

# THE EFFECT OF REAGENTS AND IODINE CONCENTRATION ON SAGO STARCH ACETYLATION AND IN VITRO DIGESTIBILITY

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## THE EFFECT OF REAGENTS AND IODINE CONCENTRATION ON SAGO STARCH ACETYLATION AND *IN VITRO* DIGESTIBILITY

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### ABSTRACT

Starch is the major carbohydrate in a normal diet. Acetic anhydride and acetic acid were used to modify sago starch. The solvent free acetylation was conducted using the process of microwave radiation heating. Iodine served as a catalyst and had a greater influence on the degree of substitution (DS) of sago starch acetate than did the 8 acetylation reagents. As the DS increased, the thermal properties ( $\Delta H_c$  and  $T_p$ ) of sago starch acetate significantly reduce. Scanning electron micrographs confirmed that the alteration of the initial morphology of sago starch became more evident as DS increased. *In vitro* digestion studies showed that acetylation increased the degree of resistant starch. Acetylation using acetic acid and low concentrations of iodine produced low DS of starch acetate, which is safe for food ingredients.

**KEYWORDS:** Sago Starch Acetate, Degree Of Substitution, Thermal Properties, Surface Morphology, *In Vitro* Digestion.

### INTRODUCTION

A few palms can effectively be used as a main starch staple (e.g. the sago palm, *Metroxylon* spp.). Sago is native to Indonesia and it is believed that sago is from the region around Sentani lake, Jayapura, Papua [1]. The greatest number of sago plantations are in Papua (1.2 million ha) and Papua New Guinea (1.0 million ha), and comprises about 90% of the total sago in the world. Sago palm contains a large amount of starch in its trunk; and has a yield that is greater than that of paddy rice [2]. Sago is an annual crop, and it produces about 25 tons of dry starch/ha/year [1]. Sago palm is environmentally friendly since it survives in swampy, acidic peat; submerged and saline soils. It is also immune to floods, draught, fire and strong winds. Starch is the most significant contributor to total dietary carbohydrate (often 70-80% of the dietary carbohydrate). Starch can be structurally modified by chemical reaction to enhance its nutritional properties (e.g. increasing resistance starch (RS)). RS has functional properties similar to fermentable dietary fiber [3, 4]. In the colon, it increases fecal bulk, lowers colonic pH and the portion fermented by the intestinal microorganism produces a range of short chain fatty acids, especially butyrate, which is useful in reducing the risk of bowel cancer. Starch modification is commonly performed using genetic, physical and chemical techniques [5]. Genetic modification of plants can yield novel starches with certain properties similar to postharvest modified starch [6-8]. Physical modification of starch is primarily heat-moisture treatments. The critical parameters that need to be controlled are the starch to moisture ratio, temperature and heating time [9]. Chemical modification involves hydroxyl group oxidation, crosslinking, stabilization and depolymerization [10]. Certain chemical reactions such as esterification, etherification or oxidation can also be used to significantly alter starch properties.

Esterification stabilizes starch by reducing the number of hydroxyl groups and decreasing the reassociation of the molecule; consequently retrogradation is slowed down and the stability of starch at low temperature increases. The reactivity of reagent is crucial to obtain certain substitution levels. Esterification can be performed using acetic, succinic anhydride, mixture between acetic and adipic acids, 1-octenylsuccinic anhydride, phosphoryl chloride, sodium trimetaphosphate, sodium tripolyphosphate, or monosodium orthophosphate [10]. The most popular technique used in esterification is acetylation via acetic anhydride. This produces a high degree of substitution (DS) of hydroxyl groups [11-21]. Acetic acid is the reagent of choice for a low degree of substitution of starch acetate, and it is safer for foodstuffs than acetic anhydride [22]. Acetylation is obtained by treating starch slurry with acetylated reagent at specific pH values. This process needs large amounts of water to maintain pH, is time consuming and yields large amounts of wastewater. A straightforward solvent-free method that requires less water and time involves the use of microwave radiation as the energy source [16, 19, 23-25]. In solvent-free acetylation, iodine is typically used as a catalyst. The effect of acetylating agent and reaction time in the solvent-free acetylation has already been studied for corn starch [16, 23], potato starch [4, 19], sago starch [26] and cellulose [24, 27, 28].

The effect of iodine as a catalyst on DS was shown to be significant for corn starch and cellulose [28, 29]. We are unaware of studies that investigate the effect of acetylation reagents and iodine concentration on the DS and *in vitro* digestion of sago starch acetate. This study, therefore, investigates the effect of acetylation reagents and iodine concentration on DS, thermal properties, surface morphology and *in vitro* digestion of sago starch acetate.

