis which were present in the trunk of both the trunking and non-trunking sago were similar in terms of the type and amount of proteins. Starch-branching enzymes (SBEs) are one of the four major enzyme classes involved in starch biosynthesis in plants and algae, and their activities play a crucial role in determining the structure and physical properties of starch granules (Tetlow & Emes, 2014). The four major enzyme classes are starch synthase (SSs), starch branching enzyme (SBEs), starch debranching enzyme (DBE) and ADP glucose pyrophosphorylase (AGPase). However, so far there is still lack of evidence that supports the involvement of the protein and the area inside the amyloplast in determining the shape and size of the starch granules. The average size of starch granules from the trunk of trunking and non-trunking sago palm were around 20-40 µm.

Meanwhile, for trunking sago palm leaves, the shape of the starch granules was disc shaped and oval and the size was smaller than in the trunk. The size of starch granules ranged from 1 μm to 5 μm as seen in Figure 4.5. Nevertheless, the shape of the starch granules from the non-trunking sago palm leaves were disc shaped and oval as shown in Figure 4.7. This shows that the starch granules extracted from non-trunking sago palm leaves have round shape which range between 1 μm to 5 μm size.

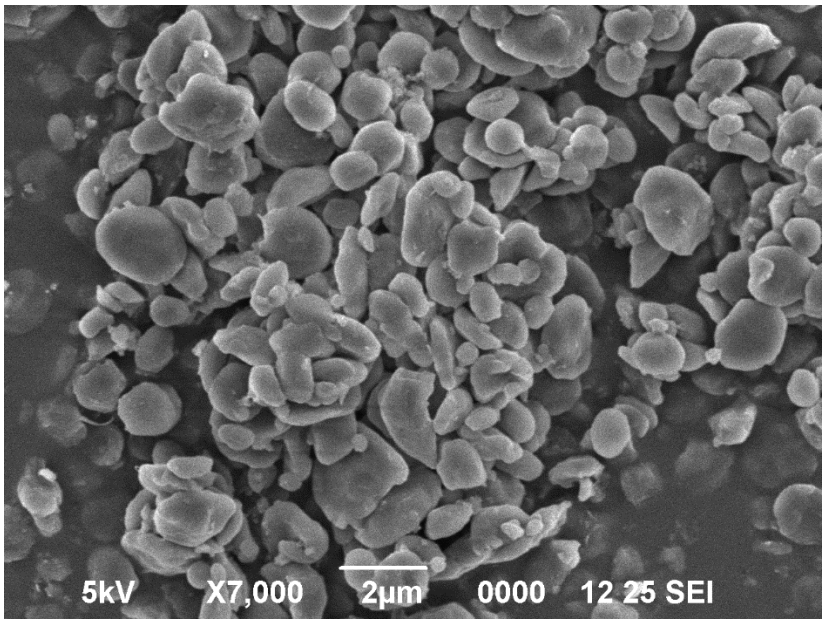


Figure 4.5: Starch granules from leaves of trunking sago palm (morning) at Dalat. The size of starch granules ranged from 2 μm.

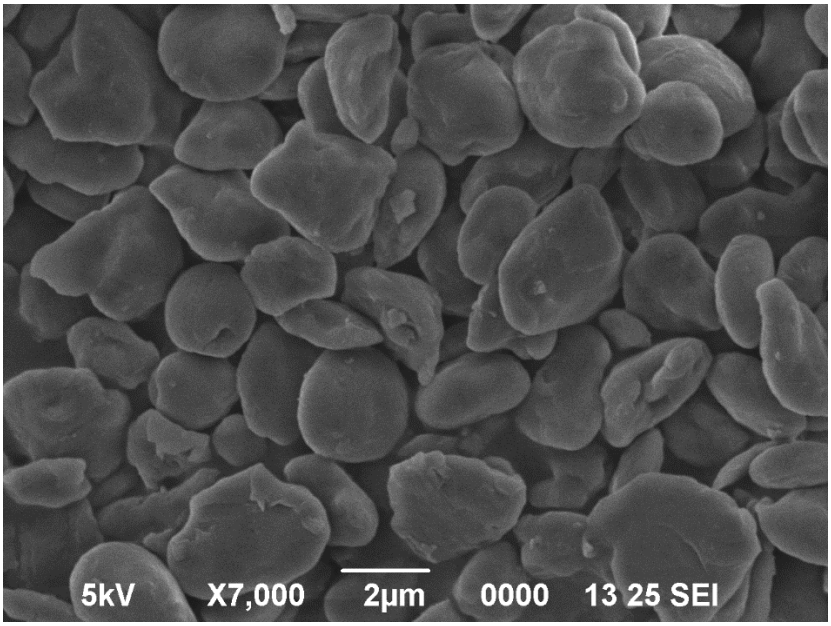


Figure 4.6: Starch granules from leaves of trunking sago palm (evening) at Dalat. The size of starch granules ranged from 3 µm.

The starch granules from leaves of trunking sago palm in the morning period (Figure 4.5) were smaller than in than evening period (Figure 4.6). The disc shape of the granules were much thinner and smaller in the morning compared to the evening period where the granules were thicker and larger in size. This was due to the starch granule biosynthesis and also starch degradation. Starch granule biosynthesis occurs in the morning while the starch degradation occurs at night (Tetlow, 2011). Hence, after the morning period, the starch granule biosynthesis has occurred resulting in the thicker and larger size granules. Meanwhile, after the night period, starch has been degraded and resulting in the thinner and smaller size granules.

In the light reactions, the energy-providing molecule ATP was synthesized using light energy absorbed by chlorophyll and accessory pigments such as carotenoids and phycobilins, and water was broken apart into oxygen and a hydrogen ion, with the electron of the hydrogen transferred to another energy molecule, NADPH. The ATP and NADPH molecules power the

second part of photosynthesis by the transfer of electrons. In these light independent or dark reactions, carbon was broken away from carbon dioxide and combined with hydrogen by the Calvin cycle to create carbohydrates. Some of the carbohydrates, the sugars, can then be transported around the organism for immediate use, the starches, can be stored for later use.

In general, starch metabolism is independent of chloroplast size for a given leaf age. The relationship among chloroplast volume, the volume of starch per chloroplast, and the size of starch granules at the end of the day was approximately the same for large and small chloroplasts (Crumpton-Taylor *et al.*, 2012).

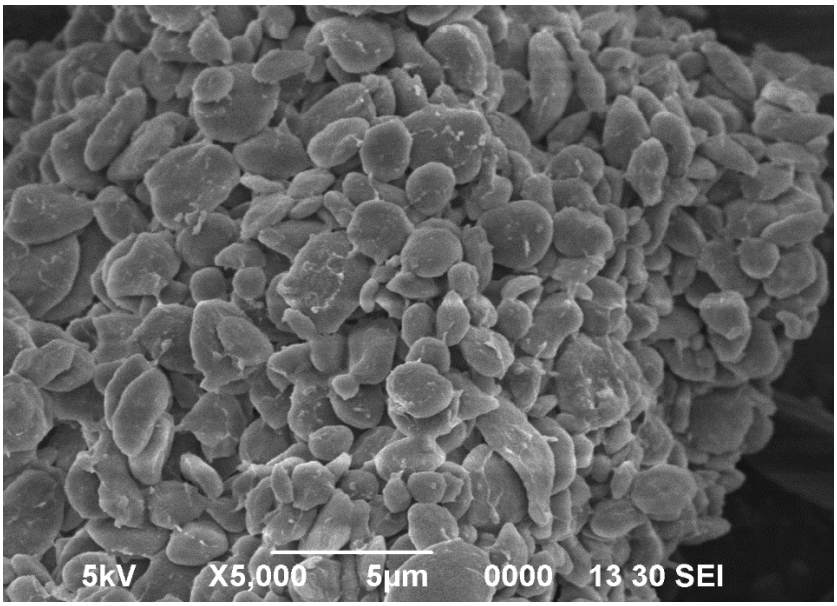


Figure 4.7: Starch granules from leaves of non-trunking sago palm (morning) at Dalat. The size of starch granules ranged from 1-5 µm.

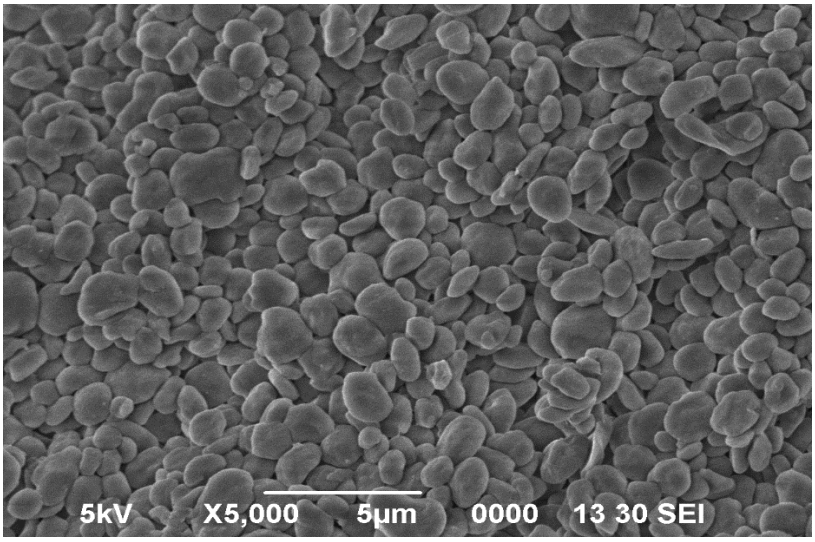


Figure 4.8: Starch granules from leaves of non-trunking sago palm (evening) at Dalat. The size of starch granules ranged from 1-5 µm.

Starch granules from leaves of non-trunking sago palm at Dalat for the morning period (Figure 4.7) were same in size as in non-trunking sago palm for the evening period (Figure 4.8). The shape of the starch granules was disc shaped and oval ranging from 1 µm to 5 µm. There was no difference between these starch granules. This shows that there is no changes in the starch granules even though starch granule biosynthesis phase was completed. This might due to the starch being transported to the trunk during night for other uses in the metabolic process. However, for non-trunking sago palm, it does not transfer and accumulates in the leaves. The starch stayed in leaves since the trunk for non-trunking is stunted.

Degradation of starch is best understood in leaves, where transitory starch is degraded at night, and in cereal endosperms where storage starch is broken down over several days after seed germination (Zeeman *et al.*, 2010). Transitory starch is accumulated during the day by leaf cell chloroplasts and broken down during the night. The structure of transitory starch differs

from that of the storage polysaccharide by a decrease in amylose content and a modification in amylopectin structure (Buleon *et al*., 1998a). The pathways in these leaves and endosperm share some components but also have major differences. The pathway in other starch-containing tissues such as tubers, roots, and non-cereal seeds is more fragmentary (Zeeman *et al.*, 2010).

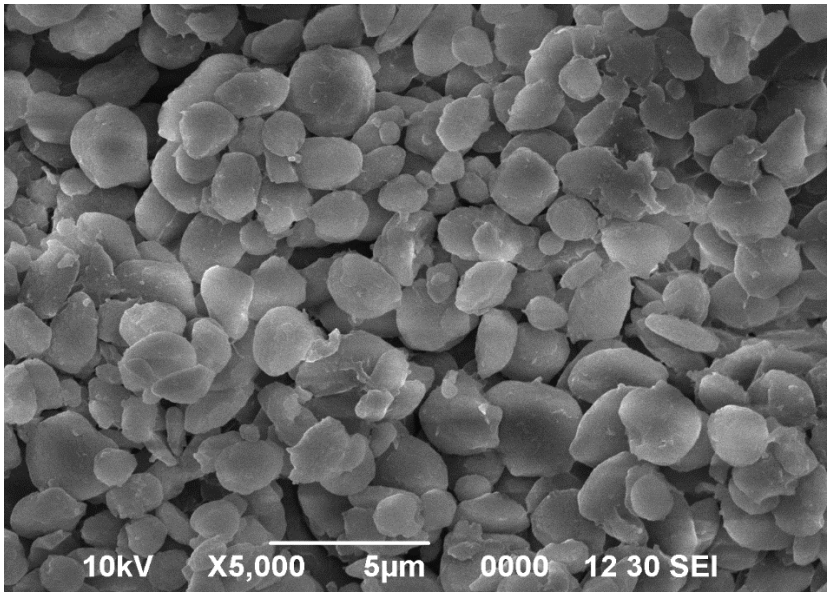


Figure 4.9: Starch granules from leaves of trunking sago palm (morning) at Sebakong. The size of starch granules ranged from 1-5 µm.

