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Extraction and Characterization of Non-Starch Polysaccharides from Different Growth Stages of Sago Starch

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Abstract: Extraction of non-starch polysaccharides from the pith of sago (*Metroxylon* spp.) at four growth stages named as Plawei (palm at maximum vegetative growth), Bubul (flowering structure), Angau Muda (well develop flowering) and lastly Angau Tua (fruiting palm) was conducted using enzyme extraction method. The extracted NSP was classified into water-soluble polysaccharides (WIP) and water-insoluble polysaccharides (WIP). The percentage yield of WSP is 0.34-0.80%, significantly lower than WIP (8.32 to 10.19%) and only WIP were used throughout the study. Fourier Transform Infrared Analysis (FTIR) were conducted to verify the WIP and absorption bands was found at 3200-3600/cm (O-H), 2850-3000/cm (C-H), 2358/cm (N-H), 1600-1550/cm (C = C in aromatic rings), 1200-1100/cm (C-O-C) and lastly 1050-1150/cm (C-O). Therefore, the predominant components of extracted NSP are cellulose, hemicellulose, pectens and lignin. The particle size distribution of NSP showed decreasing trend as the age of the plant increase from Plawei (91.16 µm), Bubul (83.66 µm), Angau Muda (63.93 µm) and Angau Tua (46.54 µm). SEM showed the structure of WIP have been ruptured due to extensive extraction, looks like 'thin folded structure'. Then, 5% of NSP were added into the flour and analysis on physico-chemical properties were conducted. In terms of proximate analysis, only moisture and crude fiber content of NSP flour showed a significant increased compared to normal flour.

Key words: Sago starch, non starch polysaccharide, fibers, flour

INTRODUCTION

Recently, with the growing of public awareness of nutrition and health care, research evidence is keep increasing on the potential of dietary fiber (DF) and its beneficial health effects for human and their positive action towards modification of dough properties in wheat dough. DF is principally the non-starch polysaccharides (NSP) of the plant origin that is not digested by endogenous enzymes within human intestinal tract, but it being marked as important component of human diet (DeVries *et al*., 1999).

Starch is the predominant constituent of the sago pith, which comprised over 80% dry weight of the pith. Besides starch, sago pith also contains small amounts of non-starch polysaccharides (NSP). On the other hand, the sago pith is composed of only a small amount of NSP (11.3%) and lignin (4.8%). Lignin is not a carbohydrate. It can be described as a group of polymerized aromatic alcohols formed in the cell wall structure from ester bound phenolic carboxylic acid (Akhbar *et al*., 2011). Non-starch polysaccharide is plant cell wall carbohydrates which are divided into cellulose and hemicelluloses which is contain arabinose, xylose, mannose, glucose and galactose residues as the principle constituent sugars (Subba Rao *et al*., 2004). The presence of lignins, on the other hand, influence the woody structural rigidity by stiffening and holding the fibres together (Tania *et al*., 2006).

Over the year, there are a lot of research had being done about characterization of non-starch polysaccharide and its benefit in food industry and health benefit to human and animals. Influence of non-starch polysaccharides isolated from wheat flour on the gelatinization and gelation of wheat starches had being done by Sasaki *et al*. (2004) and find that adding an isolated NSP rich fraction to starch markedly affected starch gelatinization and pasting properties in a positive way. Other than that, Shyama *et al*. (2007) study about functional properties of water-soluble non-starch polysaccharides from rice and ragi and their effect on dough characteristics and baking quality. In health benefit to human, NSP act as a marker to nutritional labeling of some product especially in food industries and dietary fiber supplement (Abdorreza *et al*., 2012; Bhupinder *et al*., 2011).

There are many source of dietary fiber from plant such as from root, tuber, cereals and legumes and also from palm source. The NSP content or dietary fiber mainly studied on cereals source especially in wheat. This is because wheat is one of the most common starches used in bakery product where the properties of the NSP form wheat have being in widely investigated. Usually, the study of NSP mainly focused on physiological,

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psychochemical, rheological properties and also chemical composition of the NSP and how it effect the rheological properties and characteristic of flour dough. In this paper, NSP was extracted from sago palm at different growth stages. Study on sago palm has been mainly focused on the physicochemical properties and the utilization. Very little information in known regarding the NSP from sago palm. Therefore, the purpose of this project is to investigate the characterization of extracted NSP from sago palm (*Metroxylon sagu*) at four growth stages (Plawei, Bubul, Angau Muda and Angau Tua) by analyzing their effect to psychochemical properties, morphology and functional properties when added into sago flour.

MATERIALS AND METHODS

Materials: Sago pith with four different growth rate (Plawei, Bubul, Angau Muda and Angau Tua) was supply from Craun Research Sdn. Bhd. at Kuching Sarawak. Sago flour were obtain from the processing of raw sago pith into flour. Enzyme protease, amyloglucosidase and alpha amylase was obtained from Sigma-Aldrich (M) Sdn. Bhd. Other chemical were analytical graded.

