Physicochemical Properties Of Starch From Sago (*Metroxylon Sagu*) Palm Grown In Mineral Soil At Different Growth Stages

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Abstract. A study was carried out to determine the physico-chemical properties of sago starch from sago palm grown in mineral soil at different growth stages. Four stages of sago palm, namely, Plawei (P), Bubul (B), Angau Muda (AM) and Angau Tua (AT) were studied. Sago starch granules were observed by using scanning electron microscopy (SEM) while the x-ray diffraction patterns were examined to study the starch crystallinity. The highest starch content was found at Plawei stage (94.2%) and Angau Muda stage (97.9%), respectively. The amylose content varied between 29.4 to 31.2% for each growth stages. The highest swelling power was found at the earliest growth stages (P) late growth stages (AT) which are 13.3 g/g and 13.2 g/g, respectively. Granule size distributions were similar as the palm grows to the later growth stages, where highest mean diameter of sago starches granules was found at AM. Variation of starch, amylose and proximate content was observed for starches derived from sago palm different growth stages were insignificant.

Keywords: Sago, *Metroxylon sagu*, growth stages, starch

1. Introduction

Malaysia is currently the largest world exporter of starch from sago (*Metroxylon sagu*) palm i.e. 47,000 metric ton/year where 96% from the starch was produced in Sarawak. Sago palm is characterized by a crown of compound leaves and terminating in a tall, woody and unbranch stem with non-branching roots go straight down to the soil [1]. It grows well in humid tropical lowlands, up to an altitude of 700 meters. Temperature above 25 °C and relative air humidity of 70 % are favorable. It is extremely hardy plant, thriving in swampy, acidic peat soils, submerged and saline soils where few other crops survive but
growing slowly in peat soil than mineral soil [2]. The sago palm is hapaxantic, means it flowers once and
dies shortly thereafter. The palm is mostly propagated vegetatively through its suckers in the wild as well
as cultivation. Individual sucker firstly grows into a rosette of leaves, then produce a stout trunk. During
the vegetative stage, just before flowering, the plant converts its stored nutrients into starch, which
accumulates in the trunk [2].

The matured trunk will be harvested, leaving the immature and suckers, thereby sustaining sago
production [1]. 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 of fibrous bark. The bark is progressively denser
towards the outside where the surface is covered with a thin shiny reddish-brown skin. This shell provides
most of the structural strength of the palm and protects against predatory organisms [2]. The sago starch
accumulates in the pith core of the stem of the sago palm. Trunk formation starts in the third and forth
year of growth of the palm. The vegetative phase of the sago palm takes about 7 to 15 years, during which
time the pith is saturated with starch from the base of the stem upwards[3]. The classification of sago
palm stages are tabulated as followed [4].

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

Quality of sago starches is important when starch is designated for export or when it is sold to large-scale
food processors [4]. For example, good quality sago starches give high viscosity during gelatinization [5].
The poor quality of sago starch has been attributed to a number of factors such as poor processing
conditions, presence of metal ions during processing, freshness of the raw pith, presence of polyphenol
compounds and the consequent activity of polyphenol [6]. Quality problems associated with sago starch
are inconsistent viscosity (or variable pasting properties), variable moisture content, distinct odor, low
profile viscosity, high level of fiber and dull color. Mature palms are essential for the production of high-
quality starch.
The major problem faces by the industry or food manufacturer is the variation in quality of sago starch from batch to batch. The existing data are not sufficient to cater the increasing demand for high quality sago starch from the industry. An understanding of basic properties is required to effectively utilize the starch which data is still lacking for sago palm grown in mineral soil. This study was carried out to determine the effects of different growth stages and on the physicochemical properties of starch granule extracted from sago palm. Four commercial growth stages of sago palm i.e. Plawei (palms at maximum vegetative growth), Bubul (appearance of flowering structure), Angau Muda (flowering) and Angau Tua (fruiting) were studied.

