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**Mini review****A review on acid and enzymatic hydrolyses of sago starch**

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This paper reviews reported studies on the hydrolysis of starch especially sago via acid and enzyme. The review begins with overview of sago palm and the starch industry, followed by process of extracting the starch from sago pith. Physicochemical properties of sago starch were tabulated for better understanding of hydrolysis process. Factors or process condition influencing hydrolysis process is discussed based on results from previous researches. Advantages and disadvantages of each hydrolysis is also discussed. Generally, there are very few researches dedicated on sago starch as compared to other starches. It can be concluded that, enzyme hydrolysis gives higher yield at milder process conditions. However, the reaction rate of enzyme hydrolysis is still low compared to acid hydrolysis.

**Keywords***Sago starch**Acid hydrolysis**Enzymatic hydrolysis*

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**Introduction**

In Malaysia, sago palm (*Metroxylon* spp.) is widely planted especially in Sarawak and Johor. Sago industry is so well established here in the Eastern state of Malaysia which lead to their contribution towards economic revenue with 25,000-40,000 tons of sago products being produced annually (Singhal *et al.*, 2008). The starch is processed for direct food consumption, pharmaceutical product and fermentable sugar for others different products through bioconversion. One of the processes involved is hydrolysis. Hence, the objective of this paper is to review previous studies on sago starch, specifically starch hydrolysis. This paper focuses on the two techniques to hydrolyze starch, which are acid and enzymatic hydrolyses. Both techniques have their own advantages and disadvantages that need to be considered before choosing the suitable method for treating the starch for further applications.

**Sago palm**

Sago palm (*Metroxylon sagu*) is a type of plant native to countries in tropical southeastern Asia such as Malaysia, Indonesia, Papua New Guinea and Thailand. Since ancient time, it acts as an important source of carbohydrate to the native population. Locally known as 'rumbia', Melanau communities in Sarawak consume starch obtained from sago palm as their staple food source (Mohamad Naim *et al.*, 2016).

Many scientists consider sago palm as the 'starch crop of the 21<sup>st</sup> century' (Singhal *et al.*, 2008). This is due to many characteristics that makes it a quite remarkable plant. Firstly, sago is an extremely resistant plant that able to survive in swampy, acidic peat soil (Chew *et al.*, 1999). Furthermore, the palm is immune to floods, drought, fire and strong winds. Sago forest also acts as an excellent carbon sink which helps in mitigating the greenhouse effect and global warming arising from the release of carbon dioxide into the atmosphere. Second special characteristic is that it does not need replanting since the plant itself continually produce suckers which in turn grow into adult palm. This consequently eliminates the need for recurring expensive establishment costs after every harvest of the adult palm. Thirdly, among starch-producing crops, sago palm gives the highest yield of starch with potentially up to 25 tons of starch per hectare per year. In term of per unit area, the yield could be about 3 to 4 times higher than that of rice, corn, or wheat, and about 17 times higher than that of cassava (Karim *et al.*, 2008). In short, in this age of concern for the environment and economy, sago is the crop par excellence for sustainable agriculture and profitability.

**Sago starch industry**

Sago palm is an important commercial tropical crop in Malaysia. Sarawak is the state in Malaysia where the trees are planted in abundance with 67,957 hectares of land (Mohamad Naim *et al.*, 2016)

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meanwhile in Peninsular Malaysia, Batu Pahat, Johor is the main planting area for sago palm. However it is considered as a minor crop due to the agriculture land dedicated to plant the tree is less than 1% of the total plantation area.

Malaysia is currently the third largest sago producer in the world after Indonesia and Papua New Guinea (Mohamad Naim *et al.*, 2016). This can be regarded by the fact that Indonesia is one of the largest countries in Southeast Asia with a production of 585,093 tons per year. Nonetheless, in term of productivity, Malaysia holds its own edge as Sarawak manages to become the largest world exporter due to its modern sago flour processing technology despite of having only estimated sago planting area of above 60,000 hectares (Mohamad Naim *et al.*, 2016).