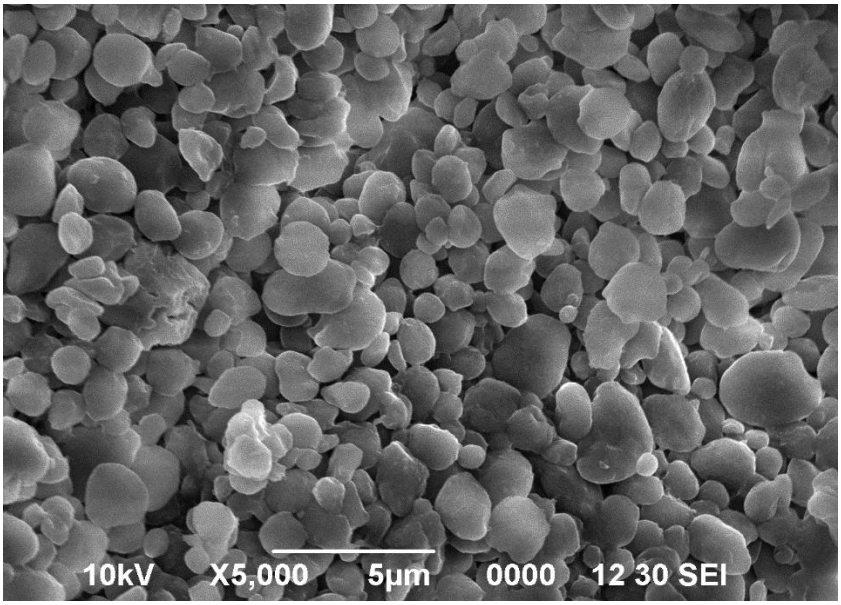


Figure 4.10: Starch granules from leaves of trunking sago palm (evening) at Sebakong. The size of starch granules ranged from 1-5 µm.

The starch granules from leaves of trunking sago palm at Sebakong for the morning period (Figure 4.9) were same as in trunking sago palm for evening period (Figure 4.10). The shape of the starch granules was disc shaped and oval ranging from 1 µm to 5 µm. There was no difference between these starch granules. The thickness of the starch granules and starch distribution also were the same for the morning and evening period. The result are the same as in the leaves of non-trunking at Dalat. The starch granules from leaves of trunking and non-trunking sago palm at Dalat and Sebakong have the same size and shape. In the morning, the size of starch granules were thinner than in the evening due to starch degradation in the evening for leaves of trunking sago palm at Dalat. However, the size of starch granules were the same during morning and evening for the leaves of non-trunking sago palm at Dalat and for the leaves of trunking sago palm at Sebakong.

Starch granules of wheat have a bimodal size distribution. The large granules are lenticular/disk shaped with an average diameter of 10-35 μm upon maturity, whereas the small granules are spherical/irregular in shape with an average diameter of 1-10 μm at maturity (Peng *et al*., 1999; Yoo & Jane, 2002; Kim & Huber, 2008). Similarly, sago starch granules showed bimodal size distribution with diameter of 20-30 μm for large granules, meanwhile the small granules with diameter of 1-10 μm. This shows that there were no difference with starch granules size in sago palm compared to wheat.

Based on measurements of leaf starch content and size of isolated granules, Howitt *et al*. (2006) reported that the distribution of starch granule sizes was the same at the end of the day and the end of the night. Thus, it can be concluded that some granules are quickly and completely degraded during the night, but others are not subject to degradation. Most granules underwent a large reduction in volume during the night, and there was relatively little loss of individual granules.

4.2 Starch granule distribution and statistical analysis (ANOVA)

The starch granule distribution was performed by using ImageJ to count the starch granule distribution in the trunk and leaves of trunking and non-trunking sago palm. By doing this, the distribution of starch granules and also the size of granules in the trunk and leaves of sago palm were compared.

For the trunk of trunking and non-trunking sago palm, the average size distribution of starch granule was around 20-40 µm.

Table 4.1: Size distribution of starch granules from the trunk of trunking and non-trunking sago palm.

Size Distribution of Starch Granule (µm)	**Number of Granules**		**F calculated**
	Trunking	**Non-trunking**	
1 – 10	39	67	1.3576
11 – 20	52	83	1.9592
21 – 30	87	69	0.4208
31 – 40	114	74	1.8038
41 – 50	47	46	0.0133

The analysis was done by using five pictures to collect the starch distribution among the group. A 95% confidence level was used because the interval that are 95% certain contains the true population value as it might be estimated from a much larger study. The size distribution of starch granule within 1-10 µm for trunking and non-trunking were 39 and 67. This shows a significant value but when doing the statistical analysis using one-way ANOVA (Appendix),

these values show no significance where F calculated < F table, 1.3576 < 5.3177 at 95% confidence level. For size distribution within 11-20 µm, this also shows significant value, 52 for trunking and 83 for non-trunking. However, when analysis was performed, the value shows no significance where F calculated < F table, 1.9592 < 5.3177 at 95% confidence level. For size distribution within 21-30 µm, the size distribution for trunking and non-trunking were 87 and 69. When analysis was performed, the value shows no significance where F calculated < F table, 0.4208 < 5.3177 at 95% confidence level. Meanwhile the size distribution within 31-40 µm, for trunking and non-trunking were 114 and 74. This shows the highest value of starch distribution in trunking sago palm. When analysis was performed, the value shows no significance where F calculated < F table, 1.8038 < 5.3177 at 95% confidence level. For the size distribution within 41-50 µm in trunking and non-trunking, the size were 47 in trunking and 46 in non-trunking. When analysis was performed, the value shows no significance where F calculated < F table, 0.0133 < 5.3177 at 95% confidence level. All of the size distribution within variable shows no significance thus can conclude that there is no significant between sizes of starch granule for trunking and non-trunking sago palm. Although the value seem differences between the trunking and non-trunking, but there is no differences in statistical analysis.

Nozaki *et al*. (2004) reported that starch concentration is related to the activities of starch synthetic enzymes in sago palm. Starch granules of wild-type plants were flat and discoid. Even when plants were transferred to continuous light to promote further starch synthesis, the granules increased in size but did not alter radically in appearance (Zeeman *et al*., 2002). The cause of the different granule morphology, and how it relates to the enzymatic deficiency is not yet clear (Zeeman *et al*., 2002).

For the leaves of trunking and non-trunking sago palm, the average size of a starch granule is around 1-10 µm. Mostly, starch granules for leaves were much smaller than in trunk. Starch granules were abundance in the smaller range size (Table 4.2).

Table 4.2: Size distribution of starch granules from the leaves of trunking and non-trunking sago palm.

Size Distribution of Starch Granule (µm)	**Number of Granules**		**F calculated**
	Trunking	**Non-trunking**	
1 – 10	83	127	4.774
11 – 20	65	71	0.1525
21 – 30	30	24	0.7423
31 – 40	17	12	0.7576

The size distribution of starch granules within 1-10 µm for trunking and non-trunking were 83 and 127. This shows the highest value of starch distribution in both trunking and non-trunking sago palm leaves. When doing the statistical analysis using one-way ANOVA (Appendix), these value shows no significance where F calculated < F table, 4.774 < 5.3177 at 95% confidence level. For size distribution within 11-20 µm, this also shows significance value, 65 for trunking and 71 for non-trunking. However, when analysis was performed, the value shows no significance where F calculated < F table, 0.1525 < 5.3177 at 95% confidence level. For size distribution within 21-30 µm, the size distribution for trunking and non-trunking were 30 and 24. When analysis was performed, the value shows no significance where F calculated < F table, 0.7423 < 5.3177 at 95% confidence level. Meanwhile the size distribution within 31-

40 µm, for trunking and non-trunking were 17 and 12. When analysis was performed, the value shows no significance where F calculated < F table, 0.7576 < 5.3177 at 95% confidence level.

In conclusion, despite the presence of starch degrading enzymes in chloroplasts, no degradation of starch was detected during periods of net starch synthesis. The starch granules themselves were found to contain varying amounts of amylose, depending on the conditions of synthesis, and exhibited very similar levels of structural organization to granules from non-photosynthetic tissues (Zeeman *et al.*, 2002).

4.3 Comparison of trunking and non-trunking sago palm by statistical analysis (ANOVA)

In statistics, analysis of variance (ANOVA) is a collection of statistical models, and their associated procedures, in which the observed variance in a particular variable is partitioned into components attributable to different sources of variation. In its simplest form, ANOVA provides a statistical test of whether or not the means of several groups are all equal, and therefore generalizes *t*-test to more than two groups. Doing multiple two-sample t-tests would result in an increased chance of committing a type I error. For this reason, ANOVAs are useful in comparing two, three, or more means.

For this work, analysis was done to determine whether there is difference or not in mean of starch content in trunking and non-trunking sago palm. For Table 4.4, the starch content in trunking and non-trunking sago palm was compared. For Table 4.6, the starch content in trunking and non-trunking sago palm in the morning period was compared. And last but not least, Table 4.8 compares the starch content in trunking and non-trunking sago palm in the evening period.

The ANOVA assesses whether the variance between the variable are significant or not. This test is suitable for different number of replicates. The calculation of ANOVA was shown in Table 4.4, Table 4.6 and Table 4.8.

Firstly, for comparison between trunk of the trunking and non-trunking sago palm, the assumption for null hypothesis was there is no difference between μ_1 and μ_2. For alternate hypothesis, there is difference between $\mu_1 = \mu_2$.

$H_0 : \mu_1 = \mu_2$

$H_1 : \mu_1 \neq \mu_2$

μ_1 = mean of expressed of the trunk of trunking sago palm

μ_2 = mean of expressed of the trunk of non-trunking sago palm

Table 4.3: Number of granules in the trunk of trunking and non-trunking sago palm.

	Trunking	**Non-trunking**
1st replicate	59	74
2nd replicate	106	53
3rd replicate	105	128

Table 4.4: ANOVA: Single factor analysis for number of granules in the trunk of trunking and non-trunking sago palm.

Groups	*Count*	*Sum*	*Average*	*Variance*
Trunking	3	270	90	721
Non-trunking	3	255	85	1497

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	37.5	1	37.5	0.033814	0.863048	7.708647
Within Groups	4436	4	1109			
Total	4473.5	5				

df= 5, p-value= 0.05

α= 0.863048

F table= 7.708647

F calculated= 0.033814

F calculated < F table : Reject H_1; Accept H_o

As a conclusion for comparison between trunk of the trunking and non-trunking sago palm, the alternate hypothesis was rejected and the null hypothesis was accepted. There were no differences or significance in mean of starch content in trunk of trunking sago palm and non-trunking sago palm. Thus, the starch granules distribution in trunk of trunking and non-trunking sago palm were not significantly different.

For comparison between leaves of the trunking and non-trunking sago palm morning period, the assumption for null hypothesis was there is no difference between μ_1 and μ_2. For alternate hypothesis, there is difference between $\mu_1 = \mu_2$.

$H_0 : \mu_1 = \mu_2$

$H_1 : \mu_1 \neq \mu_2$

μ_1 = mean of expressed of trunking sago palm in the morning

μ_2 = mean of expressed of non-trunking sago palm in the morning

Table 4.5: Number of granules in the leaves of trunking and non-trunking sago palm morning period.

	Trunking morning	**Non-trunking morning**
1st replicate	51	44
2nd replicate	43	64
3rd replicate	32	61

Table 4.6: ANOVA: Single factor analysis for number of granules in the leaves of trunking and non-trunking sago palm morning period.

Groups	*Count*	*Sum*	*Average*	*Variance*
Trunking morning	3	126	42	91
Non-trunking morning	3	169	56.33333	116.3333

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	308.1667	1	308.1667	2.972669	0.159774	7.708647
Within Groups	414.6667	4	103.6667			
Total	722.8333	5				

df= 5, p-value= 0.05

α= 0.159774

F table= 7.708647

F calculated= 2.972669

F calculated < F table : Reject H_1; Accept H_o

As a conclusion for comparison between leaves of the trunking and non-trunking sago palm morning period, the alternate hypothesis was rejected and the null hypothesis was accepted. There were no differences or significance in mean of starch content in the leaves of trunking and non-trunking sago palm for morning period. Thus, the starch granules distribution in the leaves of trunking and non-trunking sago palm for the morning period were not significantly different.

For comparison between leaves of the trunking and non-trunking sago palm evening period, the assumption for null hypothesis was there is no difference between μ_1 and μ_2. For alternate hypothesis, there is difference between $\mu_1 = \mu_2$.