2. Materials And Methods

2.1 Materials

Sago starch was supplied by CRAUN Research SDN BHD, (Kuching, Sarawak, Malaysia). The starch sample was taken from sago plantation in Bau, Sarawak. The starches were taken at different growth stages of sago palm namely Plawei, Bubul, Angau Muda and Angau Tua. All reagents and chemicals were of analytical grades purchased from commercial scientific supplier.

2.2 Proximate Composition of Starch

The moisture content of starch samples were determined by using IR-30 Moisture Analyzer (Denver Instrument, Colorado, USA). Starch (5 g) were spread uniformly on the pan and heated at 105 oC.

The protein content of each starch was determined by Macro-Kjeldahl method [7]. Each starch was analyzed in triplicate and the protein content was calculated by converting the nitrogen content with the factor 6.25 and reported in percent of protein.

The ash content of each starch was determined by using standard AOAC procedure [7]. The analysis was done in triplicate and reported in percent of ash.

The fat content of each starch was determined by using Soxhlet method [7]. Analysis was done in triplicate and reported in percent of fat.

2.3 Determination of Starch Content

Starch content in these samples was determined by using Total Starch Assay Procedure from Megazyme International Ireland Limited (2005). Starch samples (100 mg) were dispersed by using 0.2 ml ethanol (80% v/v) and stirred on a vortex mixer. α-Amylase enzyme (3 ml) was added then incubated in a boiling water bath for 6 minutes. In 50 oC water bath, 4 ml sodium acetate buffer (200 mM, pH 4) was added, followed by 0.1 ml amylglucosidase (20 U), stirred on a vortex mixer, and then incubated at 50 oC for 30 minutes. The content was transferred into volumetric flask and topped up to 100 ml, followed by centrifuged (Bench Top Centrifuge Kubota 5100, Korea) at 3000 rpm for 10 minutes. The aliquot (0.1 ml) was transferred into a test tube, added with 3 ml Glucose Determination Reagent (GOPOD) then incubated at 50 oC for 20 minutes.

The absorbance was read at 510 nm for each samples while D-glucose control was read against the reagent blank. The starch percent (w/w) on dry basis was calculated by the following equation:

\[
\text{Starch \% w/w (dry basis)} = \frac{\text{Starch\% w/w as is} \times 100}{100 - \text{Moisture content (\% w/w)}}
\]
2.4 *Determination of Amylose Content*

Amylose content of each sample and raw starch was determined in triplicate according to the procedure described by McGrance et al. [8].

2.5 *Scanning Electron Microscopy (SEM)*

The scanning electron microscopy study was carried out on starch samples using Leica Cambridge S360 Scanning Electron Microscope (Leica, Wetzlar, Germany). The starch was sprinkled on double-sided adhesive tapes mounted on the studs. The studs were placed in a container and coated with gold in an Argon gas environment. Next, the coated studs were placed on a sample holder of the Leica SEM to be scanned. The captured images were printed and saved.

2.6 *X-Ray Diffraction Analysis*

X-ray diffraction patterns of native starch and treated starch were obtained using a D5000 SIEMENS X-ray powder diffractometer equipped with a CuKα target at 40 kV and 25 mA at a diffraction angle ranging from $2\theta = 5\degree$ to $40\degree$. A step-scan was set at an angle of 0.05$\degree$ per step with a counting of 2 sec.

2.7 *Determination of Swelling Power and Solubility*

Swelling power and solubility was determined in triplicate according to the method of Leach *et al.* [9].

\[
\text{Swelling (g/g)} = \frac{\text{Weight of the wet sediment (g)}}{\text{Weight of the dry starch (g)}}
\]

\[
\text{Solubility (\%)} = \frac{\text{Weight of dried supernatant (g)} \times 100}{\text{Weight of dry starch (g)}}
\]

2.8 *Particle Size and Distribution Analysis*

The particle size and distribution study was analyzed by using the Long Bench Mastersizer S (Malvern Instrument) fitted with QSpec Dry Powder Feeder. Sago starch powder (2.0 g, dry basis) was prepared in duplicate for this analysis. The value of D [4, 3] i.e. the volume mean diameter; D (n, 0.1) i.e. 10% cut-off point as ten percent of the distribution is below this point; D (n, 0.5) i.e. volume median diameter and divides the distribution exactly in half; D (n, 0.9) i.e. 90% cut-off point as ninety percent of the distribution is below this point were measured.