Demand for sago starch will always exist because of its various applications in different industries. For example, in food industry, sago starch is the ingredient for making 'cendol', 'keropok', 'lempeng', sago pudding, tabalo biscuits (Karim *et al.*, 2008) and sago pearl (Ahmad *et al.*, 1999). Other than that, in the non-food industry, it is used as stabilizer, thickener and adhesives. The hydrolysed sago starch are also used for production of enzyme, ethanol, biohydrogen (Abd-Aziz, 2002; Azmi *et al.*, 2011; Puad *et al.*, 2015) and lactic acid for biodegradable polymer from polylactic acid which has numerous applications in pharmaceutical and packaging fields (Singhal *et al.*, 2008).

#### Sago starch processing

Extraction of sago starch can be performed either by using traditional or modern method (Karim *et al.*, 2008). The traditional method is usually practiced by individual farmers while modern method involves mechanized processing plant inside large-scale factories. However, both methods share the same principle for extraction purpose and the procedures are shown in Figure 1.

The process to extract sago starch from the logs begin with debarking process, followed by pulping to create small chips which are further disintegrated using a hammer mill. The resulting starch slurry is then passed through a series of centrifugal sieves to separate the coarse fiber or hampas. Further purification is carried out by separation in a nozzle separator to obtain pure starches. Dewatering of the starch is achieved using a rotary drum dryer, followed by hot air drying. During the processing of sago starch, three major types of by-products were generated namely bark of sago trunk, fibrous pith residue (locally known as hampas) and refuse water.

Bark and coarse residue are classified as solid

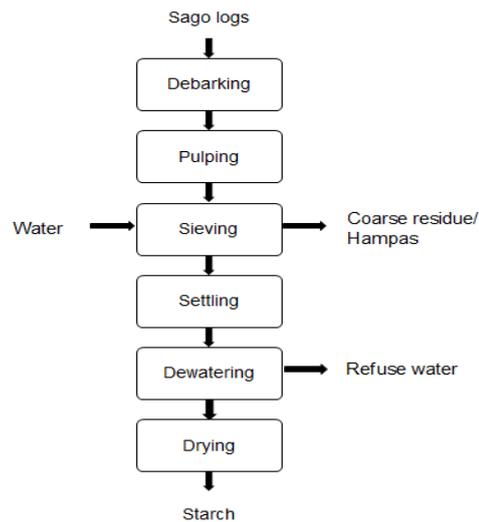


Figure 1. Schematic flow diagram for sago processing based on Awg-Adeni *et al.* (2010)

residue which is mainly composed of cellulose and lignin, whereas the refuse water is classified as liquid residue. Sago bark is usually reused as platform and footpaths around the factory and houses, respectively. Besides, the locals use the bark as timber fuel, wall materials, ceilings and fences. Meanwhile, coarse residue is usually given to animals as a feedstuff. However, the coarse residue contains approximately 30-45% starch and 30-35% fiber (Awg-Adeni *et al.*, 2012) while the refuse waste (or sago wastewater) also contains some amount of starch or carbohydrate as shown in Table 1.

#### Physicochemical properties of sago starch

Starch in general is a complex carbohydrate or polysaccharide that is made up of a large number of linked glucose molecules (monosaccharides). It is produced by most green plants as an energy store and joined together by glycosidic bonds (Habibi and Lucia, 2012). The glucose is stored mainly in the form of starch granules, in amyloplasts. Starch or amyllum is generally stored in plant cells in the form of organized grains of various sizes and shapes, and is made up of amylose and amylopectin. Amylose is a helical polymer made of  $\alpha$ -D-glucose units, bonded to each other through  $\alpha$ -1,4 glycosidic bonds. On the other hand, amylopectin is a branched glucose polymer formed by the presence of  $\alpha$ -1,6 glycosidic linkages.