$H_0 : \mu_1 = \mu_2$

$H_1 : \mu_1 \neq \mu_2$

μ_1 = mean of expressed of trunking sago palm in evening

μ_2 = mean of expressed of non-trunking sago palm in evening

Table 4.7: Number of granules in the leaves of trunking and non-trunking sago palm evening period.

	Trunking evening	**Non-trunking evening**
1st replicate	41	99
2nd replicate	29	88
3rd replicate	51	179

Table 4.8: ANOVA: Single factor analysis for number of granules in the leaves of trunking and non-trunking sago palm evening period.

Groups	*Count*	*Sum*	*Average*	*Variance*
Trunking evening	3	121	40.33333	121.3333
Non-trunking evening	3	366	122	2467

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	10004.17	1	10004.17	7.7302	0.049802	7.708647
Within Groups	5176.667	4	1294.167			
Total	15180.83	5				

df= 5, p-value= 0.05

α= 0.049802

F table= 7.708647

F calculated= 7.7302

F calculated > F table : Reject H_0; Accept H_1

As a conclusion for comparison between leaves of the trunking and non-trunking sago palm evening period, the alternate hypothesis was rejected and the null hypothesis was accepted. There were differences or significance in mean of starch content in the leaves of trunking and non-trunking sago palm for the evening period. Thus, the starch granules distribution in leaves of trunking and non-trunking sago palm for evening period was significantly different.

4.4 Composition and structure *M. sagu* starch granules

To reveal growth rings by using the method from Pilling and Smith (2003), granules were subjected to mechanical damage at low temperature and then to enzymatic digestion with α-amylase. Rings were examined by SEM on the inner surfaces of granules cracked along the major axis. Composition and structure of starch granule were viewed under scanning electron microscope followed by transmission electron microscope. By doing this, the structure of the amorphous and semi-crystalline lamella could be viewed.

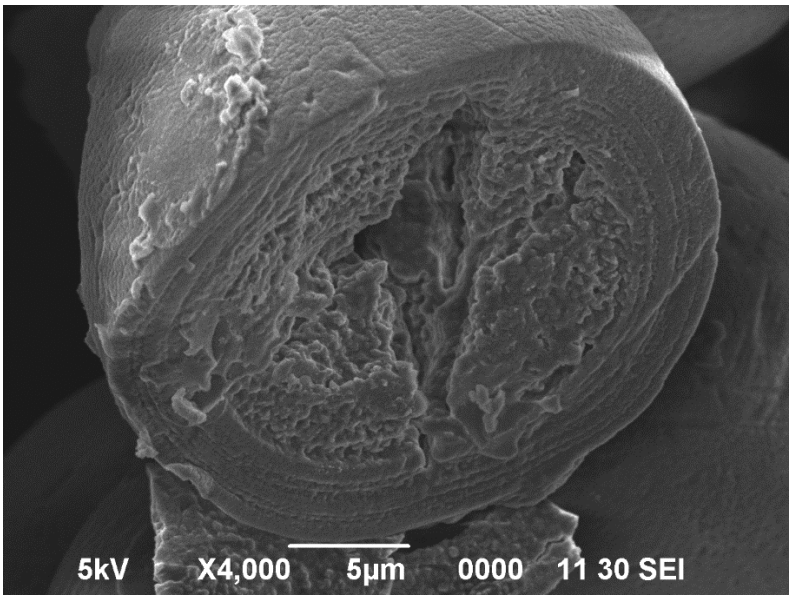


Figure 4.11: Growth ring in trunk of trunking sago starch granule. The starch granule was treated with α-amylase.

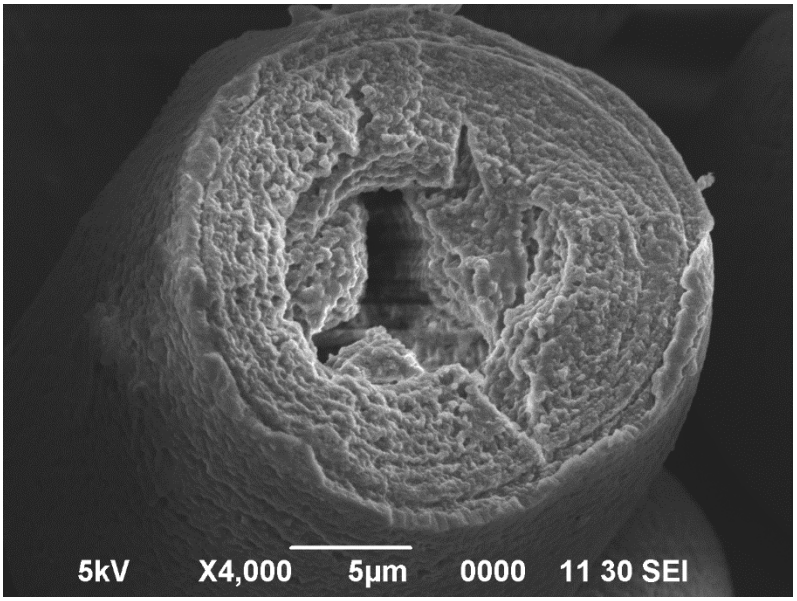


Figure 4.12: Growth rings in trunk of non-trunking sago starch granule. The starch granule was treated with α-amylase.

Growth rings were present in all of the starch granules examined (Figure 4.11 and Figure 4.12). The widths of rings in the trunk of trunking and non-trunking sago palm were also very similar. The diurnal rhythm is most likely to influence growth ring formation. It is affected by the supply of sucrose from the leaves to the tubers. The rate of supply of sucrose is higher during the day than during the night (Pilling & Smith, 2003).

At the lowest level of structure, most starch granules are made up of alternating amorphous and semi-crystalline shells which are between 100 and 400 nm thick (French, 1984). Figure 4.11 and Figure 4.12 shows the growth rings in trunk of trunking and non-trunking sago starch granules where the alternating crystalline and amorphous lamellae were observed with a thickness of 100-400 nm.

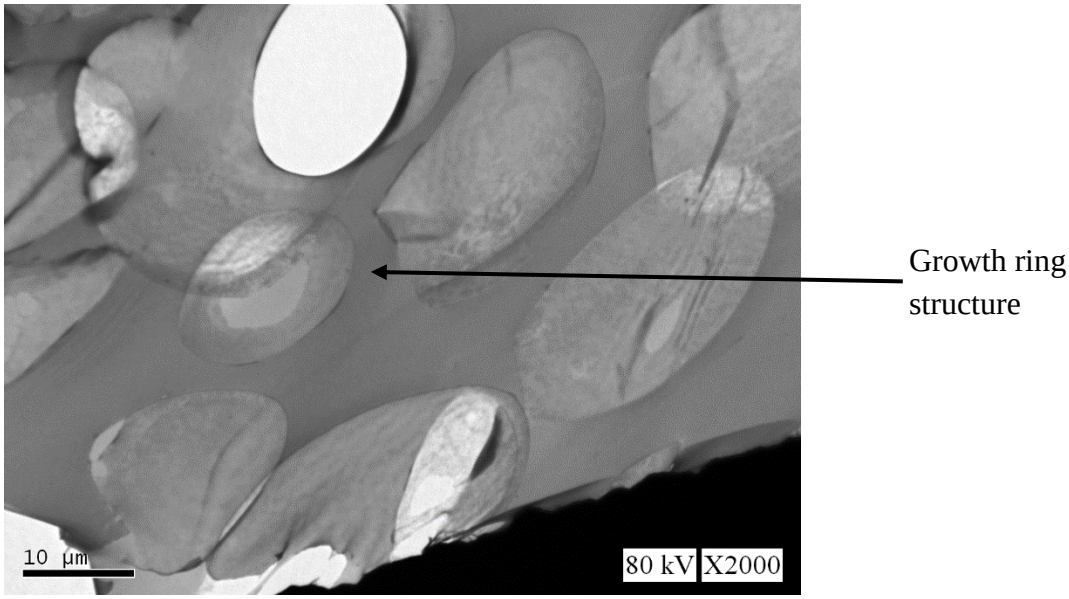


Figure 4.13: Growth rings in trunk of trunking sago palm starch granule in TEM. The starch granule was treated with α-amylase.

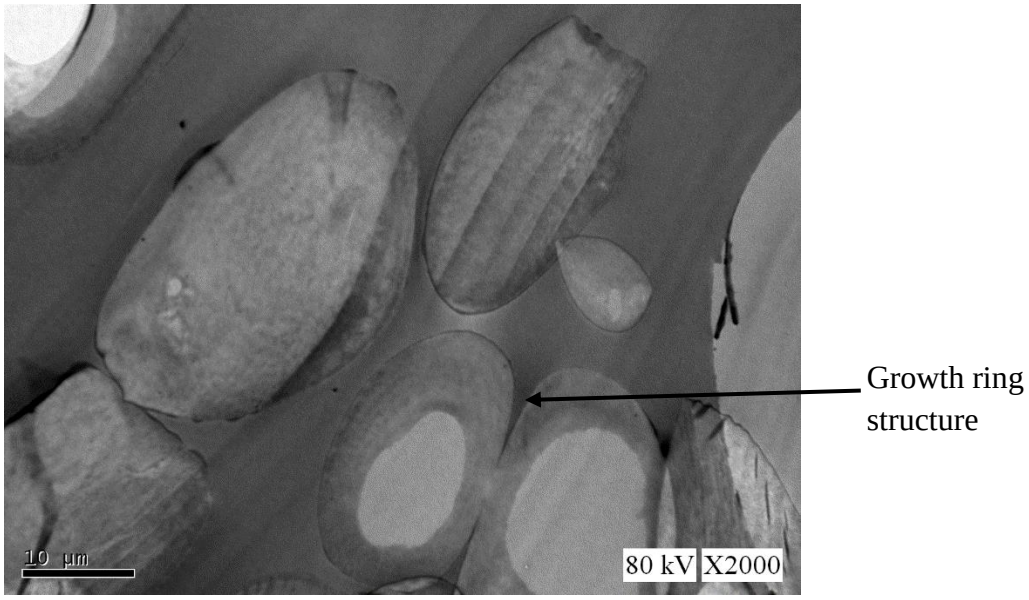


Figure 4.14: Growth rings in trunk of non-trunking sago palm starch granule in TEM. The starch granule was treated with α-amylase.

Figure 4.13 and Figure 4.14 show the growth rings in trunk of trunking and non-trunking sago palm starch granule in TEM. The alternating amorphous and semi-crystalline lamella can be seen clearly under TEM cross section (Figure 4.15).

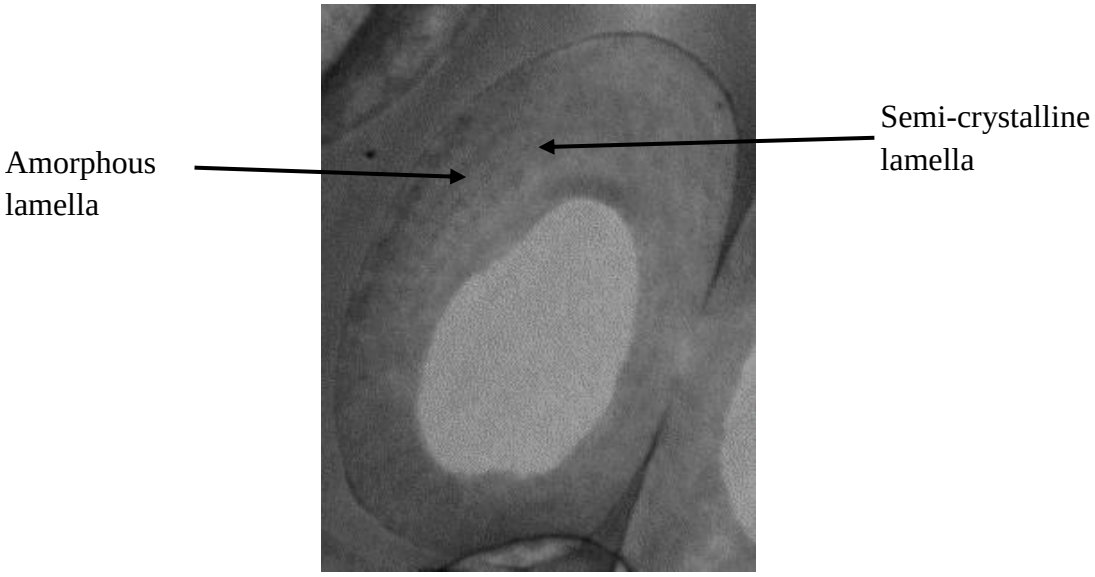


Figure 4.15: Growth rings in trunk of sago palm starch granule in TEM.