Sample preparation

Preparation of sago flour: Sago flour at four different growth rate (Plawei, Bubul, Angau Muda and Angau Tua) was prepared by using the method of (Shifeng *et al*., 2012) with slight modification. Sago pith was washed and then cuted into 5 mm thick slices by knife and dried in hot oven at 50°C for 24 h and grinded by centrifugal mill for 3 min to make sago flour. The flesh of the sago pith were pass trough a seiver of 250 µm aperture by seiving machine at a speed of 80 rpm for 15 min to get the fine flour of sago pith for each growth rate.

Extraction of non-starch polysaccharide (NSP): The extraction of non starch polysaccharide was done by using enzymatic extraction method from the simplified modification method of (Fasihuddin *et al*., 1999). In this method, starch sample was extracted into Water Soluble Polysaccharide (WSP) and Water Insoluble Polysaccharide (WIP). The sago flour (10 g) was homogenized with 300 ml sodium phosphate buffer (0.08M at pH 6.0) and mix with 750 µL α-amylase in conical flask (500 mL). The suspension then heat in boiling water bath for 20 min and shake every 5 min. After 20 min, the sample being cool to adjust the pH to 7.5 by using sodium hydroxide 0.275M and 300 µL enzyme protease was added proceed with incubation at 60°C with continuous agitation for 30 min. After adjusting pH to 4.5 using hydrochloric acid 0.325M, 350 µL enzyme amyloglucosidase was added and incubated at 60°C for 30 min. The sample then centrifuge at 3500 rpm for 10 min and washed 2 times with hot water (combine with supernatant for preparation of WSP), 2 times with 95 % ethanol and acetone. The residue then left overnight in oven at 60°C to get WIP. Preparation of WSP involve mixing of 95% ethanol (preheated to 60°C, 4 volumes) with supernatant and was left overnight at room temperature to form precipitate. The precipitate then collected by centrifuge at 3500 rpm and washed 2 times with 78 % ethanol, 95 % ethanol, acetone and proceed by dry in oven at 60°C to get WSP.

Characterization analysis of NSP

Percentage yield of NSP: NSP extracted from sago pith at four growth stages (Plawei, Bubul, Angau Muda and Angau Tua) was weight and recorded as percentage yield of Water Soluble Polysaccharide (WSP) and Water Insoluble Polysaccharide (WIP). The calculation is shown as below:

$$\text{Percentage yield (\%)} = \frac{\text{Yield of NSP}}{\text{Weight of flour (sago)}} \times 100\%$$

Determination of moisture: Moisture content of flour with or without 5% WIP extract was determine by using moisture analyzer (ANDMAX-50).

Determination of ash: Total ash of sago flour with or without 5% WIP has being measure its total ash content by using dry ashing method (AOAC 923.03).

Determination of protein: The crude protein of sago flour with or without the addition of 5% NSP were determined by using Kjeldahl Method (AOAC 955.04).

Determination of fat: Fat content from sago flour with or without 5% WIP was determined using soxhlet method (AOAC method number 920.39).

Determination of crude fiber: The content of crude fiber in sago flour with or without 5% WIP was determined by using Gravimetric Method (AOAC Number: 935.53). 1.0 to 2.0 g of dried and defatted sample are weighed into the round bottom flusk. 150 mL of distilled water and 50 ml of 5% H_2SO_4 are added into the flusk. The flask was connected to the condenser and the mixture are reflux for 30 min. 5 mL of NaOH is added and the excess acid is neutralized by using 40% NaOH (phenolphthalein is use as indicator) and then another 10 mL of NaOH 25% is added. The mixture was reflux again for 30 min. The hot mixture is filtered by using weighed filter paper. The precipitate are washed by using 1% HCl follow by hot water to remove the acids (it was tested using indicator). The precipitate are then washed by using the spirit methyl, then the filter paper is transfer into the crucible (pre weighed). The precipitate are vaporized until dry on the water bath and was transfer into the oven 105°C until constant weigh is obtained and it then being transfer into the desiccator to cool it down. The crucible and the sample are weighed. The crucible then was burned in a fume cupboard. The crucible with the ash then are transferred into a muffle furnace (450°C) until no more black spot exist. The crucible with ash were cool in desiccator and is weighed again. The calculation of crude fiber content are based on the following formula:

$$\text{Crude fiber (\%)} = \frac{(A-C)-B}{S} \times 100\%$$

S = Weight of dry and defatted sample
C = Weight of ash free filter paper
A = Weight of crucible+filter paper+dried precipitate
B = Weight of crucible+ash