An understanding of basic properties of sago starch is important to effectively utilize and process the starch. Hence, sago starch physicochemical properties are summarized in Table 2 which tabulated a compilation of data from several researchers (Ahmad *et al.*, 1999; Khatijah and Patimah, 1995; Polesi *et al.*, 2011; Uthumporn *et al.*, 2014;

Table 1. Characteristics of sago starch processing effluent (Yunus *et al.*, 2014).

Parameter	Concentration (mg/L)
Total COD	12,409 ± 262
Soluble COD	9530 ± 198
TS	6542 ± 157
TSS	1516 ± 221
VSS	2340 ± 257
TKN	124 ± 6
Soluble carbohydrates	683 ± 32

COD: Chemical Oxygen Demand, TS: total solids, TSS: total suspended solids, VSS: Volatile suspended solids, TKN: Total Kjeldahl Nitrogen

Przetaczek-Rożnowska, 2017) analysis. Generally sago starch granule is bigger than those of corn, rice and cassava but smaller than those of potato. The granule has shape of oval with size range between 20 to 60 µm (Ahmad *et al.*, 1999; Uthumporn *et al.*, 2014). The size of the granule is bigger as the sago palm ageing (Uthumporn *et al.*, 2014).

Raw sago starch has 10-20% of moisture content with 0.06% ash, 0.10 - 0.13% crude protein, 0.20 to 0.32% fiber with acidic aqueous at pH between 3.69 to 5.96. The starch content is between 72 - 94% with high amylose content (i.e. up to 45%) compared to other type of starches as presented in Table 1.

### Hydrolysis

The specific starch composition varies from plant to plant and even from species to species (Ahmad *et al.*, 1999). For example, long grain rice (e.g. basmathi) has a high amylose level and very little amylopectin which is why its grains retain their shape when cooked. On the other hand, short grain rice tends to be low in amylose and high in amylopectin (0% amylose and 100% amylopectin) hence making them sticky. Amylose content for sago starch is varied between 24% and 45% (Table 2). Reason for the difference in amylose content is most likely due to the timing of harvesting sago at different growth phase.

The proportion of amylose to amylopectin in a starch has important effects on the physical properties of the starch and affects the suitability of a food for some technological processes (Charles *et al.*, 2005; Rusendi, 1996). In the case of sago starch digestibility, some researcher suggested that sago starch is resistant to both microbial and enzyme digestion due to low hydrolysis yield (44.6%) obtained after long reaction time (72 h) (Srichuwong *et al.*, 2005). However, recent researches have proved that sago starch was able to be digested provided that pretreatment procedure was performed beforehand (Awg-Adeni *et al.*, 2010; Puad *et al.*, 2015). The following section will be devoted to a further elaboration of this subject.

### Acid hydrolysis of starch

Starch hydrolysis can be achieved by using acid or enzyme. According to Dziedzic and Kearsley (2012), acid hydrolysis was discovered at the beginning of the 19th century when a German chemist, Kirchoff showed that by boiling wheat starch with dilute sulfuric acid, a sweet syrup could be obtained. Later, potato starch was used as the starch source and sulfuric acid was replaced by hydrochloric acid and indirect heating of the reaction vessel was common place. Since then, acid has been used to a great extent for the breakdown of starch into glucose.

Researchers have conducted various studies on hydrolysis of starch using acid and the findings are summarized in Table 3. Bej *et al.* (2008) had investigated on concentrated acid hydrolysis (H<sub>2</sub>SO<sub>4</sub>) of wheat flour in a batch reactor at different temperatures and acid concentrations. A maximum conversion (42%) of starch to the reducing sugars was obtained at 95°C and pH 3. Other than that, Hoseinpour *et al.* (2010) did a study on the hydrolysis of starch using dilute sulfuric acid. The starch was almost completely converted to glucose under optimum conditions, obtained at 130°C, 1% acid and 7.5% solids loading for 30 minutes. Miao *et al.* (2011) investigated on the structure and digestion properties of waxy maize starch when undergone mild acid hydrolysis (2.2 N HCl at 35°C). The results demonstrated that the amorphous regions of starch granules are preferentially hydrolyzed and affect the slow digestion and resistance properties of waxy maize starch. In other word, the amount of rapidly digestible starch increased, whereas the amounts of slowly digestible and resistant starch decreased. Azmi *et al.* (2016) attempted optimizing hydrolyzing cassava starch mix with cassava leaves using nitric acid. They found out that starch concentration plays significant role compared to acid concentration and hydrolysis temperature. As shown in Table 3, there are very few studies reported on acid hydrolysis of sago starch. Example of work done by Abdorreza *et al.* (2012) focused on the effects of acid hydrolysis towards physicochemical and rheological properties of sago starch and Sunaryanto *et al.* (2013) using sulfuric acid on sago starch for maximum reducing sugar.