Even though some detailed information regarding the starch polymer structures has been simulated at the atomic level by computer modelling, the granule structure at the level of the amorphous and crystalline domains is less well understood (Figure 4.16). Such knowledge is important for an understanding of the physical properties of starch (Perez & Bertoft, 2010).

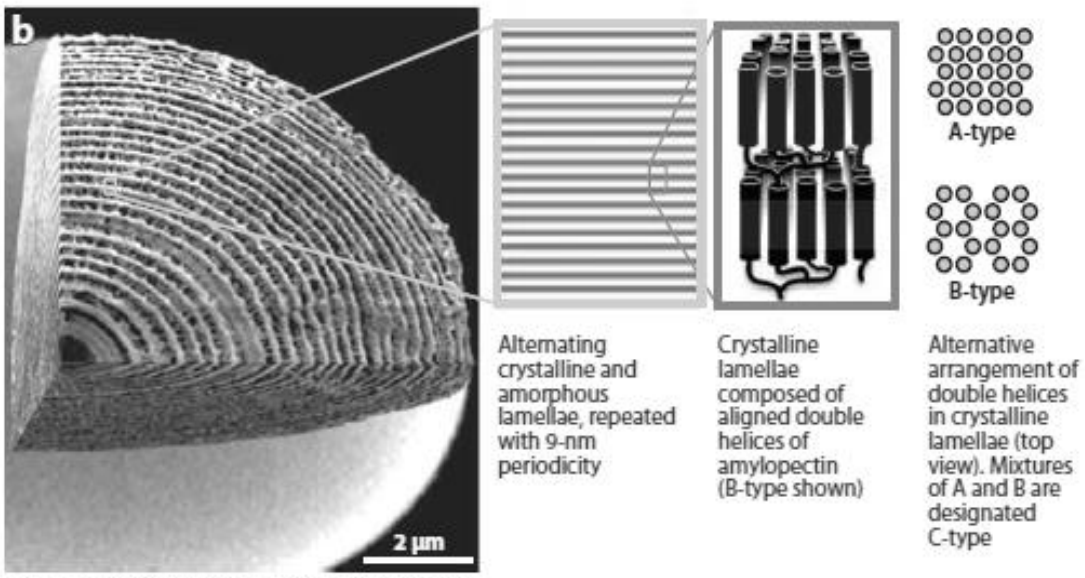


Figure 4.16: The composition and structure of starch granules (Zeeman *et al.*, 2010).

Haska and Ohta (1992) found that sago starch is resistant to enzymes compared to cereal starches. It has been reported that the raw sago starch degradation pattern is mainly surface erosion and crevassing (Wang *et al*., 1995). A deep hole or pore develops on the granule surface during enzyme hydrolysis that treated with α-amylase. Pores with sufficient size for enzymes to gain entry into the granule interior will thereby increase the rate of amylolysis reaction. Combination of α-amylase and glucoamylase could more effectively hydrolyze the raw sago starch (Monma *et al*., 1989).

Starch granules from higher plants contain alternative zones of semi-crystalline and amorphous material known as growth rings (Jenkins *et al*., 1993), which represents the periodic growth of starch granules (Buttrose, 1960; French, 1984) and have been observed under the light

microscope (Ridout *et al*., 2003) and scanning and transmission electron microscopes (SEM & TEM) (Pilling & Smith, 2003). The number and size of growth rings are influenced by the genotype of the starch granule (Li *et al*., 2003). The growth rings become closer together towards the periphery of the granule and with increasing amylose content (Li *et al*., 2003). The amorphous growth ring is largely amorphous, contains more water, and is at least as thick as the semi-crystalline growth ring (Cameron & Donald, 1992). As a conclusion, the possibility of diurnal rhythms could contribute to the control of growth ring formation in sago starch granules.

4.5 Fourier transform infrared spectroscopy (FTIR) analysis

FTIR spectroscopy was used to verify the changes in the chemical structure of starch molecules. However, in this study FTIR analysis was done to identify the chemical structure occurred in extracted starch granule from trunking and non-trunking sago palm from Dalat and trunking sago palm from Dalat and Sebakong. The absorption band between these sago palm were significantly the same.

Figure 4.17 shows the infrared spectra of trunking and non-trunking sago palm starch granules at Dalat while Figure 4.18 shows infrared spectra of trunking sago palm starch granules at Dalat and Sebakong. The FTIR spectra of the samples are in the 4000-500 cm^{-1} region. The IR spectrum of starch samples was described by seven main modes, with maximum absorbance peaks near 3500, 3000, 1600, 1400, 1000, 800 and 500 cm^{-1} (Koksel *et al.*, 2008; Sitohy *et al.*, 2000). The absorption bands in starch have been assigned and matched with the vibrational modes of the functional groups and the structures of starch molecules by many researchers.

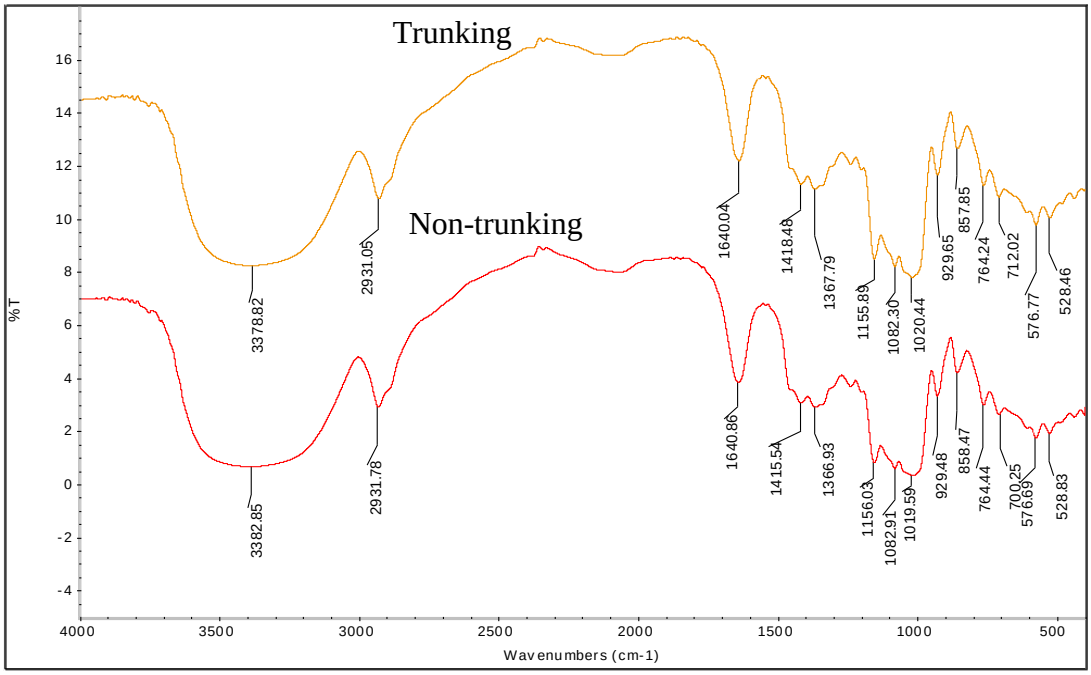


Figure 4.17: Infrared spectra of trunking and non-trunking of sago starch granules at Dalat.

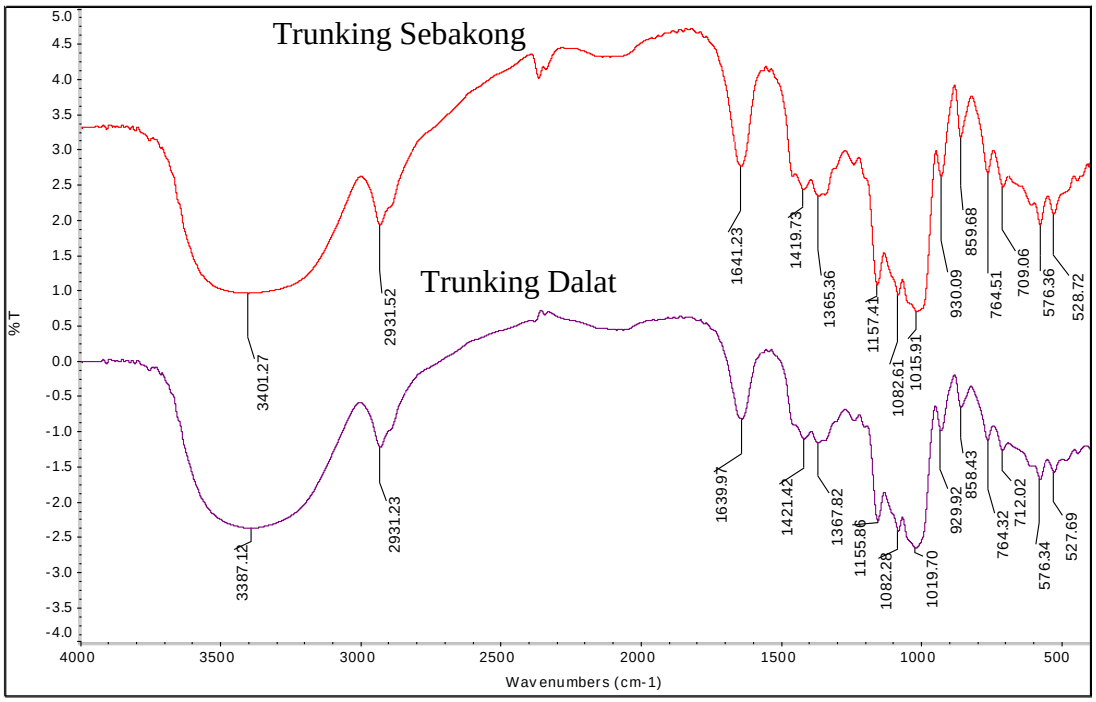


Figure 4.18: Infrared spectra of trunking of sago starch granules at Dalat and Sebakong.

The broad band in the 3200-3600 cm^{-1} region is corresponded to O-H stretching which is H-bonded group of alcohol of phenols and the band at 2850-2980 cm^{-1} is characteristic of C-H stretching vibration. The band at 1620-1580 cm^{-1} is corresponded to varying intensity of C=C stretch in aromatic compounds. The band at 1290-1180 cm^{-1} consists of antisym corresponded to C-O-C stretch ester and C-C-C stretch. The 1275-1200 cm^{-1} band is corresponded to the C-O-C stretch by cyclic ethers, and the 1250-1025 cm^{-1} band is corresponded to C-H in plane bending that have five bands in phenyl group. The band at 1050-1150 cm^{-1} represented C-O stretching vibration in the chemical structure of water-soluble polysaccharide. The bands at 1020-1050 cm^{-1} were associated with the ordered and amorphous structures of starch, respectively. Lastly, the 1600-1670 cm^{-1} bands were assigned for H_2O bending vibrations.

There were no differences between trunking and non-trunking sago palm functional group. As the predominant component of these granule are amylose and amylopectin and other major constituents are also polysaccharides, the spectra of the samples are superficially similar between the trunking and non-trunking sago palm from Dalat and trunking sago palm from Dalat and Sebakong.

CHAPTER 5

CONCLUSION

This research is mainly focused on identification the distribution of starch granules from trunk and leaves in trunking and non-trunking sago palm and to profile starch from trunking and non-trunking sago palm from Dalat area in Sarawak.

The shape of the starch granules for trunking sago palm trunk was the same as non-trunking sago palm trunk, where the shape was oval and truncated. The average size of starch granules from trunk of both trunking and non-trunking sago palm are around 20-40 μm. There were no difference in size and shape between the starch from the trunk of trunking and non-trunking sago palm. The starch granules extracted from trunking and non-trunking sago palm leaves have disc shaped and oval, where the size of granules range between 1-5 μm. The starch granules from leaves of trunking sago palm in the morning period at Dalat were smaller than in the evening period. The disc shape of the granules were much thinner and smaller in the morning compared to the evening period. Starch granules from leaves of non-trunking sago palm at Dalat for the morning period were the same as in non-trunking sago palm for the evening period. The starch granules from leaves of trunking sago palm at Sebakong for the morning period were the same as in trunking sago palm for the evening period.

The comparison of the trunk of trunking and non-trunking sago palm by statistical analysis (ANOVA) show that there were no differences of significance in mean of starch content in the trunk of trunking and non-trunking sago palm. There were also no differences in mean of starch content in leaves of trunking and non-trunking sago palm for the morning period. The

starch granules distribution in leaves of trunking and non-trunking sago palm for the evening period were significantly different.