Swelling and solubility: Swelling and solubility power of sago flour with or without 5% WIP was determine by using the modified method of Sasaki *et al.* (2004).
Swelling and solubility of the sago flour with or without 5% WIP were calculated using the following formula:

$$\text{Swelling (g/g)} = \frac{\text{Weight of wet sediment (g)}}{\text{Weight of initial dry starch (g)}}$$

$$\text{Sollubility (\%)} = \frac{\text{Weight of dried supernatant (g)}}{\text{Weight of initial dry starch (g)}} \times 100\%$$

Fourier transform infrared (FTIR) analysis: The FTIR transmittance spectra was analyzed by using Nicolet iS10 FT-IR Spectrometer modification method of Yaacob *et al.* (2010) in the range of 4000-400/cm. The powder sample was first form in the form of pallet with the mixture of sample powder (in about 0.5 mg) and mix with Potassium Bromide (KBr) until the weight reach approximately 100 mg. The absorption range value was used to analyze the existence of group of compound presence in the sample.

Particle size and distribution analysis (PSD): The analysis was done by using Long Bench Mastersizer S (Malvern Instrument) fitted with Qspec Dry Powder Feeder modification method from (Pei-Lang *et al.*, 2008). Sago starch powder with 5% of WIP and extracted WIP itself was place in the powder feeder in a enough amount for the instrument to analyze the sample. The values of mean diameter was measured as D (4, 3), D (n, 0.1), D (n, 0.5) and D (n, 0.9), respectively.

Scanning electron micrograph (SEM): The morphological features of the WIP extract together with sago flour were observed with Scanning Electron Microscopy (EVO-MA10). The dried samples were coated with gold to make the sample conductive and micrograph were recorded the images at different magnification. The morphology of the flour at four sago palm growth stages and NSP extract can be identified.

Statistical analysis: All samples were analyzed at least in triplicates. In this analysis, the effect of addition NSP to the properties of sago flour at different growth stages are the factor to compared in this project. Statistical analysis was carried out by using the SPSS (version 16.0) software data analysis with ANOVA (Duncan's multiple range test) method at $\alpha = 0.05$ (95% confidence level).

RESULTS AND DISCUSSION

Percentage yield of NSP: The extraction of NSP was carried out by using enzymatic extraction method that can divide the extraction of NSP into two types, Water Soluble Polysaccharide (WSP) and Water Insoluble Polysaccharide (WIP). Apart from sago granules, the pith of the sago trunk also contains most of the constituents, named cellulose, hemicelluloses, other cell structural materials, soluble solids and unidentified traces of other substances (Karim *et al.*, 2008). Non-Starch Polysaccharide is one of the component presences in the sago pith other than starch.
During the extraction, semi-crystalline starch is gelatinized by heating in the presence of sodium phosphate buffer and then the starch is broken down and solubilized by specific enzymes, strong acid or strong alkali. The glucose is then separated from NSP by filtration or separated from total fiber by selective precipitation of the NSP with ethanol solutions. Starch is completely removed enzymatically and NSP measured as the sum of its constituent sugars released by acid hydrolysis (Akhbar *et al.*, 2011). In this project, WIP extract is the one used for further analysis instead of WSP. This is because the percentage yield of WSP extracted is very low to be used for analyzing. In Table 2 the yield for WSP is very low compared to WIP and the percentage yield of WIP and WSP varied between the four growth stages. The highest percentage yield of WIP is occurred at Angau Muda stage in about 10.85% followed by Plawei and Angau Tua stage. At Bubul stage, however, the yield is the lowest in about 8.32%. This is supported by Pei-Lang *et al.* (2008) who stated that NSP decreased from Plawei to Angau Tua stages and increased as the palm matured to Angau Tua stage at mid height. However at base height, NSP is lowest to late Angau Tua.

Table 1: Percentage Yield (%) of extracted NSP from sago pith at different growth stages

	Percentage yield (%)	
Growth stage	WIP	WSP
Plawei	10.19 ± 0.85^{b}	0.34 ± 0.05^{a}
Bubul	8.32 ± 0.43^{a}	0.60 ± 0.04^{c}
Angau Muda	10.85 ± 0.27^{b}	0.45 ± 0.02^{b}
Angau Tua	9.51 ± 0.12^{b}	0.80 ± 0.06^{d}

WIP: Water Insoluble Polysaccharide
WSP: Water Soluble Polysaccharide
Values are mean±standard deviation (N = 3) and with different superscript letters indicate significant difference at $\alpha = 0.05$ (95% confidence level)

Table 2: Proximate value of samples with or without addition of 5% WIP in sago flour at four different growth stages