Hence, it can be concluded that acid hydrolysis is a simple method for starch hydrolysis since the resources are easily available and cheap. However, this technique does have a number of drawbacks such as relatively low yield and formation of undesirable by-products (Ramprakash and Muthukumar, 2014). Furthermore, the process have poor flexibility since the end product can only be changed by changing the

Table 2. Physicochemical properties (average values) of starches compiled from several researchers

Starch type	Granule size ( $\mu\text{m}$ )	Moisture (%)	Ash (%)	Crude fat (%)	Crude protein (%)	Crude Fiber (%)	Aqueous pH	Starch content	Amylose (%)
Sago	20-	10.6	0.06	0.10	0.05	0.20	3.69-	72 - 94	24-
	60 <sup>a,b,c,f</sup>	20.0 <sup>a,b,c</sup>	0.43 <sup>a,b,c</sup>	0.13 <sup>a,b,c</sup>	0.25 <sup>a,b,c</sup>	0.32 <sup>a,b,c</sup>	5.96 <sup>a</sup>	a,b,c	a,b,c
Pea		7.9 <sup>a</sup>	0.10 <sup>a</sup>	0.10 <sup>a</sup>	0.25 <sup>a</sup>	0.20 <sup>a</sup>	5.89 <sup>a</sup>	76 <sup>d</sup>	40 <sup>d</sup>
Potato	15-85 <sup>e</sup>	18.5 <sup>a</sup>	0.25 <sup>a</sup>	0.12 <sup>a</sup>	0.63 <sup>a</sup>	0.28 <sup>a</sup>	6.22 <sup>a</sup>	-	25 <sup>j</sup>
Corn	5-20 <sup>e</sup>	12.2 <sup>a</sup>	0.20 <sup>a</sup>	0.20 <sup>a</sup>	0.88 <sup>a</sup>	0.24 <sup>a</sup>	5.90 <sup>a</sup>	-	25 <sup>j</sup>
Rice	3-10 <sup>e</sup>	10 - 13 <sup>g</sup>	0.39	0.07	5.96	7.07	-	78 -82 <sup>g</sup>	3.36
			0.82 <sup>g</sup>	1.74 <sup>g</sup>	8.14	8.47	-		
Cassava	5 - 25 <sup>e,i</sup>	0.91 <sup>h</sup>	2.21 <sup>h</sup>	0.69 <sup>h</sup>	29.30 <sup>h</sup>	8.14 <sup>h</sup>	4.8	58.79 <sup>h</sup> -	20 <sup>i</sup>
								96 <sup>i</sup>	

<sup>a</sup>(Ahmad *et al.*, 1999), <sup>b</sup>(Khatijah and Patimah, 1995), <sup>c</sup>(Uthumporn *et al.*, 2014), <sup>d</sup>(Polesi *et al.*, 2011), <sup>e</sup>(Karim *et al.*, 2008) and <sup>f</sup>(Wang *et al.*, 1996), <sup>g</sup>(Thomas *et al.*, 2013), <sup>h</sup>(Taiga *et al.*, 2008), <sup>i</sup>(Hermiati *et al.*, 2011), <sup>j</sup>(Mishra and Rai, 2006)

degree of hydrolysis and the equipment used must be capable of withstanding acid at the temperature of 140-150°C. Moreover, if the product of hydrolysis is intended for further usage (i.e. substrate for the fermentation process), then removal of undesirable by-product such as furan will be required (Klinke *et al.*, 2004).

