Effect of Acetylation on Physicochemical Properties and Resistant Starch Content of Metroxylon sagu Starch

Siti Aisyah Zaman[1], Awang Zulfikar Rizal Awang Seruji[2], and Shahrul Razid Sarbini[1]

***Abstract*—**Physicochemical properties and resistant starch content of acetyylated Metroxylon sagu starch with different acetylation levels has been investigated. The ASS was prepared by acetylating native sago starch suspended in alkaline solution (35% (w/w), pH 8-8.4) using vinyl acetate (VA) at different concentrations ranged from 1% to 2.5% (w/w of starch) at 30°C. Solubility, swelling power, amylose and resistant starch content were evaluated to characterize the effect of acetylation on sago starch. The RS content of each modified starch were measured based on the method approved by AOAC 2002.02. Amylose were determined using amylase/amylopectin assay kit (Megazyme, Wicklow, Ireland). Solubility and swelling power increases with increasing level of acetylation. Acetylation at 2% (w/w) vinyl acetate showed the lowest amylose content but the highest resistant starch content at 11.03% and 73.79% respectively. The results suggested that acetylation is a suitable chemical modification to increase resistant starch content without sacrificing functional physicochemical properties of the sago starch.

***Index Terms*—** Starch. Acetylation, Resistant starch, Amylose.

I. Introduction

Metroxylon sagu or commonly known as sago is one of the major agricultural commodity in Sarawak, with an annual export estimated at 60000-70000 tonnes, worth up to USD 10 millions. Compared to other industrial starch sources, the depth of the research involving this indigenous tropical species can be considered scarce. Sago starch were obtained from its trunk, different to other common starches that are derived from cereals, tubers, roots and legumes and its starch yield per unit is also relatively higher than that of rice, corn, wheat and cassava.

Resistant starch (RS) is defined as the sum of starch and the product of starch digestion that resist digestion in the small intestine of a normal human being [1]. It may reach the colon where it will be fermented by the gut microbiota, producing a variety of products which includes the short chain fatty acids that is physiologically beneficial towards the host. There are 4 classes of resistant starch; RS type I, physically inaccessible starch; RS type II, resistant starch granules; RS type III, retrograded starch and RS type IV, chemically modified resistant starch.

Modification of sago starch to increase the RS content will be valuable to increase the competitiveness of sago as an agricultural commodity. It will allow the introduction of sago starch as not only staple food but as a potential nutritional food ingredient. With its colourless and odourless characteristics, it will be suitable as food ingredients as it will not influence the final taste and colour. Resistant starch has also been shown to increased swelling power and peak viscosity, gel formation and water holding capacity, thus making it a desirable ingredient to be used in food [2].

The objective of the study is to evaluate the effect of different levels of acetylation on physicochemical properties of sago starch. It is also to evaluate the potential of using acetylation process to increase the resistant starch content from its native counterpart.

II. Procedure

A. Materials

Unless stated otherwise, all reagents and chemicals used were purchased from Sigma laboratories (Gillingham, Dorset, UK).). Fibersym® was provided by MGP Ingredients, Inc (Atchison, KS, US)

B. Sago starch production

Sago log were obtained from the sago plantation of CRAUN Research Inc., located at Paya Paloh, Sarawak. The logs were debarked using an automated rasper. The resulting powdered-like shredding were washed thrice with tap water and left for an hour to allow the starch to sediment. The resulting starch are then dried at low temperature for 2 days, grinded using blender and sieved through 0.3mm sieve (ABSS, Victoria, Australia).

C. Production of acetylated starch

The sago starch slurry was prepared by dispersing the starch in distilled water (100 g starch, 35 % w/v). Vinyl acetate (1%,1.5%,2%,2.5%, gram of vinyl acetate to dry weight of starch) was added to the slurry with simultaneously stirring at 35^0C while maintaining pH within a range 8.0–8.4. The reaction was allowed to proceed for additional 5 min before the reaction was ended by decreasing the pH to 5. The resulting starch is then vacuum filtered, washed with 5 volumes of water and oven dried. Once dry, the starch powders were sieved through 0.3mm sieve (ABSS, Victoria, Australia).

Siti Aisyah Zaman1[1] and Shahrul Razid Sarbini[1] Department of Crop Science Universiti Putra Malaysia Bintulu Campus Jalan Nyabau97008 Bintulu, Sarawak Malaysia.

Awang Zulfikar Rizal Awang Seruji[2] Craun Research Inc. Jalan Sultan Tengah 93055 Kuching, Sarawak Malaysia.