The growth rings formation in the trunk of trunking and non-trunking sago palm were the same; they were made up of alternating amorphous and semi-crystalline layers. The width of rings in the trunk of trunking and non-trunking sago palm were also very similar.

FTIR analysis showed there were no differences between trunking and non-trunking sago palm functional group. The spectra of the samples are superficially similar between the trunking and non-trunking sago palm from Dalat and trunking sago palm from Dalat and Sebakong.

Although physical characteristics of trunking and non-trunking palm such as height and diameter show differences between them, analysis of the starch granules showed more similarity between them. Based on this work, most likely starch biosynthesis is not the key determining factor for the growth of palm. The result from study shows that harvesting should be done in evening. The trunk give good quality of sago starch compared to leaves. Only trunking sago palm contribute to the starch industry compared to non-trunking sago palm. Researchers and scientists will get the information from this finding and contribute to the sago starch industry. Further study using different approach can be applied to determine the pathway affected in the synthesis of starch and formation of non-trunking phenomena.

REFFERENCES

Agricultural Statistic of Sarawak. (2011). Sarawak exports of sago starch, Department of Agriculture Sarawak.

Appelqvist, I.A.M. & Debet, M.R.M. (1997). Starch-biopolymer interactions: A review. *Food Reviews International, 13,* 163-224.

Asatsuma, S., Sawada, C., Itoh, K., Okito, M., Kitajima, A., & Mitsui, T. (2005). Involvement of α-amylase I-1 in starch degradation in rice chloroplasts. *Plant Cell Physiology, 46,* 858–869.

Awg-Adeni, D.S., Abd-Aziz, S., Bujang, K., & Hassan, M.A. (2010). Bioconversion of sago residue into value added products. *African Journal of Biotechnology, 9,* 2016-2021.

Baldwin, P.M. (2001). Starch granule-associated proteins and polypeptides: A review. *Starch/Stärke, 53*, 475-503.

Ball, S., Guan, H-P., James, M., Myers, A., Keeling, P., Mouille, G., Buléon, A., Colonna, P., & Preiss J. (1998). From glycogen to amylopectin: A model for the biogenesis of the plant starch granule. *Cell, 86*:349-52.

Banks, W. & Greenwood, C.T. (1975). Intermediate material. In W. Banks & C. T. Greenwood (Eds.), Starch and its components (pp. 51-66). Edinburgh: Edinburgh University Press.

Beatty, M.K., Rahman, A., Cao, H., Woodman, W., Lee, M., Myers, A.M., & James, M.G. (1999). Purification and molecular genetic characterization of ZPU1, a pullulanase-type starch-debranching enzyme from maize. *Plant Physiology, 119,* 255-266.

Bertoft, E., Qin, Z., & Manelius, R. (1993). Studies on the structure of pea starches. Part 4: Intermediate material of wrinkled pea starch. *Starch/Stärke, 45,* 420-425.

Blauth, S.L., Yao, Y., Klucinec, J.D., Shannon, J.C., Thompson, D.B., & Guilitinan, M.J. (2001). Identification of Mutator insertional mutants of starch-branching enzyme 2a in corn. *Plant Physiology, 125*, 1396-1405.

Blazek, J., Salman, H., Rubio, A.L., Gilbert, E., Hanley, T., & Copeland, L. (2009). Structural characterization of wheat starch granules differing in amylose content and functional characteristics. *Carbohydrate Polymers*, *75*, 705-711.

Blennow, A., Bay-Smidt, A.M., Wishmann, B., Olsen, C. E., & Møller, B.L. (1998). The degree of starch phosphorylation is related to the chain length distribution of the neutral and the phosphorylated chains of amylopectin. *Carbohydrate Research, 307,* 45-54.

Blennow, A., Engelsen, S.B., Nielson, T.H., Baunsgaard, L., & Mikkelsen, R. (2002). Starch phosphorylation: a new front line in starch research. *Trends in Plant Science, 7*, 445-449.

Bujang, K.B. (2006). *Potentials of Bioenergy from the Sago Industries in Malaysia*. EOLSS - Encyclopedia of Life Support Systems. UNESCO - IOBB, Brisbane, Australia.

Buléon, A., Colonna, P., Planchot, V., & Ball, S. (1998a). Starch granules: structure and biosynthesis. *International Journal of Biological Macromolecules, 23*, 85-112.

Buléon, A., Gérard, C., Riekel, C., Vuong, R., & Chanzy, H. (1998b). Details of the crystalline ultrastructure of C-starch granules revealed by synchrotron microfocus mapping. *Macromolecules, 31*, 6605-6610.

Buleon, A., Véronése, G., and Putaux, J.L. (2007). Self-association and crystallization of amylose. *Australian Journal of Chemistry, 60*, 706-718.

Burton, R.A., Jenner, H., Carrangis, L., Fahy, B., Fincher, G.B., Hylton, C., Laurie, D.A., Parker, M., Waite, D., van Wegen, S., Verhoeven, T., & Denyer, K. (2002). Starch granule initiation and growth are altered in barley mutants that lack isoamylase activity. *The Plant Journal, 31*, 97-112.

Buttrose, M.S. (1960). Submicroscopic development and structure of starch granules in cereal endosperms. *Journal of Ultrastructure Research, 4*, 231-257.

Cao, H., Imparl-Radosevich, J., Guan, H., Keeling, P.L., James, M.G., & Myers, A.M. (1999). Identification of the soluble starch synthase activities of maize endosperm, *Plant Physiology, 120*, 205-216.

Cameron, R.E., & Donald, A.M. (1992). A small-angle X-ray scattering study of the annealing and gelatinization of starch. *Polymer, 33*, 2628-2635.

Caspar, T., Lin, T-P., Kakefuda, G., Benbow, L., Preiss, J., & Somerville, C. (1991). Mutants of *Arabidopsis* with altered regulation of starch degradation. *Plant Physiol*, *95*, 1181-1188.

Cecil, J.E. (1986). Increasing profits from sago processing. *The development of the sago palm and its products.* Report of the FAO/BPPT consultation, Jakarta, Indonesia, 16-21 January 1984. FAO, Rome. pp. 112-120.

Chong, S., & James, T.C. (2015). The sago terminology among Melanau of Sarawak. *Mediterranean Journal of Social Science, 6*, 136-144.

Cisneros, F.H., Zevillanos, R., & Cisneros-Zevallos, L. (2009). Characterization of Starch from two Ecotypes of Andean Achira Roots (*Canna edulis*). *Journal of Agriculture and Food Chemistry, 57*, 7363-7368.

Commuri, P.D. & Keeling, P.L. (2001). Chain-length specificities of maize starch synthase I enzyme: studies of glucan affinity and catalytic properties. *The Plant Journal, 25*, 475-486.

Corner, E.J.H. (1966). *The Natural History of Palms*. London: Weidenfeld & Nicolson.

Crumpton-Taylor, M., Grandison, S., Png, K.M.Y., Bushby, A.J., & Smith, A.M. (2012). Control of starch granule numbers in *Arabidopsis* chloroplasts. *Plant Physiology, 158*, 905-916.

De Fekete, M.A.R., Leloir, L.F. & Cardini, C.E. (1960). Mechanism of starch biosynthesis. *Nature, 187*, 918-919.

Delatte, T., Trevisan, M., Parker, M.L., & Zeeman, S.C. (2005). *Arabidopsis* mutants *Atisa1* and *Atisa2* have identical phenotypes and lack the same multimeric isoamylase, which influences the branch point distribution of amylopectin during starch synthesis. *Plant Journal, 41*, 815-830.

Delrue, B., Fontaine, T., Routier, F., Decq, A.,Wieruszeski, J.M. & Ball, S. (1992). Waxy Chlamydomonas reinhardtii: monocellular algalmutants defective in amylose biosynthesis and granule-bound starch synthase activity accumulate a structurally modified amylopectin. *Journal of Bacteriology, 174*, 3612-3620.

Dian, W., Jiang, H., & Wu, P. (2005). Evolution and expression analysis of starch synthase III and IV in rice. *Journal of Experimental Botany, 56*, 623-632.

Dinges, J.R., Colleoni, C., James, M.G., & Myers, A.M. (2003). Mutational analysis of the pullulanase-type debranching enzyme of maize indicates multiple functions in starch metabolism. *The Plant Cell, 15*, 666-680.

Edner, C., Li, J., Albrecht, T., Mahlow, S., Hejazi, M., Hussain, H., Kaplan, F., Guy, C., Smith, S.M., Steup, M., & Ritte, G. (2007). Glucan, water dikinase activity stimulates breakdown of starch granules by plastidial β-amylases. *Plant Physiology, 145*, 17-28.

Fannon, J.E., Huber, R.J., & BeMiller, J.N. (1992). Surface pores of starch granules. *Cereal Chemistry, 69*, 284-288.

Flach, M. (1983). The sago palm. *FAO plant production and protection paper* 47, AGPC/MISC/80. Rome, Italy: Food and Agriculture Organization of the United Nations.

Flach, M. (1997). *Sago palm metroxylon sagu Rottb*. Italy: International Plant Genetic Resources Institute.

Flach, M., & Schuiling, D.L. (1989). Revival of an ancient starch crop: A review of the agronomy of sago palm. *Agroforestry System, 7*, 259-81.

FAO. (2002). *World Agriculture: Towards 2015/2030*. Rome.

French, D. (1984). Organization of the starch granules. In J.N. BeMiller, & J.F. Paschall (Eds.), *Starch: Chemistry and Technology* (pp. 183-247). Orlando: Academic Press.

Fujita, N. Yoshida, M., Asakura, N., Ohdan, T., Miyao, A., Hirochika, H., & Nakamura, Y. (2006). Function and characterization of starch synthase I using mutants in rice. *Plant Physiology, 140*, 1070-1084.

Fujita, N., Hasegawa, H., & Taira, T. (2001). The isolation and characterization of a waxy mutant of diploid wheat (*Triticum monococcum L.*). *Plant Science, 160,* 595-602.

Fujita, N., Toyosawa, Y., Utsumi, Y., Higuchi, T., Hanashiro, I., Ikegami, A., Akuzawa, S., Yoshida, M., Mori, A., Inomata, K., Itoh, R., Miyao, A., Hirochika, H., Satoh, H., & Nakamura, Y. (2009). Characterization of pullulanase (PUL)-deficient mutants of rice (Oryza sativa L.) and the function of PUL on starch biosynthesis in the developing rice endosperm. *Journal of Experimental Botany, 60*, 1009-1023.

Fujita, N., Yoshida, M., Kondo, T., Saito, K., Utsumi, Y., Tokunaga, T., Nishi, A., Satoh, H., Park, J.H., Jane, J.–L., Miyao, A., Hirochika, H., & Nakamura, Y. (2007). Characterization of SSIIIa-deficient mutants of rice: the function of SSIIIa and pleiotropic effects by SSIIIa deficiency in the rice endosperm. *Plant Physiology*, *144*, 2009-2023.

Fulton, D.C., Edwards, A., Pilling, E., Robinson, H.L., Fahy, B., Seale, R., Kato, L., Donald, A. M., Geigenberger, P., Martins, C., & Smith, A.M. (2002). Role of granule-bound starch synthase in determination of amylopectin structure and starch granule morphology in potato. *The Journal of Biological Chemistry, 277*, 10834-10841.

Fulton, D.C., Stettler, M., Mettler, T., Vaughan, C.K., Li, J., Francisco, P., Gil, M., Reinhold, H., Eicke, S., Messerli, G., Dorken, G., Halliday, K., Smith, A.M., Smith, S.M., & Zeeman, S.C. (2008). BETA-AMYLASE 4, a noncatalytic protein that is required for starch breakdown, acts upstream of three active β-amylases in *Arabidopsis* chloroplasts. *Plant Cell, 20*, 1040-1158.

Gao, M., Wanat, J., Stinard, P. S., James, M. G., & Myers, A.M. (1998). Characterization of dull1, a maize gene coding for a novel starch synthase. *The Plant Cell, 10*, 399-412.

Glaring, M.A., Koch, C.B., & Blennow, A. (2006). Genotype-specific spatial distribution of starch molecules in the starch granule: A combined CLSM and SEM approach. *Biomacromolecules, 7,* 2310-2320.

Grennan, A.K. (2006). Abiotic stress in rice. An "omic" approach. *Plant Physiology,* 140, 1139-1141.

Griffith, W. (1844). On the root parasites referred by authors to *Rhizantheae* and their allies. *Proceedings of the Linnean Society of London, 1*, 216-221.