#### Enzymatic hydrolysis of starch

In the last decades, the starch industry has transformed from using acid in the hydrolysis process to enzyme. The acid was largely replaced with by  $\alpha$ -amylase and glucoamylase which gives 95% more yield of glucose (Hua and Yang, 2016). Unlike acid hydrolysis, there are commonly three stages involved in the conversion of starch by enzymatic hydrolysis. The first stage is gelatinization which is to break down the intermolecular bonds of starch with heat in the presence of water. Starch granules are quite resistant to the penetration by both water and hydrolytic enzymes due to the formation of hydrogen bonds within the same molecule and with other neighboring molecules. However, these intra- and inter- hydrogen bonds are weakened during gelatinization. During this stage, the temperature of aqueous suspension of starch is elevated, the water is absorbed and the starch granules is expanded (Albani, 2008), dissolving starch granules to form a viscous suspension or slurry. This allows disruption or burst of the starch granules and exposes it to enzyme attack. Second stage is liquefaction which is the the partial hydrolysis of the starch to oligosaccharides by action of  $\alpha$ -amylase, with concomitant loss in viscosity (liquefy). Liquefaction resulted of a mixture product of oligo- and polysaccharide or maltodextrin from starch (Bednarska, 2015; Hua and Yang, 2016). The third stage is saccharification by glucoamylase or pullulanase which this produces mixture of glucose and maltose (Hua and Yang, 2016).

Generally, enzyme in starch processing is divided into four categories which are endohydrolase, exohydrolase, debranching, transferase and isomerase (Bednarska, 2015; Hua and Yang, 2016). The mechanism of reaction of these five categories of enzyme and the enzymes that involved in starch processing are describe elsewhere (Hua and Yang, 2016). This review focuses mostly on hydrolysis using enzymes belong to endo- and exohydrolase. Table 3 summarizes previous studies on enzymatic hydrolysis of various kinds of starches.

Based on Kuddus *et al.* (2012), bacterial  $\alpha$ -amylase randomly attacks only the  $\alpha$ -1,4 bonds and it belongs to the liquefying category. On the other hand, the fungal  $\alpha$ -amylase belongs to the saccharifying category and attacks the second linkage from the non-reducing terminals (i.e. C4 end) of the straight segment, resulting in the splitting off two glucose units at a time. The bond breakage is thus more extensive in saccharifying enzymes than in liquefying enzymes. Finally, the amyloglucosidase (i.e. glucoamylase) selectively attacks the last bond on the non-reducing terminals. This type of enzyme can act on both  $\alpha$ -1,4 and the  $\alpha$ -1,6 glycosidic linkages resulting in the splitting off of simple glucose units. Fungal amylase and amyloglucosidase may be used together to convert starch to simple sugars.

#### Factors affecting enzymatic hydrolysis

Enzymes are biological catalysts that help to accelerate chemical reactions and without it, the reactions would take place at a considerably slower rate. Unlike chemical catalyst, they are highly selective and specifically catalyze specific reaction. However, in order for it to work properly, several factors affecting enzymatic hydrolysis need to be considered. Accordingly, the factors that affecting the hydrolysis yield have been identified through several researches as summarized in Table 3. Among