### MATERIALS AND METHODS

#### Materials

Sago pith at the *plawai* growth stage (estimated age 10 years) from Manokwari, West Papua was sun dried then crushed into powder. Before starch isolation, a number of characteristics were measured (e.g. protein content [30] and proximate analysis [31]). The proximate analyses of sago pith included moisture, ash, and fixed carbon, which were 4.5%, 0.32%, and 84.20%, respectively. The amount of crude protein and total starch in sago pith were 0.62% and 75.88%. Acetic acid ( $\geq 99\%$ ) and acetic anhydride ( $\geq 98\%$ ) were analytical grade from Sigma-Aldrich (St. Louis, USA). Sodium thiosulfate, ethanol, iodine, and potassium hydroxide were analytical grade supplied by Merck (Darmstadt, Germany). Hydrochloric acid was obtained from Fluka (Steinheim, Germany).

#### Starch isolation and acetylation

Sago starch was obtained by soaking sago pith in water for 6 hours with a ratio of solid to water of 1:5. The mixture was then filtered, and the filtrate was centrifuged at 1000 g for 20 min to obtain sago starch. Sago starch was dried in a vacuum oven (Lab-Line Duo-Vac Oven, Lab-Line Instrument Inc, Melrose Park, ILL, USA) at 60°C for 3h. Before the acetylation process, sago starch was dried in an oven (Memmert GmbH, Germany) and its MC then measured at 3.4 %. A detailed procedure of starch acetate synthesis and degree of substitution determination has been previously described [26]. Constant variables considered in this experiment were reaction temperature (100°C), medium stirring (equipment setting), microwave power (100 W), the mole ratio of starch to acetylation agent (1:4) and reaction time (10 min). Two variables, the ratio of acetic anhydride to acetic acid and iodine concentration were kept at two levels, high level (+1) and lower levels (-1) as listed in Table 1. An analysis of variable interaction based on two-level full factorial design of experiment (DOE) was calculated by variance (ANOVA) technique using Minitab 16 software.

#### Thermal properties analysis

Thermal properties of sago starch acetate were analyzed using a differential scanning calorimetry (DSC Jade, Perkin Elmer). Samples of sago starch acetate (3.5 mg) and distilled water (70%) were placed in 40- $\mu$ L aluminum pans (TA Instruments, USA). The samples were sealed and allowed to stand for 1 h at room temperature before heating in the DSC. Sample pans were heated from 25 to 100°C at 10°C/min. The calibration of DSC was performed using indium and an empty aluminum pan as the reference. Thermal stability was studied using a Perkin Elmer Diamond TG/DTA Instrument (Perkin Elmer, Japan). In order to determine the

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decomposition temperature. 10 sago starch acetate ( $\pm 6$  mg) was placed on a platinum pan and heated from 30 to 950°C at a heating rate of 10°C/min. Air flow through the system was at 20 mL/min and atmospheric pressure, during the entire measurements.

### Scanning electron microscopy analysis

The scanning electron micrographs of native and sago starch acetate were taken with a Cambridge scanning electron microscope (S-360) at an accelerating voltage of 20 kV. Granules of native starch and sago starch acetate were sprinkled onto a double-sided tape attached to a stub and then coated with gold.

### In vitro study

To study the effect of DS on susceptibility of sago starch acetate to pancreatic amylase (starch digestibility, SD), a number of trials were carried out in vitro [32]. Sago starch acetate was incubated with 1000 units of  $\alpha$ -amylase for 16 h at 37°C. Total starch (TS) was analyzed enzymatically using amyloglucosidase from *A. niger* (Sigma) [33-36]. Sago starch acetate (25 mg) was dispersed in 6 ml of 2 M potassium hydroxide solution and shaken vigorously for 30 min at room temperature. 60  $\mu$ l of amyloglucosidase was added to the solubilized starch. Glucose concentration in the supernatant was determined using a glucose oxidase-peroxidase kit (Sigma). Resistant starch was calculated as the difference between TS and SD.

## RESULTS AND DISCUSSION

### Starch isolation and acetylation

The isolation of starch from sago pith yielded 78.5%, and the moisture content was 11.2%. The results of amylose and amylopectin analysis of sago starch were 23.9% and 76.1%, respectively.