D. Measurement of solubility and swelling power

A 2 g (W0) sample were weighed in a centrifuge tube and distilled water was added until the weight reaches 180 g. The mixture was stirred to allow complete mixing before heated at 85°C for 30 minutes in a shaking water bath before they were centrifuged for 1 minute at 2200 rpm. The resulting supernatant were poured into a pre-weighed test tube and dried at 105°C until a constant weight is reached (W1). The sediment paste weight (W3) was recorded. Solubility and swelling power were calculated as follows;

Solubility percentage (%) = (W1/W0) X 100
Swelling power (g/g) = (W3 X 100) / (W0 X (100 – solubility)

E. Amylose content determination

Amylose portion was analysed using amylose/amylopectin assay kit (Megazyme, Wicklow, Ireland) based on a method by Yun and Matheson (1990), with slight modification. The samples were pretreated with dimethyl sulphoxide with heat to remove lipid. The precipitated sample was then dissolved in an acetate/salt solution. Amylopectin was then precipitated with the addition of Concanavalin A, and removed by centrifugation. The amylose contained in the supernatant was analysed using glucose oxidase/peroxidise, after enzymatic hydrolization to D-glucose. The total starch, in a separate aliquot of the acetate/salt solution was also similarly hydrolysed to D-glucose and analysed with GOPOD. Percentage of amylase in the starch sample is the ratio of GOPOD absorbance at 510 nm of the supernatant of the amylose to that of the total starch sample.

F. Resistant starch determination

Resistant starch is analysed using Megazyme resistant starch assay based on the method of AOAC 2002.02 and AACC 38-40. The basic principle is that the sample is first digested with alpha amylase and amyloglucosidase (AMG), the residue pellet is then solubilized with potassium hydroxide and the resulting RS pellet will be recovered. Finally, the resistant starch is hydrolyzed to glucose by AMG.

G. Statistical analysis

Each experimental process was done in triplicate. Statistical analysis was performed using SPSS for Windows, version 20.0. One-way analysis of variance (ANOVA) and posthoc Tukey's test were used to determine the significant difference between substrate. Differences were deemed significant when $P < 0.05$.

III.. Result and Discussion

A. Solubility and swelling power

Water solubility and swelling power are two functional properties of starch when dispersed in water[3]. The swelling power of starch is measured as the ratio of the wet weight of the sediment gel to its dry weight[4], while solubility is defined and calculated as the percent of dry matter in the supernatant[5]. Solubility and swelling power of starch, once dispersed in water, are often dependent on temperature and pH of the water. In cases of modified starches, the type and degree of modification will influence and changes its solubility and swelling power properties compared to its native form. Figure 1 illustrate the solubility and swelling properties of native and acetylated starches respectively.

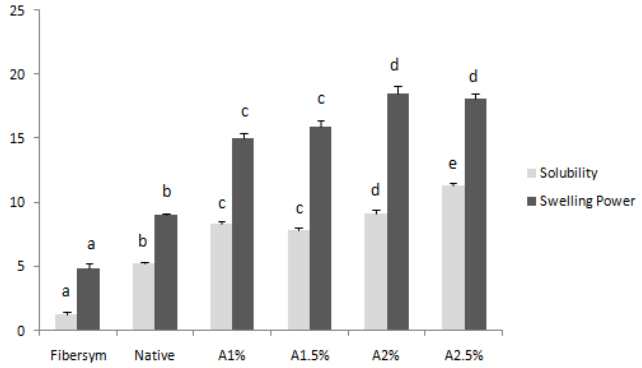

Fig. 1: Solubility And Swelling Power Of Fibersym, Native Sago Starch (Native), And Acetylated Starches (A1%, A1.5%, A2%, A2.5%). The A1%, A1.5%, A2%, A2.5% Respectively Represent 1%, 1.5%, 2% And 2.5% Of The Percentage Of Vinyl Acetate Used In Modification. Significant Differences Among Substrates ($P< 0.05$) Were Indicated With Different Letters Above Bars

All levels of acetylation significantly increase the solubility and swelling capacity of sago starch as opposed to its native form ($P < 0.05$). Swelling is a property of the amylopectin, and its value decreased together with amylose content. As sago starch has relatively lower amylose content compared to amylopectin, this may explain the generally lower swelling power of native starch. However, sago starch solubility and swelling power has been recorded to be higher than wheat starch [6]. This is due to its amylose fraction's higher molecular mass compared to wheat [7]. This is corroborated by our result, where Fibersym, a commercialized cross-linked wheat starch, showed a relatively low solubility and swelling power.