Table 3 Summary of studies on acid hydrolysis of starch

Starch Type	Starch pretreatment	Acid	pH	Temperature	Reaction time	Main findings	Reference
Wheat flour	NR	Concentrated sulfuric acid	2-5	75-95°C	15-105 min	Maximum conversion (42%) of starch to reducing sugar obtained at 95°C and pH 3	Bej et al. (2008)
Potato starch	NR	Dilute sulfuric acid, 0-1% (v/v)	NR	70-150°C	0-40 min	Optimum conditions for glucose production obtained at 130°C, 1% acid and 7.5% solids loading for 30 minutes.	Hoseinpour et al. (2010)
Waxy maize starch	NR	Hydrochloric acid, 2.2 N (or 2.2 M)	NR	35°C	3, 8 and 15 days	Amorphous regions of starch granules are preferentially hydrolyzed, increasing amount of rapidly digestible starch	Miao et al. (2011)
Cassava starch (1.5 - 3.5w/v%) and leaves (2.5%)	NR	Nitric acid, 0.12 - 0.22 M	NR	121°C	19 - 30 min	Maximum glucose yield of 0.96 g/g was obtained when 2.5% (w/v) of cassava leaves with 2.5% (w/v) of starch was hydrolyzed using 0.20 M nitric acid at 160 °C of temperature for 10 min.	Azmi et al. (2016)
Sago starch	NR	Hydrochloric acid, 0.14 M	NR	30-90°C	6-24 h	Acid hydrolysis can decrease the gelling point and improve the solubility of sago starch in water.	Abdorreza et al. (2012)
Sago starch	NR	Sulfuric acid, 2.5%	1.0 - 2.0	121°C	120 min	6.6% (w/v) reducing sugar.	Sunaryanto et al. (2013)

NR = Not Reported

the factors are; type of starch, starch or substrate concentration and viscosity, enzyme concentration, temperature, pH, reaction duration, agitation rate, and starch pretreatment.

Types of starch influence the degree of hydrolysis and the reducing sugar produced. Uthumporn *et al.* (2010) studied on hydrolysis of granular starch at sub-gelatinization temperature of 35°C for 24 h using mixture of alpha-amylase and glucoamylase. They observed that sago has the highest resistance to enzymatic degradation compared to corn, mung bean and cassava starches. This is due to the presence of pores on starch surfaces which are likely to become center of enzymatic attack. Uthumporn *et al.* (2010) study was also consistent with other studies from O'Brien and Wang (2008), Wang *et al.* (1996), Zhang and Oates (1999), Regy and Padmaja (2013).

Substrate concentration and viscosity is related to one another and therefore they are discussed together here. Firstly, substrate concentration is the amount of substrate per total solution while viscosity is a property of fluid that indicates resistance to flow. Generally, increasing the concentration of a dissolved or dispersed substance will lead to increase in viscosity. Starch is a source for thickening agent or thickener which functions to increase the viscosity of a liquid without substantially changing its other properties. Wee *et al.* (2011) reported the effect of high substrate concentration towards the yield of reducing sugar after hydrolysis using glucoamylase. It was concluded that as the substrate concentration keep increasing, yield of reducing sugar will decrease due to high viscosity of starch solution that resulted in the poor mixing of samples. Furthermore, Uribe and Sampedro (2003) stated that solvent viscosity results in friction against proteins in solution, and

this should result in decreased motion, as well as inhibiting catalysis.

Enzyme concentration is the amount of enzyme used per total solvent, and it is an important parameter to look at. If enzyme concentration is too low, reaction will take place at a slower rate and resulted in the low yield. If enzyme concentration is too high, it can lead to underutilized of the enzyme and this situation should be avoided since commercial enzymes are currently expensive. From the same study as before, Wee *et al.* (2011) observed that a higher yield of reducing sugar was obtained when enzyme concentration increases but further increase in concentration did not influence the yield. Hence, it is clear that providing a proper amount of enzyme for the reaction is very important.

On the other hand, most of the studies used multiple enzymes which reflect the intended purpose of the reaction (i.e. liquefaction or saccharification). Hence,  $\alpha$ -amylase and glucoamylase were the main enzymes involved in most studies (Table 4). Interestingly, pullulanase, a debranching enzyme, has been utilized in some studies which serve to prevent the reverse reaction of glucose condensation catalyzed by glucoamylase (Findrik *et al.*, 2010). However, study conducted by Wee *et al.* (2011) showed that by using only single enzyme which was glucoamylase, an approximately 60% of sugar yield from sago starch can be obtained.

Temperature is one of the crucial factors in the enzymatic hydrolysis. This is because many enzymes are adversely affected at the high temperatures and are completely destroyed at 100°C. Besides, each enzyme has its own optimum temperature for it to work properly and become active. The activity of an enzyme is decreased when the temperature of

Table 4 Summary of studies on enzymatic hydrolysis.