Stages	Sample	Moisture content (%)	Ash content, dry basis (%)	Fat content, dry basis (%)	Protein content (%)	Crude fiber content (%)
Plawei	F	9.78 ± 0.12^{d}	2.28 ± 0.00^{a}	0.77 ± 0.06^{a}	1.29 ± 0.00^{a}	0.51 ± 0.06^{a}
	FN	10.58 ± 0.08^{e}	2.65 ± 0.09^{ab}	0.99 ± 0.21^{ab}	1.52 ± 0.06^{bc}	1.30 ± 0.1^{c}
Bubul	F	8.21 ± 0.14^{a}	2.49 ± 0.06^{a}	0.59 ± 0.69^{a}	1.44 ± 0.19^{ab}	0.78 ± 0.0^{b}
	FN	9.55 ± 0.05^{c}	2.80 ± 0.06^{ab}	1.17 ± 0.08^{abc}	1.50 ± 0.04^{bc}	1.62 ± 0.06^{d}
Angau Muda	F	9.38 ± 0.07^{b}	3.45 ± 0.01^{bc}	0.91 ± 0.14^{ab}	1.34 ± 0.07^{ab}	0.69 ± 0.03^{ab}
	FN	9.80 ± 0.06^{d}	3.81 ± 0.07^{c}	2.02 ± 0.63^{c}	1.47 ± 0.01^{abc}	1.59 ± 0.03^{d}
Angau Tua	F	9.30 ± 0.03^{b}	4.19 ± 1.09^{c}	0.66 ± 0.01^{a}	1.65 ± 0.00^{cd}	0.65 ± 0.14^{ab}
	FN	9.76 ± 0.05^{d}	4.10 ± 0.10^{c}	1.82 ± 0.40^{bc}	1.81 ± 0.03^{d}	1.39 ± 0.01^{c}

F: Flour without addition 5% WIP FN: Flour with addition 5% WIP

Values are mean±standard deviation (N = 2) and with different superscript letters indicate significant difference at α = 0.05 (95% confidence level)

Table 3: Sago starch and WIP distribution range at four different growth stages

		Diameter (µm)		
Sago flour	Sample	Mean D{4,3}	Median D{n,0.5}	Range
Plawei	FN	153.53 ± 9.05^{d}	42.16 ± 1.37^{c}	15.73^{b}-539.58^{e}
	N	190.72 ± 23.97^{e}	91.16 ± 15.15^{e}	22.68^{e}-548.86^{e}
Bubul	FN	53.14 ± 4.62^{ab}	34.35 ± 0.04^{ab}	14.36^{b}-84.16^{ab}
	N	90.60 ± 5.42^{c}	63.93 ± 0.99^{d}	19.22^{c}-167.26^{c}
Angau Muda	FN	55.58 ± 3.18^{ab}	34.42 ± 1.01^{ab}	14.16^{b}-93.24^{ab}
	N	143.88 ± 9.41^{d}	83.66 ± 0.72^{e}	23.10^{d}-374.22^{d}
Angau Tua	FN	37.50 ± 3.79^{a}	29.63 ± 0.17^{a}	11.31^{a}-57.02^{a}
	N	60.82 ± 4.70^{b}	46.54 ± 0.58^{c}	14.68^{b}-121.32^{bc}

FN: Flour with addition 5% WIP N: Water Insoluble Polysaccharide (WIP)

Values are mean±SD deviation (N = 2) and with different superscript letters indicate significant difference at α = 0.05 (95% confidence level)

Table 4: Swelling power (g/g) and solubility (%) of sago starch at different growth stages with or without addition 5% of WIP

Type of flour	Type of sample	Swelling power, g/g	Solubility, (%)
Plawei	F	12.55 ± 0.17^{ab}	6.59 ± 0.25^{ab}
	FN	13.75 ± 0.34^{b}	6.03 ± 0.4^{a}
Bubul	F	12.64 ± 0.44^{a}	6.47 ± 0.06^{ab}
	FN	13.32 ± 0.10^{a}	6.03 ± 0.4^{a}
Angau Muda	F	12.21 ± 0.31^{a}	7.36 ± 0.47^{bc}
	FN	12.40 ± 0.8^{a}	6.62 ± 0.92^{ab}
AngauTua	F	12.37 ± 0.68^{a}	7.98 ± 0.31^{c}
	FN	12.39 ± 0.0^{a}	5.62 ± 0.43^{a}

F: Flour without addition 5% WIP FN: Flour with addition 5% WIP

Values are mean±SD deviation (N = 2) and with different superscript letters indicate significant difference at α = 0.05 (95% confidence level)

WIP is consider as insoluble dietary fibre (IDF) is the main structural component of plant cell wall that insoluble in concentrated alkali, insoluble in water but soluble in concentrated acid which includes celluloses, some hemicelluloses and lignin (Ishiaku *et al*., 2002). According to Pei-Lang *et al*. (2006), the WIP and Total non-starch polysaccharide (TNSP) content in the sago pith are important for mobilization of starch in preparation of fruiting stages of sago palm.