Guan, H.Li, P., Imparl-Radosevich, J., Preiss, J., & Keeling, P. (1997). Comparing the properties of Escherichia coli branching enzyme and maize branching enzyme. *Archives of Biochemistry and Biophysics, 342*, 92-98.

Guan, H.-P. & Preiss, J. (1993). Differentiation of the properties of the branching isozymes from maize (Zea mays). *Plant Physiology, 102*, 1269-1273. Guichard, E. (2002). Interactions between flavor compounds and food ingredients and their influence on flavor perception. *Food Reviews International, 18*, 49-70.

Han, X.Z. & Hamaker, B.R. (2002). Association of starch granule properties with starch ghosts and remnants revealed by confocal laser scanning microscopy. *Cereal Chemistry, 79*, 892-896.

Han, Y.T., Sun, F.J., Rosales-Mendoza, S., & Korban, S.S. (2007). Three orthologs in rice, Arabidopsis, and Populus encoding starch branching enzymes (SBEs) are different from other SBE gene families in plants. *Gene, 401*, 123-130.

Haska, N., & Ohta, Y. (1992). Mechanism of hydrolysis of the treated sago starch granules by raw starch digesting amylase from *Penicillium brunneum* no. 4. *Starch/Stärke, 44*, 25-28.

Hizukuri, S. (1996). Starch: Analytical aspects. In A. C. Eliasson (Ed.), Carbohydrates in foods (pp. 347-429). New York: Marcel Dekker Inc.

Hizukuri, S., Tabata, S., & Nikuni, Z. (1970). Studies on starch phosphates. Part I. Estimation of glucose-6-phosphate residues in starch and the presence of other bound phosphate(s). *Starch/Stärke, 22*, 338-343.

Howitt, C.A., Rahman, S., & Morell, M.K. (2006). Expression of bacterial starch binding domains in *Arabidopsis* increases starch granule size. *Functional Plant Biology, 33*, 257-266.

Huber, K.C. & BeMiller, J.N. (2001). Location of sites of reaction within starch granules. *Cereal Chemistry, 78*, 173-180.

Huber, K.C., & BeMiller, J.N. (2000). Channels of maize and sorghum starch granules. *Carbohydrate Polymers, 41*, 269-276.

Hussain, H., Mant, A., Seale, R., Zeeman, S., Hinchliffe, E., Edwards, A., Hylton, C., Bornemann. S., Smith, A.M., Martin. C., & Bustos, R. (2003). Three Isoforms of Isoamylase Contribute Different Catalytic Properties for the Debranching of Potato Glucans. *The Plant Cell, 15*, 133-149.

Inouchi, N., Glover, D.V., Takaya, T., & Fuwa, H. (1983). Development changes in fine structure of starches of several endosperm mutants of maize. *Starch/Stärke, 35*, 371-376.

Ito, T., Arai, Y., & Hisajima, S. (1979). Utilization of sago starch. Japan. *Journal of Tropical Agriculture, 23*, 48-56.

James, M.G., Robertson, D.S., & Myers, A.M. (1995). Characterization of the maize gene sugary1, a determinant of starch composition in kernels. *The Plant Cell, 7*, 417-429.

James, M.G., Denyer, K., & Myers, A.M. (2003). Starch synthesis in the cereal endosperm. *Current Opinion in Plant Biology, 6,* 215-222.

James, M.G., Robertson, D.S., & Myers, A.M. (1995). Characterization of the maize gene *sugary1*, a determinant of starch composition in kernels. *Plant Cell, 7*, 417-429.

Jane, J.L. (2009). Structural features of starch granules II: In J. BeMiller, & R. Whistler, (Eds.), Starch: Chemistry and technology (pp. 193-236). NewYork: Elsevier Inc.

Jane, J.L., Chen, Y.Y., Lee, L.F., McPherson, A.E., Wong, K.S., Radosavljevic, M., & Kasemsuwan, T. (1999). Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chemistry, 76*, 629-637.

Jane, J.L., Kasemsuwan, T., Leas, S., Zobel, H., & Robyt, J.F. (1994). Anthology of starch granule morphology by scanning electron microscopy. *Starch/Stärke, 46*, 121-129.

Jayakody, L. & Hoover, R. (2002). The effect of lintnerization on cereal starch granules. *Food Research International, 35*, 665-680.

Jeffery, G.H., Bassett, J., Mendham, J., & Denney, R.C. (1989). *Vogel's Textbook of Quantitative Chemical Analysis*. 5th edition. New York: John Wiley & Sons.

Jenkins, P.J., Cameron, R.E., & Donald, A.M. (1993). A universal feature in the structure of starch granules from different botanical sources. *Starch/Stärke, 45*, 417-420.

Jeon, J.S., Ryoo, N., Hahn, T.–R., Walia, H., & Nakamura, Y. (2010). Starch biosynthesis in cereal endosperm. *Plant Physiology and Biochemistry, 48*, 383-392.

Jong, F.S. (1995). *Research for the development of sago palm (Metroxylon sagu Rottb.) cultivation in Sarawak, Malaysia.* PhD thesis, Wageningen Agricultural University, the Netherlands. Jong Foh-Shoon, Dept. Agriculture, Kuching, Sarawak, Malaysia.

Juszczak, L. (2003). Surface of triticale starch granules - NC-AFM observations. *Electronic Journal of Polish Agricultural Universities, 6*.

Juszczak, L., Fortuna, T., & Krok, F. (2003a). Non-contact atomic force microscopy of starch granules surface. Part I. Potato and tapioca starches. *Starch/Stärke, 55*, 1-7.

Juszczak, L., Fortuna, T., & Krok, F. (2003b). Non-contact atomic force microscopy of starch granules surface. Part II. Selected cereal starches. *Starch/Stärke, 55*, 8-16.

Kaplan, F., & Guy, C.L. (2005). RNA interference of *Arabidopsis* β-amylase 8 prevents maltose accumulation upon cold shock and increases sensitivity of PSII photochemical efficiency to freezing stress. *Plant Journal, 44*, 730-743.

Karim, A.A., Tie, A.P.T., Manan, D.M.A., & Zaidul, I.S.M. (2008). Starch from the sago (*Mextroxylon sagu*) palm tree- Properties, prospects, and challenges as a new industrial source for food and other uses. *Comprehensive review in food science and food safety, 7*, 215-228.

Kawabata, A., Sawayama, S., Nagashima, N., & del Rosario, R.R. (1984). Physico-chemical properties of starches from cassava, arrowroot and sago. pp. 259-271 *in* Tropical Root Crops: *Post Harvest Physiology and Processing* (I. Uritani and E.D. Reyes, eds.). Japan Sci. Soc. Press, Tokyo. Kiew, R. 1977. Taxonomy, ecology.

Kim, H.,-S. & Huber, K. C. (2008). Channels within soft wheat starch A- and B-type granules. *Journal of Cereal Science, 48,* 159-172.

Koksel, H., Masatcioglu, T., Kahraman, K., Ozturk, S., & Basman, A. (2008). Improving effect of lyophilization on functional properties of resistant starch preparations formed by acid hydrolysis and heat treatment. *Journal of Cereal Science, 47*, 275-282.

Kosar-Hashemi, B., Li, Z., Larroque, O., Regina, A., Yamamori, M., Morell, M.K. & Rahman, S. (2007). Multiple effects of the starch synthase II mutation in developing wheat endosperm. *Functional Plant Biology, 34*, 431-438.

Kubo, A., Fujita, N., Harada, N., Matsuda, T., Satoh, H., & Nakamura, Y. (1999). The starch-debranching enzymes isoamylase and pullulanase are both involved in amylopectin biosynthesis in rice endosperm. *Plant Physiology, 121*, 399-410.

Laby, R.J., Kim, D., & Gibson, S.I. (2001). The *ram1* mutant of *Arabidopsis* exhibits severely decreased β-amylase activity. *Plant Physiology, 127*, 1798-1807.

Leloir, L. F., De Fekete, M. A. R., & Cardini, C. E. (1961). Starch and oligosaccharide synthesis from uridine diphosphate glucose. *The Journal of Bological Chemistry, 236,* 636-641.

Li, J.H., Vasanthan, T., Hoover, R., & Rossnagel, B. (2003). Starch from hull-less barley: Ultrastructure and distribution of granule-bound proteins. *Cereal Chemistry, 80*, 524-532.

Li, J.H., Vasanthan, T., Rossnagel, B., & Hoover, R. (2001). Starch from hull-less barley: I. Granule morphology, composition and amylopectin structure. *Food Chemistry, 74*, 395-405.

Li, Q.F., Zhang, G.Y., Dong, Z.W., Yu, H.X., Gu, M.H., Sun, S.S., & Liu, Q.Q. (2009). Characterization of expression of the OsPUL gene encoding a pullulanase-type debranching enzyme during seed development and ermination in rice. *Plant Physiology and Biochemistry, 47*, 351-358.

Lim, E.T. (1991). *A comparative study on the performance of Metroxylon sagu and Metroxylon rumphii grown on gleyed mineral soil and organic soil* [MSc thesis]. Faculty of Food Science and Biotechnology, Univ. Putra Malaysia.

Lim, S.T., Kasemsuwan, T., & Jane, J.L. (1994). Characterization of phosphorous in starch by 13P nuclear magnetic resonance spectroscopy. *Cereal Chemistry, 71*, 488-493.

Lineback, D. R. (1986). Current concepts of starch structure and its impact on properties. *Journal of the Japanese Society of Starch Science, 33*, 80-88.

Maddelein, M.L., Libessart, N., Bellanger, F., Delrue, B., D‟Hulst, C. & Ball, S. (1994). Toward an understanding of the biogenesis of the starch granule: determination of granule-bound and soluble starch synthase functions in amylopectin synthesis. *Journal of Biological Chemistry, 269*, 25150-25157.

Matheson, N.K. (1996). The chemical structure of amylose and amylopectin fractions of starch from tobacco leaves during development and diurnally–nocturnally. *Carbohydrate Research, 282*, 247-262.

Mathur, P.N., Riley, K.W., Rao, V.R., & Zhou, M. (1998). *Conservation and sustainable use of sago (Metroxylon sago) genetic resources*. Pekanbaru, Riau, Indonesia: Riau Univ. Training Centre.

McClatchy, W., & Cox, P.A. (2006).Use of the sago palm *Metroxylon warburgii* in the Polynesian Island, Rotuma. *Economic Botany*, *46*, 305-309.

Meade, G.P., & Chen, J.C. (1997).*Cane sugar handbook: A manual for cane sugar manufacturers and their chemists*. New York: Wiley.

Monma, M., Yamamoto, Y., Kagei, N., & Kainuma, K. (1989). Raw starch digestion by α-amylase and glucoamylase from *Chalara paradoxa*. *Starch/Stärke, 41*:382-385.

Moore, H.E. Jr. (1973). *The major groups of palms and their distribution*. Gentes Herb, *11*, 27-141.

Morell, M.K., Kosar-Hashemi, B., Cmiel, M., Samuel, M.S., Chandler, P., Rahman, S., Buléon, A., Batey, I.L. & Li, Z. (2003). Barley sex6 mutants lack starch synthase IIa activity and contain a starch with novel properties. *Plant Journal, 34*, 173-185.

Morrison, W.R. (1988). Lipids in Cereal Starches: A Review. *Journal of Cereal Science, 8*, 1-15.

Morrison, W.R. (1995). Starch lipids and how they relate to starch granule structure and functionality. *Cereal Foods World, 40*, 437-446.

Myers, A.M., Morell, M.K., James,M.G., & Ball, S.G. (2000). Recent progress towards understanding the biogenesis of the amylopectin crystal. *Plant Physiology, 122*, 989-997.

Nabeya, K., Nakamura, S., Nakajima, T., & Goto, Y. (2016). Growth behavior of sucker derived from transplanted sago palm (*Mextroxylon sagu* Rottb.). *Plant Production Science, 19*, 340-347.

Nakamura, T., Yamamori, M. Hirano, H., Hidaka, S., & Nagamine, T. (1995). Production of waxy (amylose-free) wheats, *Molecular and General Genetics, 248*, 253-259.

Nakamura, Y. (2002). Towards a better understanding of the metabolic system for amylopectin biosynthesis in plants: rice endosperm as a model tissue. *Plant and Cell Physiology, 43*, 718-725.

Nakamura, Y., Umemoto, T., Ogata, N., Kuboki, Y., Yano, M., & Sasaki, T. (1996). Starch debranching enzyme (R-enzyme or pullulanase) from developing rice endosperm: purification, cDNA and chromosomal localization of the gene. *Planta, 199*, 209-218.