Table 1. Two variables considered for Design of Experiment (DOE)

Parameters	Low level (-1)	High level (+1)
Mole ratio of acetic anhydride to acetic acid (A)	1:0	0:1
Iodine catalyst (%mole of starch) (B)	0.15	5

Table 2. The effect of two level variables on the DS

Standard Run	A	B	DS
7	-	+	1.185
8	+	+	0.587
4	+	+	0.543
5	-	-	0.177
3	-	+	1.190
6	+	-	0.018
2	+	-	0.014
1	-	-	0.158

Table 3. Statistical analysis results

Factors/ Interactions	Effect	Coef	SE Coef	T	P
Constant		0.4840	0.006044	80.08	0.000
A	-0.3870	-0.1935	0.006044	-32.01	0.000
B	0.7845	0.3922	0.006044	64.90	0.000
A*B	-0.2355	-0.1177	0.006044	-19.48	0.000
S = 0.0170953					
R-Sq = 99.93%					
R-Sq (adj) = 99.88%					

Table 4. The effect of DS on the thermal properties

Sample	Gelatinisation		
	$\Delta H_G$ [J/g]	$T_D$ [°C]	$T_p$ [°C]
Native	16.3	69.1	72.6
DS 0.016	15.2	65.6	70.1
DS 0.168	12.7	58.1	67.3
DS 0.565	11.5	53.3	60.8
DS 1.188	10.1	50.7	57.1

Table 5. The effect of DS on the in vitro starch digestibility.

Starch	TS (%DM)	SD (%DM)	RS (%DM)
Native	78.70 $\pm$ 0.07	67.81 $\pm$ 0.57	10.89
DS 0.014	78.86 $\pm$ 0.11	66.85 $\pm$ 0.85	12.01
DS 0.018	79.05 $\pm$ 0.08	66.70 $\pm$ 0.41	12.35
DS 0.158	79.52 $\pm$ 0.15	63.15 $\pm$ 1.30	16.37

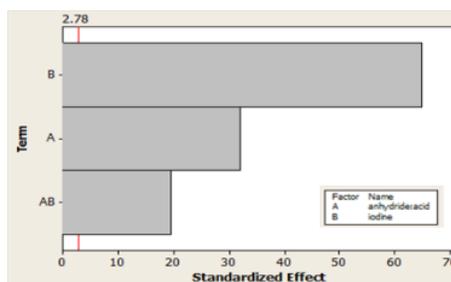


Figure 1. Pareto chart of the standardized effects (response is DS, Alpha = 0.05)

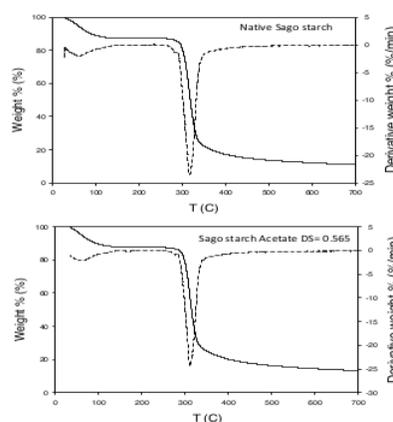


Figure 2. Thermogravimetric curves of native sago starch (upper) and sago starch acetate (bottom). Weight loss curve (-). Derivative of weight loss (-)

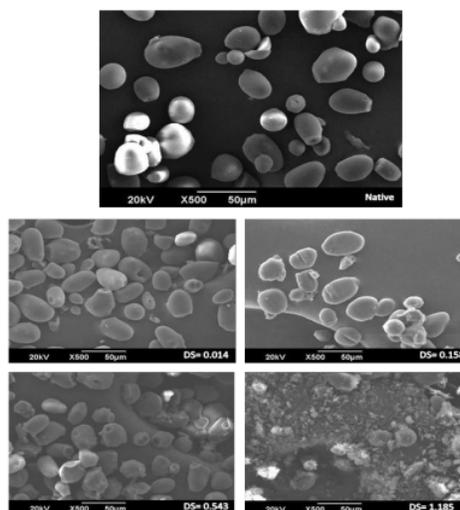


Figure 3. Scanning electron micrograph of native and sago starch acetate

The moisture content of sago starch from Manokwari, West Papua was found to be similar to sago starch from Serawak, Malaysia, likewise the amylose and amylopectin content [2,37]. Sago starch acetate was synthesized using a facile preparation method with acetic anhydride and acetic acid as acetylation reagent and iodine as catalyst. This method saves considerable time when compared to the conventional method ( $\pm 6$  h) because it used microwaves as the heating source. The result of DS from the matrix of two variables of two levels full factorial DOE is in Table 2, and the result of statistical analysis is in Table 3. The lowest DS was 0.014 that was obtained using only acetic acid as acetylation reagent and a low concentration of catalyst; whereas the highest DS was 1.190 and obtained when using only acetic anhydride as acetylation reagent and high concentration of iodine catalyst. The influence of catalyst concentration on DS is more significant than the ratio of acetic anhydride to acetic acid (Figure 1). For the same ratio of acetylation reagent, higher concentrations of iodine gave higher DS. Starch can be regarded as a polyalcohol; therefore, iodine serving as Lewis acid catalyst activates the carbonyl carbon of the acetylation reagent to make it more reactive in the absence of a solvent [38]. The mechanism has been reported by Biswas et al. [24] and Li et al. [38] iodine facilitates the initial step for the reaction of acetic anhydride with hydroxyl group. The interaction of both variables has less effect compared to the independent variable on the determination of DS.