Acetylated starch shows an increase in swelling power and solubility. There's a slight decrease of solubility as the degree of acetylation increases, but final degree of acetylation shows a significantly higher solubility ($p<0.05$). The same results are shown in acetylated corn, potato and breadfruit starches [8-10]. This may be due to the introduction of bulky acetyl group. As a result of steric hindrance, structural organization of the starch occurs. Starch molecules repulse, facilitating an increase in water percolation within the amorphous regions of the granules, increasing its swelling power [9]. The stuctural reorganization could also weaken the starch granules, increasing the rate of amylose leaching, therefore increasing its solubility.

B. Amylose and resistant starch content

Different chemical modification of starch influence amylose and resistant starch content differently. These two characteristics of starch are often inter-connected. Depending on the type of starch and its botanical origin, the impact of

the amylose content on RS content, and vice versa, varies. The data are shown in the Table 1.

TABLE I
AMYLOSE AND RESISTANT STARCH CONTENT OF SAGO STARCH (NATIVE) AND ACETYLATED STARCHES

Modification	Percentage of modification	Amylose content (%)	Resistant starch content (%)
Fibersym	-	36.77^{i}	80.11^{j}
Native	-	19.69^{abc}	68.99^{h}
Acetylation (A)	1%	29.69^{def}	61.33^{efg}
	1.5%	21.92^{bcd}	69.94^{hi}
	2%	11.03^{a}	73.79^{i}
	2.5%	33.19^{ef}	50.62^{c}

Acetylation improved resistant starch content of native sago at 2% acetylation. However, it also showed the lowest amylose content. Other levels of acetylation, though did not contribute to a increase in RS, showed a significant increase of amylose content at 1% and 2.5%. Fibersym showed the highest RS and amylose content. From the results, we can conclude that there are no definitive pattern linking amylose content and resistant starch content. Though amylose content is often associated with RS content, amylopectin also plays an integral part in starch indigestibility, particularly in high amylopectin containing starch. The formation of RS from amylopectin could be attributed to several factors. This includes an increased entanglement of the molecules in the gel network, or an increased molecular order by the helix formation of the outer short chains of amylopectin, or by an organization of these helices in three dimensional, partially crystalline structures.

Acetylated starch shows an increase of resistant starch content compared to native sago starch at 1.5% and 2% (vinyl acetate to dry weight of sample), before decreasing again at 2.5%, which is the FDA limit on vinyl acetate allowed in food. Ostergard et al. [11] postulated that the increase on RS content is due to the positioning of acetyl group on C2 of the glucose unit where it sterically hinder the proper positioning of the substrate into the active site of amylase, thus increasing its resistance. This would make it difficult for the glutamic acid residue in the catalytic site to donate a proton to the glycosidic bond oxygen. Another study by Xu et. al. [12] with acetylated rice starch shows the highest RS content at 5.3% acetyl content with RS content of 69.45%. They suggested that the level of acetyl group substituted into the starch structure does not influence the overall RS produced. As this can explain the narrow changes in RS content between 1%, 1.5% and 2% acetylated starch, however, the RS content dropped drastically at 2.5% acetylated starch to only 50.6%, much lower to the native starch.

IV. CONCLUSION

Acetylation of native sago starch significantly increases its solubility and swelling power at all levels of modification. It also significantly increase resistant starch content at 2% acetylation and amylose content at 1% and 2.5% acetylation. It is also worthy to note that native sago, without any modification, shows a relatively high resistant starch content. The high RS content of the native sago starch and its acetylated counterpart should be emphasised to increase the competitiveness of sago among other agricultural commodities. Studies on the possibility of resistant starch as non-digestible dietary ingredient i.e. prebiotic, could be the new focal point of future research on starch industry [13,14].

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Siti Aisyah Zaman is a graduate student within Faculty of Agriculture and Food Sciences at University Putra, Malaysia. She was born on September 1990 at Kuching, Malaysia. She received her Bachelor of Science (Biotechnology) with honors at University Malaysia Sarawak, Malaysia in 2012. She is currently pursuing a Master of Science in Food Biotechnology.

Ms Zaman previous research experiences include being a graduate research assistant at Institute of Medical Research, Kuala Lumpur, Malaysia and CRAUN Research Institute, Kuching, Malaysia. She also has a background in editorial work as a Technical Proceedings Editor for the 2nd International Symposium on Tropical Forest Ecosystem Science and Management Conference, University Putra, Malaysia and Assistant Editor for Natural Resources and Biodiversity System, a book publication by University Putra Malaysia Press. She has one published articles in Critical Review for Biotechnology and another chapter in book publication in Recent Progress in Medicinal Plants, Studium Press LLC, US. Her current research interest is in food biochemistry, biotechnology and human nutrition**.**