2.9 *Statistical Analysis*

The sago palm growth stages and heights are the factors that are being compared in this research. The comparison was done among the growth stages (i.e., Plawei, Bubul, Angau Muda and Angau Tua) at different heights (base and middle). Statistical analysis was carried out by using one-way ANOVA analysis using the Duncan’s multiple range tests to compare means (Steel and Torrie, 1960) in SPSS 11.5 Programs. Triplicate analysis were done for each samples while the significance different was determine at $\alpha = 0.05$ (95 % confidence level).

3. *Results And Discussion*

3.1 *Proximate Composition of Starch*

The results of moisture, protein, ash and fat content for sago starches are presented in Table 1. There are no significant values of moisture content of sago starch as the palm grows from Plawei to Angau Tua. It
is speculated that sago starch granules becomes more hygroscopic as the palm grows to the latest growth stages. Protein content of sago starch in this study was very low (less than 0.1%) when compared to the value of 0.1–0.3% [11] and 0.4–1.4% [12]. The highest protein content was shown at Bubul stage which is 0.09% and no significant value of protein content observed when the palm grows from Bubul towards Angau Tua.

Table 1 Chemical values of sago starch derived from sago palm at different growth stages at two different heights

| Samples     | Moisture, % | Protein, % | Ash, %  | Fat, %  |
|-------------|-------------|------------|---------|---------|
| Plaweiz     | 15.62 ± 0.73| 0.05 ± 0.007| 0.14 ± 0.03| 0.17 ± 0.02 |
| Bubul       | 15.54 ± 0.48| 0.09 ± 0.013| 0.12 ± 0.01| 0.21 ± 0.03 |
| Angau Muda  | 16.96 ± 0.63| 0.07 ± 0.001| 0.18 ± 0.02| 0.17 ± 0.01 |
| Angau Tua   | 16.03 ± 0.64| 0.08 ± 0.006| 0.14 ± 0.02| 0.24 ± 0.03 |

Values are means ± SD (n = 3).

Ash content in this study (0.1–0.3%) was in relation to the previous researchers i.e. 0.06–0.4% [11] and 0.2–0.7% [12]. The value increased significantly as the palm grows from Plaweiz (0.1%) to Angau Muda stage (0.2%). Ahmad et al. [11] reported that ash contents of sago starches were low when compared to potato (0.3%) and corn (0.2%).

The highest value for fat content was found at Angau Tua stage (0.24%) and Bubul stage (0.23%). Commericially starches normally contain trace amounts of fatty acid glycerides usually less than 0.1% which can be removed by using Soxhlet extraction using ether or hexane [11]. The formation of starch-lipid or starch-surfactant complexes improves the textural properties of various foods [13] therefore high fat content value in this study will value-add the benefit of sago starch in various food products.

3.2 Determination of Starch Content

Starch content for sago starches in this study is given in Table 2. From the result, no significant different of starch content values except for the latest growth stages i.e. Angau Muda (94%) and Angau Tua (92%). The result in this study agrees to the previous finding where most sago starch accumulates below 6 m height of sago palm trunk [14]. These specify the initiation of an upward mobilization of starch from the base to the upper portion of the trunk, for the conversion of the starch to other forms of energy for flower and fruits development [15].
Table 2 Starch content values of sago starch derived from sago palm at different growth stages at two different heights

| Samples       | Starch Content, % | Amylose Content, % |
|---------------|-------------------|--------------------|
| Plawei        | 94.24 ± 1.56      | 30.79<sup>ab</sup> ± 0.82 |
| Bubul         | 91.69 ± 0.55      | 30.30<sup>ab</sup> ± 1.10 |
| Angau Muda    | 93.91 ± 0.32      | 31.19<sup>b</sup> ± 0.92 |
| Angau Tua     | 91.84 ± 1.61      | 30.58<sup>ab</sup> ± 1.01 |

Values are means ± SD (n = 3).