Starch Type	Starch pretreatment	Enzyme	pH	Temperature	Reaction time	Agitation Speed	Main findings	Author (Year)
Native granular starches from • Corn • Cassava • Mung bean • Sago	NR	• $\alpha$ -amylase ( <i>Aspergillus kawachi</i> ) • Glucoamylase ( <i>Aspergillus niger</i> )	4.0 - 4.5	35°C	24 h	150 rpm	DE values for corn (53%), mung bean (36%), cassava starch (35%) and sago starch (16%).	Uthumporn et al. (2010)
• Corn • Potato • Hylon	Multiple-step Annealing (Temperature begin from 40°C to temperature before starch gelatinization)	• $\alpha$ -amylase • Glucoamylase	• 6.9 • 4.5	50°C	36 h	145 rpm	Annealing affect positively on hydrolysis of sago starch using enzyme with maximum degree of hydrolysis (DH) from waxy corn (68%).	O'Brien and Wang (2008)
• Sago • Corn • Tapioca	Dissolve in sodium acetate buffer then incubate in oven	• Glucoamylase • $\alpha$ -amylase	5.0	35°C	40 h		• Raw sago starch is resistant to actions of enzyme • Significant synergism observed for glucoamylase and $\alpha$ -amylase	Wang et al. (1996)
Six varieties of sweet potato	Phosphate buffer (pH 7.1)	• $\alpha$ -amylase (porcine pancreatic)		37°C	26 h	• 160 times/min	• DH varies from 49% to 63% depend on susceptible of starch towards $\alpha$ -amylase.	O'Brien and Wang (2008)
Sago	Drying	Glucoamylase	3.5 - 5.5	45 - 65°C	NA		61°C, pH 4.5 is optimal conditions for glucoamylase with 60% yield	Wee et al. (2011)
Maltose		Dextrozyme glucoamylase pullulanase)	(i.e. 5.5 +	40 and 65°C			Dextrozyme was more active at 65°C, but operational stability decay was observed during the prolonged use in the reactor at this temperature.	Findriker et al. (2010)
Cocoyam	Dilute acid (HCl at 0.5-1.5% w/w	Amyloglucosidase 1% (v/v)	5.5 - 6.5	80 - 100°C (acid) 55 - 65°C (enzyme)	5 - 5 min (acid) 55 - 65 min (enzyme)		Reaction at 80°C for 10 min resulted in 72.06 g/L of reducing sugar while 75.22 g/L of reducing sugar was obtained when reacted at temperature of 90°C for the same duration.	Amenaghawon et al. (2016)
• Sweet potato • Potato • Cassava	• Crusher • Juice mixer • Homogenizer • High speed planetary mill	• Glucoamylase • Cellulase	4.5	40°C	20 h		Two step hydrolysis give highest glucose yield	Kumakura and Kaetsu (1983)
• Starch pith	Heating using autoclave or microwave in; • Water • Dilute sulfuric acid	• $\alpha$ -amylase • dextrozyme • cellulase • xylanase	and	• 95°C • 50°C • 50°C		Nil	Max. degree polymerization by; • Autoclave = 3 (134g/L TS) • Microwave = 4.4 (146g/L TS)	Sunarti et al. (2012)
Sago	No	• $\alpha$ -amylase • Dextrozyme (glucoamylase pullulanase)	5.0	• 85°C • 61°C	• 140 min • 60 h		• Dextrozyme produced more reducing sugar yield • Substrate concentration play role for hydrolysis	Sunaryanto et al. (2013)
Cassava	No	• Pectinase • $\alpha$ -amylase • amyloglucosidase	5.5 and 4.0	• 45°C • 95°C • 60°C	• 60 min • 60 min • 4-72 h		• 98% conversion efficiency • 160 g/L sugar	Collares et al. (2012)
Sago hampas	Oven dry	Dextrozyme (glucoamylase pullulanase)	4.0	60°C	60 min for each step		• High substrate load will result in difficult enzymatic hydrolysis process • 3 cycles of hydrolysis is practical	Awg-Adeni et al. (2012)
• Arrowroot • Cassava • Curcuma • Dioscorea • Sweet potato • Xanthosoma • Corn	Dry, sieve	• $\alpha$ -amylase • Glucoamylase	• 7.0 • 4.0	• 90°C • 60°C	• 1 h • 48 h		Glucose formed after saccharification was higher for cassava, arrowroot and sweet potato starch	Regy and Padmaja (2013)