Nelson, O.E. & Rines, H.W. (1962). The enzymatic deficiency in the waxy mutant of maize. *Biochemical and Biophysical Research Communications, 9*, 297-300.

Nielsen, T.H., Wischmann, B., Enevoldsen, K., & Moller, B.L. (1994). Starch phosphorylation in potato tubers proceeds concurrently with de novo biosynthesis of starch. *Plant Physiology, 105*, 111-117.

Niittyla, T., Comparot-Moss, S., Lue, W-L., Messerlie, G., Trevisan, M., Seymour, M.D., Gatehouse, J.A., Villadsen, D., Smith, S.M., Chen, J., Zeeman, S.C., & Smith, A.M. (2006). Similar protein phosphatases control starch metabolism in plants and glycogen metabolism in mammals. *Journal of Biological Chemistry, 281*, 11815-11818.

Nishi, A., Nakamura, Y., Tanaka, N. & Satoh, H. (2001). Biochemical and genetic analysis of the effects of amylose-extender mutation in rice endosperm. *Plant Physiology, 127*, 459-472.

Nozaki, K., Nuyim, T., Shinano, T., Hamada, S., Ito, H., Matsui, H., & Osaki, M. (2004). Starch properties of the sago palm (*Mextroxylon sagu* Rottb.) in different soils. *Plant Foods for Human Nutrition, 59*, 85-92.

Ohdan, T., Francisco Jr., P.B., Sawada, T., Hirose, T., Terao, T., Satoh, H., & Nakamura, Y. (2005). Expression profiling of genes involved in starch synthesis in sink and source organs of rice. *Journal of Experimental Botany, 56*, 3229-3244.

Park, H., Xu, S., & Seetharaman, K. (2011). A novel in situ atomic force microscopy imaging technique to probe surface morphological features of starch granules. *Carbohydrate Research, 346*, 847-853.

Patron, N.J., Smith, A.M., Fahy, B.F., Hylton, C.M., Naldrett, M.J., Rossnagel, B.G., & Denyer, K. (2002). The altered pattern of amylose accumulation in the endosperm of low-amylose barley cultivars is attributable to a single mutant allele of granule-bound starch synthase I with a deletion in the 5'-non-coding region. *Plant Physiology, 130*, 190-198.

Peng. M., Gao, M., Abdel-Aal, E.S.M., Hucl, P., & Chibbar, R.N. (1999). Separation and characterization of A- and B-type starch granules in wheat endosperm. *Cereal Chemistry, 76*, 375-379.

Peng, M., Gao, M., Båga, M., Hucl, P., & Chibbar, R.N. (2000). Starch branching enzymes preferentially associated with A-type starch granules in wheat endosperm. *Plant Physiology, 124*, 265-272.

Peng, M., Hucl, P., & Chibbar, R.N. (2001). Isolation, characterization and expression analysis of starch synthase I from wheat (*Triticum aestivum* L.). *Plant Science, 161*, 1055-1062.

Perez, S., & Bertoft, E. (2010). The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review. *Starch/Stärke, 62*, 389-420.

Pilling, E., & Smith, A.M. (2003). Growth ring formation in the starch granules of potato tubers. *Plant Physiology, 132*, 365-371.

Rauwerdink, J.B. (1986). An essay on *Metroxylon*, the sago palm principles. *The Journal of Palm Society, 30*, 165-180.

Rekha, S.S., Kennedy, J.F., Sajilata, M.G., Kaczmarek, A., Knill, C.J., & Putri, F.A. (2008). *Carbohydrate Polymers, 72*, 1-20.

Ridout, M.J., Parker, M.L., Hedley, C.L., Bogracheva, T.Y., & Morris, V.J. (2003). Atomic force microscopy of pea starch granules: Granule architecture of wild-type parent, r and rb single mutants, and the rrb double mutant. *Carbohydrate Research, 338*, 2135-2147.

Roldán, I., Lucas, M.M., Delvallé, D., Planchot, V., Jimenez, S., Perez, R., Ball, S., D"Hulst, C., & Mérida, A. (2007). The phenotype of soluble starch synthase IV defective mutants of Arabidopsis thaliana suggests a novel function of elongation enzymes in the control of starch granule formation. *Plant Journal, 49*, 492-504.

Ruddle, K., Johnson, D., Townsend, P.K., & Rees, J.D. (1978). *Palm sago: A tropical starch from marginal lands*. Honolulu, Hawaii: Univ. Press of Hawaii.

Sandhu, K.S., Sing, N., & Kaur, M. (2004). Characteristics of the different corn types and their grain fractions: Physicochemical, thermal, morphological, and rheological properties of starches. *Journal of Food Engineering, 64*, 119-127.

Sano, Y. (1984). Differential regulation of waxy gene expression in rice endosperm. *Theoretical and Applied Genetics, 64*, 467-473.

Santacruz, G.A.A., Ordinola, A.V., Gomez, B.M., Torres, L.M.G., Espino, L.F.D., & Vazquez, F.P.G. (2004). Development of long-term and reliable *in vitro* plant regeneration systems for elite malting barley varieties: Optimizing media formulation and explant selection. *Journal of Biotechnology, 10*, 19522-19533.

Sastrapradja, S., & Mogea, J.P. (1977). Present uses and future development of Metroxylon sagu in Indonesia. In: Tan K, editor. *Sago-76: Papers of the First International Sago Symposium*. Kuala Lumpur, Malaysia: Kemajuan Kanji Sdn. Bhd. pp. 112-117.

Sawada, T., Francisco Jr., P., Aihara, S., Utsumi, Y., Yoshida, M., Oyama, Y., Tsuzuki, M., Satoh, H., & Nakamura, Y. (2009). Chlorella starch branching enzyme II (BEII) can complement the function of BEIIb in rice endosperm. *Plant and Cell Physiology, 50*, 1062-1074.

Scheidig, A., Frohlich, A., Schulze, S., Lloyd, J.R., & Kossmann, J. (2002). Down-regulation of a chloroplasttargeted β-amylase leads to a starch-excess phenotype in leaves. *Plant Journal, 30*, 581-591.

Schuiling, D.L., & Jong, F.S. (1996). *Metroxylon sagu* Rottboell. pp. 121-126. In: Flach, M. and F. Rumawas (eds.). Plant Resources of South-East Asia No. 9.Plants yielding non-seed carbohydrates. Prosea Foundation, Bogor, Indonesia.

Sim, S.L., Oates, C.G., & Wong, H.A. (1991). Characterisation and comparison of sago starches obtained from Metroxylon sagu processed at different times. *Starch/Stärke, 43*, 459-466.

Singh, N., & Kaur, L. (2004). Morphological, thermal, rheological and retrogradation properties of potato starch fractions varying in granule size. *Journal of the Science of Food and Agriculture, 84*, 1241-1252.

Singh, N., Singh, J., Kaur, L., Sodhi, N.S., & Gill, B.S. (2003). Morphological, thermal, and rheological properties of starches from different botanical sources. *Food Chemistry, 81*, 219-231.

Smith, A.M., Denyer, K. & Martin, C. (1997). The synthesis of the starch granule. *Annual Review of Plant Physiology and Plant Molecular Biology, 48*, 67-87.

Smith, K. (2015). *The Sago Palm: The Food and Environmental Challenges of the 21st Century.* Japan: Kyoto University Press.

Sitohy, M.Z., Labib, S.M., El-Saadany, S.S., & Ramadan, M.F. (2000) Optimizing the conditions for starch dry phosphorylation with sodium mono- and dihydrogen orthophosphate under heat and vacuum. *Starch/Staerke, 52*, 95-100.

Stark, J.R. & Lynn, A. (1992). Starch granules large and small. *Biochemistry of Plant Polysaccharides, 20*, 7-12.

Sujka, M., & Jamroz, J. (2009). α-amylosis of native potato and corn starches-SEM, AFM, nitrogen and iodine sorption investigations. *LWT-Food Science and Technology*, *42*, 1219-1224.

Takashima, Y., Senoura, T., Yoshizaki, T., Haniada, S., Ito, H., & Matsui, H. (2007). Differential chain-length specificities of two isoamylase-type starch-debranching enzymes from developing seeds of kidney bean. *Bioscience Biotechnology and Biochemistry, 71*, 2308–2312.

Takeda, Y., Guan, H-P., & Preiss, J. (1993). Branching of amylose by the branching isoenzymes of maize endosperm. *Carbohydrate Research, 240*, 253-263.

Tester, R.F. (1997). Starch: The polysaccharide fraction. In P. J. Frazier, P. Richmond, & A. M. Donald (Eds.), Starch: Structure and functionality (pp. 163-171). Cambridge: The Royal Society of Chemistry.

Tester, R.F., Karkalas, J., & Qi, X. (2004). Starch-composition, fine structure and architecture. *Journal of Cereal Science, 39*, 151-165.

Tetlow, I.J. (2006). Understanding storage starch biosynthesis in plants: a means to quality improvement. *Canadian Journal of Botany, 84*, 1167-1185.

Tetlow, I. J. (2011). Starch biosynthesis in developing seeds. *Seed Science Research, 21*, 5-32.

Tetlow, I.J., Emes, M.J. (2014). A review of starch-branching enzyme and their role in amylopection biosynthesis. *IUBMB Life, 8*, 546-558.

Tetlow, I.J., Wait, R., Lu, Z., Akkasaeng, R., Bowsher, C.G., Esposito, S., Kosar-ashemi, B., Morell, M.K. & Emes, M.J. (2004). Protein phosphorylation in amyloplasts regulates starch branching enzyme activity and protein-protein interactions. *Plant Cell, 16*, 694-708.

Thomas, D. J. & Atwell, W.A. (1999). Starch analysis methods. In D. J. Thomas & W. A. Atwell (Eds.). Starches (pp. 13-24). St. Paul: American Association of Cereal Chemists Inc.

Tie, A.P.T., Mohamed, A.M.D., & Karim, A.A. (2006). Sago starch and composition of associated components in palms of different growth stages. *Carbohydrate polymers, 63*, 283-286.

Tomlinson, P.B. (1990). *The Structural Biology of Palms*. Oxford: Clarendon Press.

Trethewey, R.N., & Smith, A.M. (2000) Starch metabolism in leaves. *In* RC Leegood, TD Sharkey, S von Caemmerer, eds, *Photosynthesis: Physiology and Metabolism*. Kluwer Academic Publishers, Dordrecht: Netherlands. pp. 205-231.

Tsai, C.Y. (1974). The function of waxy locus in starch synthesis in maize endosperm. *Biochemical Genetics, 11,* 83-96.

Uhl, N.W., & Dransfield, J. (1987). Genera *Palmarum. A classification of palm based in the work of Harold E. Moore, Jr. Lawrence*. Kans.: Allen Press.

Umemoto, T., & Aoki, N. (2005). Single-nucleotide polymorphisms in rice starch synthase IIa that alter starch gelatinisation and starch association of the enzyme. *Functional Plant Biology, 32,* 763-768.

Utrilla-Coello, R.G., Agama-Acevedo, E., Rosa, A. P. B., Rodríguez-Ambriz, S.L., & Bello-Pérez, L.A. (2010). Physicochemical and enzyme characterization of small and large granules isolated from two maize cultivars. *Cereal Chemistry, 87,* 50-56.

Vasanthan, T., & Hoover, R. (1992). A comparative study of the composition of lipids associated with starch granules from various botanical sources. *Food Chemistry, 43,* 19-27.

Vasanthan, T., & Bhatty, R.S. (1995). Starch purification after pin milling and air classification of waxy, normal and high amylose barleys. *Cereal Chemistry*, *72*, 379-384.

Velde, F., Riel, J., & Tromp, R.H. (2002). Visualization of starch granule morphologies using confocal scanning laser microscopy (CLSM). *Journal of the Science of Food and Agriculture, 82*, 1528-1536.

Waduge, R.N., Hoover, R., Vasanthan, T., Gao, J., & Li, J. (2006). Effect of annealing on the structure and physicochemical properties of barley starches of varying amylose contents. *Food Research International, 39*, 59-77.

Waduge, R.N., Xu, S., & Seetharaman, K. (2010). Iodine absorption properties and its effect on the crystallinity of developing wheat starch granules. *Carbohydrate Polymers, 82*, 786-794.

Wan Sulaiman, W.H., Ipor, I., Bulan, P. & Ismail, J. (2004). Report on comparative study of sago palm in smallholding and plantation in Mukah & Dalat-ecophysiological responses to different edaphic condition. *Craun Research*, 81.

Wang, Q., Monroe, J., & Sjolund, R.D. (1995). Identification and characterization of a phloem-specific β-amylase. *Plant Physiology*, *109*, 743-750.