The starch content was found to be decreased as the palm grows from Angau Muda to Angau Tua for both heights. Nozaki et al. [13] reported that starch concentration is related to the activities of starch synthetic enzymes in sago palm. Higher amylase activity in Angau Tua stage increased the starch degradation thus reducing the starch concentration inside the sago palm trunk.

3.3 Determination of Amylose Content

From the results in Table 2, no significant different observed for amylose content at different growth stages, where the values varied between 30.3 to 31.2%. The amylose content in this study agreed well with the published values of 24 – 31% [6, 11, 16]. As shown in the results, the difference in the amylose content was observed as the outcome of harvesting the sago palm at different growth stages. Amylose content (29 – 31%) of starch from sago palm grown in mineral soil in this study is higher than amylose content (23 – 27%) from palm grown in peat soil [3].

3.4 Scanning Electron Microscopy (SEM)

The scanning electron micrographs of sago starches at different growth stages are presented in Figure 1. The starch granules are oval in shape, with some truncated side. From the micrographs, it is estimated that the granule size ranges from approximately 20 – 60 μm. At Plawei stage, most of the starch granules accumulated at the base of the palm contained larger size granules compared to the middle part of the palm. As the palm grows to Bubul stage, smaller starch granules accumulated at the base of the palm while the distribution of small and large starch granules is similar from the base towards the middle heights of the palm at Angau Muda stage. This result is in accordance with the particle size distribution study (Table 7). At Angau Tua stage most of the starch granules have large starch granules.
Figure 1: SEM pictures of sago starch at different growth stages Figure a: Plawei stage, Figure b: Bubul stage, Figure c: Angau Muda stage, Figure d: Angau Tua stage (500 ×).

Figure 2: X-ray diffraction pattern for sago starches derived from sago palm at different growth stages (AT = Angau Tua, AM = Angau Muda, B = Bubul, P = Plawei).

3.5 X-Ray Diffraction Analysis

X-ray diffraction pattern for sago starches grown in mineral soil at different growth stages are displayed in Figure 2. The sago starches samples show C-type x-ray diffraction patterns with the reflection intensity at
5.5°, 10°, 11.1°, 15°, 17.2°, 17.9°, 23.5°, and 26.5° 2θ angles. 2θ values of 17.2° was the characteristic of the B pattern while one peak appeared at 23.5° was the characteristic of the A pattern. Pukkahuta and Varavinit [17] also reported C-type diffraction patterns as reported by for native sago starches. Ahmad et al. [11] also reported that the crystalline type of sago starch is intermediate to that of cereal or potato starches.

No clear difference could be seen between the starch samples from different growth stages, meaning that the crystallinity and the polymorphic type did not differ despite some differences in amylose content. Ahmad et al. [11] explained that the differences of amylose content are probably not significant enough to induce a change in the crystalline structure. It is well-known that an increase in the amylose content induces an increase in the B-type amount and a decrease in the crystallinity. However, it should be mentioned that C-type patterns are obtained for starches of relatively high amylose content (around 30 %) similar to those of the sago starches studied.

3.6 Determination of Swelling Power and Solubility

The swelling power and solubility of sago starches in this study are presented in Table 3. The results show no specific trend for swelling power and solubility value at different growth stages. The swelling power of starches varies between 11.9 – 13.3g/g. Swelling power provides evidence of non-covalent bonding between starch molecules. Several factors such as amylose-amylopectin ratio, chain length and molecular weight distribution, degree/length of branching and conformation the degree of swelling and solubility inside starch granule [18]. Moorthy [13] pointed the possible relationship between cooking quality and swelling volume, thus the consistent volume of swelling for sago starches in this study suggesting its good cooking quality.

Table 3 Swelling power and solubility of sago starch at different growth stages at two different heights

| Sample   | Swelling Power, g/g | Solubility, % |
|----------|---------------------|---------------|
| Plawei   | 13.32 ± 0.13        | 1.80 ± 0.29   |
| Bubul    | 11.92 ± 0.21        | 1.48 ± 0.06   |
| Angau    | 13.01 ± 0.11        | 2.50 ± 0.04   |
| Muda     |                     |               |
| Angau    | 12.83 ± 0.83        | 2.83 ± 0.36   |
| Tua      |                     |               |

Values are means ± SD (n = 3).