Proximate analysis: Table 2 showed that the proximate value of samples with or without the addition of 5% WIP in sago flour at four different growth stages. From Table 2, the moisture content was significantly affected when 5% of WIP was added into the flour. According to Karim *et al*. (2008), the moisture content of sago flour commercial range from 10.6-20.0% and untreated sago flour contain 8.21-9.81% moisture lower than the commercial flour, because untreated sago flour does not under goes chemical treatment during processing that can increase the moisture content of flour. The addition of 5% of WIP was slightly increase the moisture content of the sago flour at all stages but does not have significant changes among the four stages. This is due to the most important attributes of WIP that is exhibiting significantly higher water solubility that ultimately leads to higher water or moisture absorption capacities and great water-holding capacity (Choct *et al*., 1997). Therefore, the moisture content was increase as the WIP is added into the flour where the small amount of moisture being trapped by the WIP that present in the flour.

Ash content slightly increase as the palm matured from Plawei to Angau Tua. Angau Tua has the higher (4.19%) and Plawei has the least value (2.28%) of ash compared to the others. Incensement value of ash content also can be observed when the flour in corporate with WIP at all stage except for Angau Tua where the ash content was slightly reduced from 4.19 to 4.04% when corporate with WIP. The increased amount of ash content when WIP was added is explained by the presence of more cell wall material (DeVries *et al*., 1999). This could due to the properties of WIP as one of the cell wall component that also contain minerals.

From the result, no significant changes of fat values were found in Plawei and Bubul stages. Angau Muda stages showed the highest fat value compared to the others starch in about 0.91% and significantly increase to 2.02% when WIP was added. Most of the lipid comes from cell membranes and, in the starchy endosperm. Increasing of fat content when WIP is added may be due to the fat content in WIP that left during extraction and it can be concluded that the WIP extract was not 100% pure WIP.

The highest value of crude protein can be observed in Angau Tua stages where the value of F is 1.65% and Plawei is shown the lowest protein content. This may be due to the fact that protein content increased as the sago palm matured from Plawei to Angau Tua but decreased at Angau Muda stages. Plawei is the youngest stage (10 years old) which contain lowest amount of protein while Angau Tua (14 years old) is the late stage of sago palm that contain the highest amount of protein. Addition of NSP into sago flour increase the protein content of each sago flour at all growth stages. Increasing of protein content when WIP is added may be due to the protein content in WIP that left during extraction and it can be concluded that the WIP extract was not 100% pure WIP.

Crude fibre (CF) refers to the remnants of plant material after extraction with acid and alkali and includes variable portions of the insoluble NSP or WIP (Choct *et al*., 1997). The result showed the crude fiber in sago flour increased when NSP was added into the flour. The highest value of crude fiber content was at Bubul stages (0.78%) and increased to 1.62% when added with WIP. This is understandable as the crude fiber itself is also WIP component that give the health nutritional benefits especially as a dietary source in human body as they cannot be digested by human enzyme that give bulking effect in human digestive system (Salyavit *et al*., 2003).

Fourier transform infrared analysis (FTIR): FT-IR spectroscopy was used to verify the changes in the chemical structure of starch molecule resulting from various starch modification (Singh *et al*., 2012). However, in this study FT-IR analysis was done to identify the chemical structure occurred in NSP extracted from four different growth of sago palm flour.

WIP is cellulose part of plant cell wall that structured by α and β from parallel glucan chain in a flat-ribbon conformation with glucosyl units that locked in opposite orientation by intermolecular hydrogen bond (Subba Rao *et al*., 2004). The absorption band between the four growths stages of sago palm are significantly the same. The broad band in the 3200-3600/cm region is due to O-H stretching which is H-bonded group of alcohol of phenols and band at 2850-2980/cm is characteristics of C–H stretching vibration. At Plawei growth stages, there is a band 2358.35/cm that sharp and narrow compared to others due to N-H stretch. Band at 1620-1580/cm due to varies intensity of C = C stretch in aromatic compounds. Band at 1290-1180/cm consist of antisym due to C-O-C stretch ester and C-C-C stretch. At 1275-1200/cm band the C-O-C stretch by cyclic ethers and 1250-1025/cm due to C-H in plane bending that have 5 bands in phenyl group. Lastly, band at 1050-1150/cm represent C-O stretching vibration in the chemical structure of WIP.

As the predominant component of these fibers is cellulose and other major constituents (hemicelluloses and pectins) are also polysaccharides, the spectra of the samples are superficially similar between the four stages of sago palm. This band is also known as the “crystallinity band”, indicating that a decrease in its intensity reflects reduction in the degree of crystallinity of the samples (Kawaljit *et al*., 2007).