the reaction differed from its optimum temperature. Amenaghawon *et al.* (2016) conducted a study of enzymatic hydrolysis towards cocoyam starch and found that the rate of hydrolysis was faster at a higher temperature. Reaction at 80°C for 10 min

resulted in 72.06 g/L of reducing sugar while 75.22 g/L of reducing sugar was obtained when reacted at temperature of 90°C for the same duration. Hence, this means increasing the temperature of a system will increase the number of collisions of enzyme and

substrate, thus increasing the rate of reaction.

The pH of a solution can have several effects towards the structure and activity of enzymes. As explained by Khanna (2010), the pH can have an effect of the state of ionization of acidic or basic amino acids. If the state of ionization of amino acids in a protein is altered then the ionic bonds that help to determine the 3D shape of the protein can be changed. This can lead to altered protein recognition or an enzyme might become inactive. Furthermore, changes in pH may not only affect the shape of an enzyme but it may also change the shape or charge properties of the substrate so that either the substrate cannot bind to the active site or it cannot undergo catalysis. In general, enzyme has its own optimum pH value and the value is not the same for each enzyme.

Reaction duration and agitation rate are related to each other. Since enzyme action involves the collision between the substrate and enzyme, agitation will result in faster time needed to complete the reaction. Mussatto *et al.* (2008) studied on the effect of agitation speed, enzyme loading and substrate concentration towards enzymatic hydrolysis of cellulose. Later, it was found that agitation speed did not significantly affect glucose yield. From here, it can be concluded that the amount of glucose yield does not depend so much on the agitation rate since substrate concentration is the limiting factor. However, agitation provides a proper mixing of the reactants which ultimately shorten the time needed to complete the reaction.

Sago starch have a low digestibility and they are resistant to both microbial and enzyme digestions. Granule size could be one of the factors that contribute to this phenomenon and hence the need of pretreatment comes to the fore. Pretreatment such as annealing process (O'Brien and Wang, 2008), autoclaving and microwave in water or dilute acid (Sunarti *et al.*, 2012), or mechanical pretreatment such as crusher, viz juice mixer, homogenizer and high speed planetary mill (Kumakura and Kaetsu, 1983) influence the efficiency of enzymatic hydrolysis process (Table 4) and plays important role in preparing the starch for enzyme attack and degradation.

### Outlook

Sago palm is an important carbohydrate source especially to tropical southeastern Asia. The trunk is processed through several processes to obtain the starch. The starch extraction and washing processes resulted with solid and liquid residues which also rich of starch for further process. The knowledge of physicochemical properties of the starch is important

to effectively process the starch. In general, sago starch granule has bigger size and is resistance to enzyme degradation compared to other types of starch. Furthermore the presence of pores on the granule surface of other types of starch is susceptible to enzyme attack. Thus, pretreatment sometimes is required prior to hydrolysis process especially when using enzyme.

Starch hydrolysis can be accomplished using acid or enzyme. Not many researches are devoted to sago starch as compared to other starches. Despite of that, several physical factors have been studied to maximize the production yield. Acid hydrolysis is a simple method, easily available and cheap. However, few drawbacks such as relatively low yield, high process temperature and formation of undesirable byproducts shifted the option to enzyme. Enzyme is highly selective and reaction specific produce less unwanted byproduct, give higher yield to glucose at milder process which requires less energy. However, it gives low reaction rate and high sugar monomers which create difficulty for separation and yet to be economical production.

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