When starch is heated in excess water, the crystallite structure is disrupted and water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of amylose and amylopectin [19]. This causes an increase in granule swelling and solubility. The results showed significant difference for swelling power as the sago palm matured from plawei (P) to bubul (B) stage and from B to angau muda (AM) stage.
Swelling power has been reported to be influenced by strongly bonded micellar networks and amyllopectin molecular structure [20]. A low swelling power of starches may be attributed to the presence of a large number of crystallite formed by the association between long amyllopectin chains. Crystallite formation increases granular stability, thus reducing the extent of granular swelling [20]. The percent solubility of sago starch granules increases significantly from B to AM stage.

3.7 Particle Size and Distribution Analysis

Table 4 shows the mean particle size and distribution analysis of starch from sago palms from plawei (P) stage to angau tua (AT) stage. The largest mean diameter was observed at angau muda (AM) stage which was 33.3 μm. The calculation of particle size involved the basic assumption that all particles are spherical and of uniform density. In this case, the density was taken to be 1.0 g/cm$^3$ [15].

The distribution of granule size observed from the D (n, 0.1) and D (n, 0.9) values shows that the starch granule size increased as the palm matured to the latest growth stages. From the results obtained, it was observed that most of the granules has smaller size granules for all growth stages except for bubul stage. This result is different compared to sago palm grown in peat soil at different growth stages [3] where the starch granules distributed more towards higher size at base height.

Table 4  Particle size and distribution of sago starches derived from sago palm at different growth stages at two different heights

| Sample  | Diameter (µm) | Distribution (µm) | Range       |
|---------|---------------|------------------|-------------|
|         | Mean          | Median           | D (n, 0.1)  | D (n, 0.9)  | D(n,0.9) - D(n,0.1) |
|         | D [4, 3]      | D [n,0.5]        |             |             |                   |
| Plawei  | 26.8 ± 0.07   | 26.7 ± 0.03      | 20.9 ± 0.31 | 33.6 ± 0.14 | 12.7 ± 0.44       |
| Bubul   | 29.0 ± 0.04   | 28.3$^{b}$ ± 0.03| 20.9 ± 0.05 | 38.5 ± 0.15 | 17.6 ± 0.19       |
| Angau Muda | 33.3 ± 0.03 | 32.5$^{c}$ ± 0.02 | 24.7 ± 0.18 | 43.4 ± 0.08 | 18.7 ± 0.11       |
| Angau Tua | 32.2 ± 0.51  | 31.8$^{d}$ ± 0.22 | 23.4 ± 0.23 | 42.3 ± 1.09 | 18.9 ± 0.86       |

D [4, 3] is the volume mean diameter; D (n, 0.1) is the 10% cut-off point as ten percent of the distribution is below this point; D (n, 0.5) is the volume median diameter and divides the distribution exactly in half; D (n, 0.9) is the ninety percent cut-off point as ninety percent of the distribution is below this point. Values are means ± SD (n = 3).

4. Conclusions

The differences in some physico-chemical properties of sago starches were contributed by the difference in the growth stages. This study revealed that starch at angau muda (AM) stage have significant difference i.e. high starch content, swelling power, solubility and mean diameter compared to the other
growth stages. Proximate analysis showed low protein, ash and fat content for all starch samples. Starch content is high with more than 90% value with the highest value during AM stage. SEM micrographs showed that sago starch granules varied between 20 – 60 μm. X-ray diffraction study depicted that sago starches exhibit C-type of starch crystallization. The swelling power is significantly high during B and AM stage while the solubility increased significantly towards AM stage. Starches at B stage were found to retrograde easily as shown by the highest setback value. AM stage showed the highest mean diameter of starch granules while the particle distribution is similar for most of growth stages. When comparing to sago starches grown in peat soil, sago starches grown in mineral soil was found to have lower swelling power and enthalpy of gelatinization, and larger mean size diameter of starch granules. Both sago palm from peat soil and mineral soil showed the highest starch content during AM stage.

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