Watcharatewinkul, Y., Puttanlek, C., Rungsardthong, V., Uttapap, D. (2009). Pasting properties of a heat-moisture treated canna starch in relation to its structural characteristics. *Carbohydrate Polymers, 75,* 505-511.

Wattebled, F., Dong, Y., Dumez, S., Delvall´e, D., & Planchot, R., Berbezy, P., Vyas, D., Colonna, P., Chatterjee, M., Ball, S., & D'Hulst, C. (2005). Mutants of *Arabidopsis* lacking a chloroplastic isoamylase accumulate phytoglycogen and an abnormal form of amylopectin. *Plant Physiology, 138,* 184-195.

Weber, A., Servaites, J.C., Geiger, D.R., Kofler, H., Hille, D., Gröner, F., Hebbeker, U., & Flügge, U.I. (2000). Identification, purification, and molecular cloning of a putative plastidic glucose transporter. *Plant Cell, 12,* 787-801.

Weise, S., Weber, A.P.M., & Sharkey, T.D. (2004). Maltose is the major form of carbon exported from the chloroplast at night. *Planta, 218,* 474-482.

Wu, C.Y., Colleoni, C., Myers, A.M., & James, M.G. (2002). Enzymatic properties and regulation of ZPU1, the maize pullulanase-type starch debranching enzyme. *Archives of Biochemistry and Biophysics, 406,* 21-32.

Yamamori, M., Fujita, S., Hayakawa, K., Matsuki, J., & Yasui, T. (2000). Genetic elimination of starch granule protein, SGP-1, of wheat generates am altered starch with apparent high amylose. *Theoretical and Applied Genetics, 101*, 21-29.

Yamanouchi, Y. & Nakamura, Y. (1992). Organ specificity of isoforms of starch branching enzyme (Q-enzyme) in rice. *Plant and Cell Physiology, 33,* 985-991.

Yin, Y., Qi, J., Li, W., Cao, L., & Wang, Z. (2012). Formation and developmental characterization of A- and B-type starch granules in wheat endosperm. *Journal of Integrative Agriculture, 11*, 73-81.

Yoo, S-H., & Jane, J.L. (2002). Structural and physical characteristics of waxy and other wheat starches. *Carbohydrate Polymers, 49,* 297-305.

Yu, T-S., Zeeman, S.C., Thorneycroft, D., Fulton, D.C., Lue, W-L., Dunstan, H., Hegemann, B., Tung, S-Y., Umemoto, T., Chapple, A., Tsai, D-L., Wang, S-M., Smith, A.M., Chen, J., & Smith, S.M. (2005). α-Amylase is not required for breakdown of transitory starch in *Arabidopsis* leaves. *Journal of Biology Chemistry, 250,* 9773-9779.

Yuryev, V.P., Krivandin, A.V., Kiseleva, V.I.,Wasserman, L.A., Genkina, N.K., Fornal, J., Blaszczak, W., & Schiraldi, A. (2004). Structural parameters of amylopectin clusters and semi-crystalline growth rings in wheat starches with different amylose content. *Carbohydrate Research, 339,* 2683-2691.

Zeeman, S.C., Northrop, F., Smith, A.M., & Rees, T. (1998). A starch-accumulating mutant of *Arabidopsis thaliana* deficient in a starch-hydrolysing enzyme. *Plant Journal, 15,* 357-365.

Zeeman, S.C., Pilling, E., Tiessen, A., Kato, L., Donald, A.M., & Smith, A.M. (2002). Starch synthesis in Arabidopsis; granule synthesis, composition and structure. *Plant Physiology, 129*, 516-529.

Zeeman, S.C., Smith, S.M., & Smith, A.M. (2004). The breakdown of starch in leaves. *New Phytologist, 163*, 247-261.

Zeeman, S.C., Smith, S.M., & Smith, A.M. (2007). The diurnal metabolism of leaf starch. *Biochemistry Journal, 401*, 13-28.

Zeeman, S.C., Kossmann, J., & Smith, A.M. (2010). Starch: Its metabolism, evolution, and biotechnological modification in plants. *Plant Biology, 61*, 209-234.

Zhang, C., Jiang, D., Liu, F., Cai, J., Dai, T., & Cao, W. (2010). Starch granule size distribution in superior and inferior grains of wheat is related to enzyme activities and their gene expressions during grain filling. *Journal of Cereal Science, 51*, 226-233.

Zhang, X., Colleoni, C., Ratushna, V., Sirghie-Colleoni, M., James, M.G., & Myers, A.M. (2004). Molecular characterization demonstrates that the Zea mays gene sugary2 codes for the starch synthase isoform SSIIa. *Plant Molecular Biology, 54*, 865-879.

Zhou, M., Robards, K., Glennie-Holmes, M., & Helliwell, S. (1998). Structure and pasting properties of oat starch. *Cereal Chemistry, 75*, 273-281.

APPENDIX

Size distribution of starch granules from the trunk of trunking and non-trunking sago palm for 0-10 µm.

0-10µm	**Trunking Dalat**	**Non-trunking Dalat**
1st replicate	0	9
2nd replicate	5	4
3rd replicate	6	25
4th replicate	16	9
5th replicate	12	20

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
T DALAT	5	39	7.8	39.2
NT DALAT	5	67	13.4	76.3

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	78.4	1	78.4	1.357576	0.277522	5.317655
Within Groups	462	8	57.75			
Total	540.4	9				

Size distribution of starch granules from the trunk of trunking and non-trunking sago palm for 11-20 µm.

11-20µm	**Trunking Dalat**	**Non-trunking Dalat**
1st replicate	4	16
2nd replicate	8	17
3rd replicate	18	30
4th replicate	14	9
5th replicate	8	11

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
T DALAT	5	52	10.4	30.8
NT DALAT	5	83	16.6	67.3

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	96.1	1	96.1	1.959225	0.199159	5.317655
Within Groups	392.4	8	49.05			
Total	488.5	9				

Size distribution of starch granules from the trunk of trunking and non-trunking sago palm for 21-30 µm.

21-30µm	**Trunking Dalat**	**Non-trunking Dalat**
1st replicate	15	14
2nd replicate	12	6
3rd replicate	36	22
4th replicate	10	9
5th replicate	14	18

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
T DALAT	5	87	17.4	111.8
NT DALAT	5	69	13.8	42.2

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	32.4	1	32.4	0.420779	0.534722	5.317655
Within Groups	616	8	77			
Total	648.4	9				

Size distribution of starch granules from the trunk of trunking and non-trunking sago palm for 31-40 µm.

31-40µm	**Trunking Dalat**	**Non-trunking Dalat**
1st replicate	17	13
2nd replicate	44	9
3rd replicate	20	20
4th replicate	12	20
5th replicate	21	12

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
T DALAT	5	114	22.8	152.7
NT DALAT	5	74	14.8	24.7

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	160	1	160	1.803833	0.216105	5.317655
Within Groups	709.6	8	88.7			
Total	869.6	9				

Size distribution of starch granules from the trunk of trunking and non-trunking sago palm for 41-50 µm.

41-50µm	**Trunking Dalat**	**Non-trunking Dalat**
1st replicate	11	12
2nd replicate	10	7
3rd replicate	12	12
4th replicate	9	6
5th replicate	5	9

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
T DALAT	5	47	9.4	7.3
NT DALAT	5	46	9.2	7.7

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.1	1	0.1	0.013333	0.910918	5.317655
Within Groups	60	8	7.5			
Total	60.1	9				

Size distribution of starch granules from the leaves of trunking and non-trunking sago palm for 1-10 µm.

1-10µm	**Trunking Dalat**	**Non-trunking Dalat**
1st replicate	19	20
2nd replicate	19	21
3rd replicate	12	26
4th replicate	16	20
5th replicate	17	40

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
T DALAT	5	83	16.6	8.3
NT DALAT	5	127	25.4	72.8

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	193.6	1	193.6	4.774353	0.060387	5.317655
Within Groups	324.4	8	40.55			
Total	518	9				

Size distribution of starch granules from the leaves of trunking and non-trunking sago palm for 11-20 µm.

11-20µm	**Trunking Dalat**	**Non-trunking Dalat**
1st replicate	15	9
2nd replicate	11	11
3rd replicate	11	16
4th replicate	14	25
5th replicate	14	10

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
T DALAT	5	65	13	3.5
NT DALAT	5	71	14.2	43.7

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.6	1	3.6	0.152542	0.706312	5.317655
Within Groups	188.8	8	23.6			
Total	192.4	9				

Size distribution of starch granules from the leaves of trunking and non-trunking sago palm for 21-30 µm.

21-30µm	**Trunking Dalat**	**Non-trunking Dalat**
1st replicate	6	3
2nd replicate	6	5
3rd replicate	5	4
4th replicate	9	3
5th replicate	4	9

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
T DALAT	5	30	6	3.5
NT DALAT	5	24	4.8	6.2

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.6	1	3.6	0.742268	0.414012	5.317655
Within Groups	38.8	8	4.85			
Total	42.4	9				

Size distribution of starch granules from the leaves of trunking and non-trunking sago palm for 31-40 µm.

31-40µm	**Trunking Dalat**	**Non-trunking Dalat**
1st replicate	7	4
2nd replicate	3	3
3rd replicate	2	2
4th replicate	4	2
5th replicate	1	1

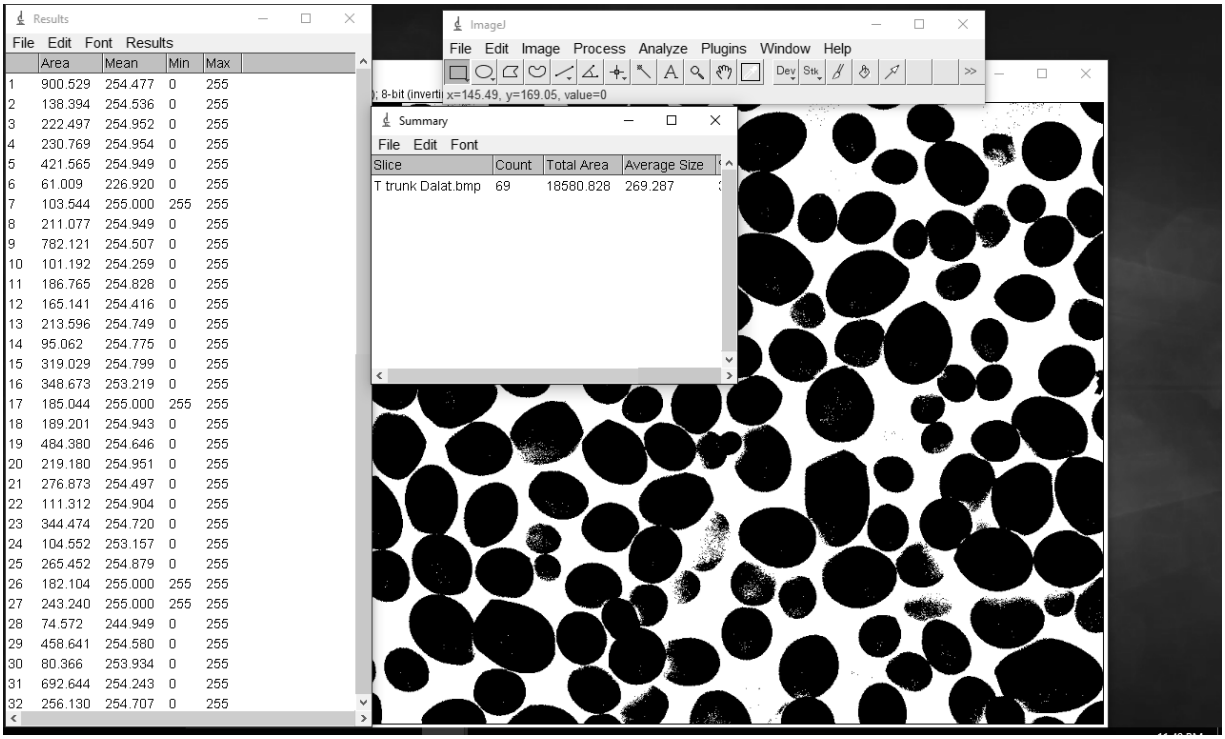
Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
T DALAT	5	17	3.4	5.3
NT DALAT	5	12	2.4	1.3

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.5	1	2.5	0.757576	0.409444	5.317655
Within Groups	26.4	8	3.3			
Total	28.9	9				



The result from ImageJ for the size distribution of starch granule for trunking and non-trunking sago palm.