Particle size and distribution (PSD): Table 3 shows the value of diameter range of particle size of sago flour with 5% WIP (FN) and NSP (N) itself at four growth stages. From the result obtained in table below, the sago starch granule distribution range varied between the four growth stages and the sago flour with addition of 5% NSP (FN) and NSP itself and it can observed that sago flour with addition of 5% NSP have the bigger distribution range in the four type of growth stages.

Plawei stages showed the distribution range between 15.73-539.58 µm and slightly have an bigger distribution range of WIP between 22.68-548.86 µm. The distribution range of FN decrease as the palm grow from Bubul to Angau Tua but still have the higher distribution range when WIP is added compared to FN at every stages. According to Karim *et al*. (2008), sago starch granules have a broad size range between 10 and 50 µm. The difference in distribution of FN in every stages may due to the presence of NSP in the flour where it can observed that the image of FN and WIP from SEM showed the WIP clump and folded together. The structure of the WIP was not in a uniform shape like granules but more presence like ‘thin folded’ structure and hence the value distribution range of WIP itself is bigger and not have constant size distribution range than FN value. Since the distribution range of WIP is bigger, the distribution range of sago flour also increase when incorporated with WIP (FN).

Scanning electron microscopy (SEM): SEM was conducted to observed the structure of the WIP extracted and the sago starch granule itself compared to the commercial sago starch as a control. Figures 2a showed the image of native sago starch, 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 the number of truncated oval granules. The granules surface usually smooth but have pitting of granules on some sago starch granules.