### Thermal properties

Figure 2 shows the thermal decomposition profile of native sago starch and starch acetate. The first thermal decomposition is dehydration, and occurs in the temperature interval of 50 – 120°C for free moisture and 120 – 210°C for bound water. The decomposition of native sago starch and sago starch acetate occurs in the same temperature interval of 210 – 550°C; however, the major decomposition of sago starch acetate (310.02°C) occurs at lower temperature compared to native sago starch (316.02°C). This result is similar to that of corn starch acetate [16]. Substitution of hydroxyl groups by acetyl group causes the rupture of the amylopectin double helices, consequently the decomposition temperature become lower [39]. Thermal studies of gelatinization of sago starch acetate revealed that acetylation reduced  $T_o$  (the mean onset temperature) and  $T_p$  (the peak temperature) (Table 4). The peak temperature of gelatinization of native sago starch is slightly higher compared to commercial sago starch, as analyzed by Ahmad et al. [2]. Sago starch acetate with higher DS reduced in the enthalpy of gelatinization as also observed for other sources of starches [39]. This phenomenon is due to the incorporation of the ester group into a starch molecule which increases the free volume inside the chain and reduces the tension provoked by the intermolecular hydrogen bond [16].

### Scanning electron microscopy

The scanning electron micrographs of native sago starch and sago starch acetate are shown in Figure 3. Native sago starch has an irregular oval shape, and there is no significant difference between native and commercial sago starch. The diameter of sago granules is in the range of 20-40  $\mu\text{m}$  [2]. Figure 3 shows the effect of DS on the starch granule surface, and it is shown that the effect of acetylation in the absence of a solvent significantly influences the surface morphology [29, 39]. Using acetic anhydride as an acetylation reagent and increasing the iodine concentration caused the DS increase from 0.158 to 1.185, while using acetic acid as acetylation reagent causes the DS increase from 0.014 to 0.0587. The influence of iodine as a catalyst on the surface morphology of starch after acetylation is more significant compared to those of acetylation reagent. Iodine serves as a Lewis acid and acts as an oxidizing agent and the action of acid could cause an exo-corrosion all over the starch granule surface [29, 40]. Other authors explained that acid can provoke exfoliation on the surface of the starch granule and the effect of an oxidizing agent causes the surface of starch become rougher [41, 42].

### In vitro starch digestibility

Acetylated starches are also called resistant starches (RS) since they do not undergo complete saccharification. They can reach the large intestine and become a source of carbon to microflora colonizing it. This type is suitable for diabetics since it is only slowly digested and the blood sugar level remains stable. The maximum DS allowed by FAO/WHO for food products is 0.1 (the maximum acetyl content is 2.5%) [10,43]. The utilization of acetic acid and low concentration of iodine produces low DS of starch acetate. Acetic acid is a safe reagent for food products compared

to the other acetylation agents [22]. The observations in the present study suggest that acetylation of sago starch increases RS (Table 5) due to the alteration in the structure during an acetylation reaction. Crystallinity in native starch is mainly responsible for resistance to enzyme hydrolysis, therefore, when crystalline regions are decreased as a result of chemical modification, the hydrolysis of native starch increased. However, chemically modified starch shows the opposite. The hydroxyl group of glucose substituted by carboxymethyl group could possibly hinder the proper positioning of the substrate into the active site of  $\alpha$ -amylase and also effectively restrict enzyme attack on adjacent glycosidic bonds of unsubstituted glucose residues [4].

### CONCLUSION

In the acetylation of sago starch from sago pith, iodine concentration as catalyst more significantly affects DS, thermal properties, surface morphology and *in vitro* digestion compared to the acetylating agent. High concentration of iodine and acetic anhydride as the acetylating agents result on high DS, lower thermal properties, porous surface and higher RS. For food products, low concentration of iodine and acetic acid as acetylating agent produce a form sago starch acetate that fulfill the requirements for a safe food as stated by FAO/WHO.

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