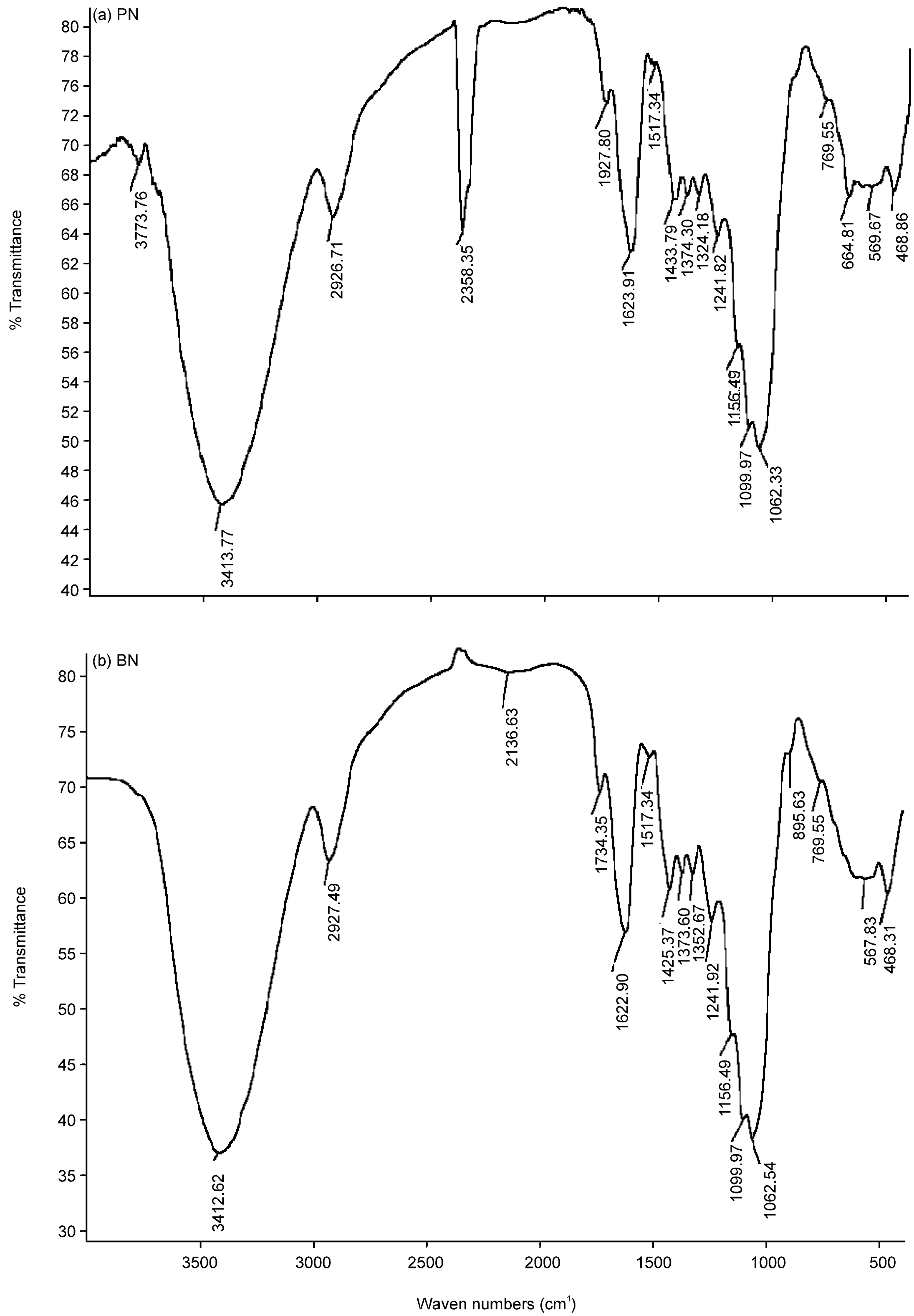

Fig. 1(a-d): Continued

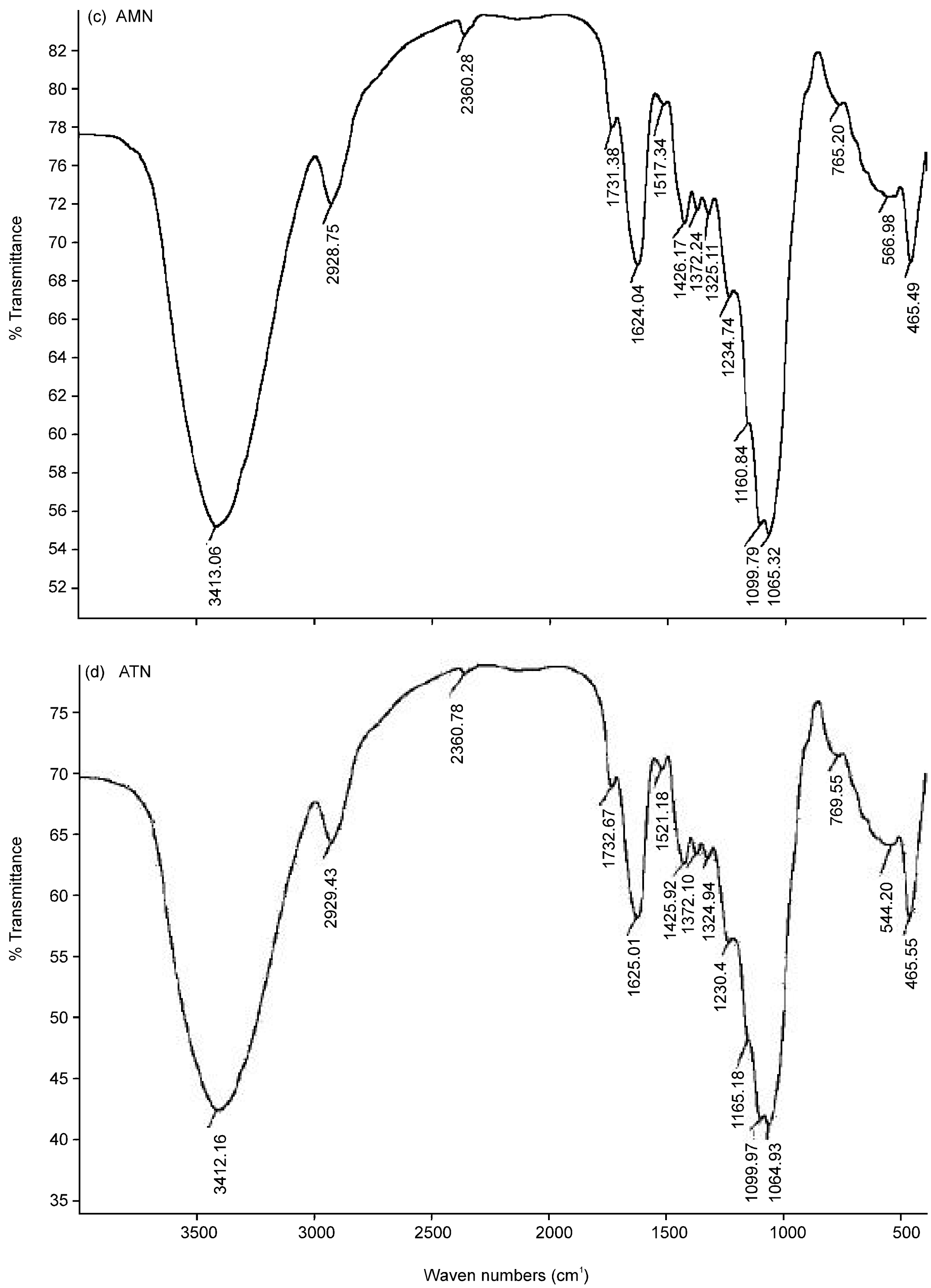

Fig. 1: FT-IR spectrum of WIP at Plawei stages (1a), Bubul (1b), Angau Muda (1c), and Angau Tua (1d)

The shape of the sago starch granules was almost similar at all stages but differ in range of particle size distribution. In Fig. 2b showed that the sago starch granules and WIP. The structure of WIP was very differ to the structure of sago starch and more looks like 'thin folded structure'. The size and structure of single WIP is hard to define as WIP more tend to clump with other WIP as they folded together to form a group of WIP as

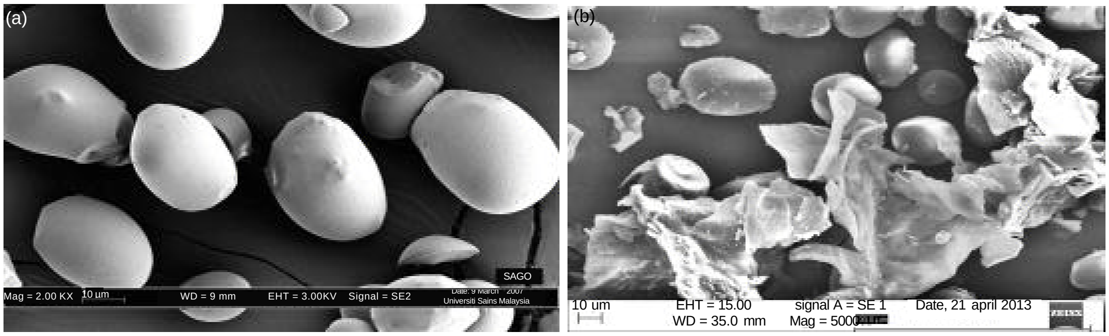

Fig. 2(a-b): Scanning Electron Microscopic images of native sago starch (2a) and sago starch with 5% WIP (2b) (500x magnification)

showed in Fig. 2b. This is because, during the extraction of NSP, the structure of granules have being ruptured due to the treatment with the enzyme, acid, alkaline and causing the release of starch from within plant cell wall.

Swelling and solubility: The swelling power of four different growth stages of sago palm was studied by heating the flour and flour with 5% insoluble NSP at 90°C of temperature in 30 min. The result obtained showed that there were almost no significant changes of swelling power value of sago flour at all stages but slightly increase of swelling power and decrease solubility index when starch alone and of WIP mixture is compared. WIP contribute to water absorption of flour and considered to significantly increase swelling power by only a 3% addition to starches due to its great water-holding capacity (Sasaki *et al.*, 2004).

The difference in amylose content had a strong impact on swelling power. Starch swelling is mainly a property of amylopectin inversely correlated with the amylose content of the sago starch at different growth stages. Sago starch contains 27% amylose and 73% amylopectin. However, amylose content of the starch are further increases with growth. It is report that, relative crystallinity of starch is higher in a 14.5-year-old palm than a 9-year-old palm (Singh *et al.*, 2012).

Cellulose is a component of insoluble NSP which is insoluble in water, alkalines and dilute acids. Same with solubility of starch alone had significantly higher solubilized starch than the starch mixed with WIP because the WIP rich fraction inhibited the starch and amylose solubilization (Sasaki *et al.*, 2004; Mohamed *et al.*, 2008). Solubility of NSP, in turn, depends on the chemical structure of the WIP and their association with the rest of the cell wall components (Choct *et al.*, 1997). Size of the molecule, whether it is branched or linear, the presence of charged groups, surrounding structures and concentration are all important factors affecting their solubility in aqueous media. The higher solubility for these is most probably due to the lower molecular mass of their amylose fraction.

The difference in amylose content had strong impact on swelling power because starch swelling is mainly property of amylopectin inversely correlated with amylose content of sago starch at different growth stages. According to Karim *et al.* (2008), sago starch contain 27% amylose and 73% amylopectin. However amylose content of the starch are increase as the sago palm mature to Angau Tua. Therefore, as the growth stages increase from Plawei to Angau Tua, swelling power will decrease.

Conclusion: This research is mainly focused on extraction and characterization of non-starch polysaccharide from sago pith (Metroxylon sago) at four different growth stages and the influenced of NSP extracted on sago starch. The percentage yield of WIP extract is higher than percentage yield of WSP. NSP tend to affect the functional and psychochemical, properties of sago flour when added into the flour. There is not much significant difference occurred in proximate, swelling power and solubility of sago flour between four growth stages. Morphology size and shape of starch granule and WIP is significantly different as the NSP structure is the glucose that separated from NSP by filtration or separated from total fiber by selective precipitation of the NSP with ethanol solutions and starch is completely removed enzymatically. FTIR analysis showed the presence of absorption bands at 3200-3600/cm (O-H), 2850-3000/cm (C-H), 2358/cm (N-H), 1600-1550/cm (C = C in aromatic rings), 1200-1100/cm (C-O-C) and lastly 1050-1150/cm (C-O). Therefore, the predominant components of extracted NSP are cellulose, hemicellulose, pectins and lignin.

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