

Physicochemical Properties of Sago Starch Modified by Acid Treatment in Alcohol

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Abstract: Sago starch is usually chemically modified to overcome and improve the unstable properties of native sago starch during processing. In this study, sago starch was treated in hydrochloric acid in the presence of methanol, ethanol and 2-propanol at 45⁰C for 1 hour. Granule size, granular structure and solubility of the modified starch were investigated. Results showed that recovery yields of the modified starch were in excess of 57% with a maximum recovery of 94%, and the average granule size of the starch ranged from 24.8 to 30.1 µm. Images under light microscope revealed some internal fissures in starch granules after acid-alcohol treatment. Acid concentration had a pronounced effect on the degree of polymerization. The degree of polymerization progressively decreased as the carbon number of the alcohol increased. This study showed that sago starch can be modified through hydrolysis and alcoholysis to give various limit dextrins with high solubility.

Key words: sago starch, acid hydrolysis, alcoholysis, starch degradation

INTRODUCTION

Starch modification is a process where the alteration of starch structure is by affecting the hydrogen bond in a controllable manner. Usually, starch degradation can be done by several methods such as chemical degradation, physical alteration, genetic modification or enzymatic transformation. Chemically modified starches are of significant importance in many industrial applications. It can be used to improve functional properties of food products and used in the production of glue, coatings, chemicals and building materials [1]. Some chemical methods were used to produce simple carbohydrates through hydrolysis, cross linking or oxidation. Acid hydrolysis has been used to modify starch for over 150 years. This process involves suspending starch in an aqueous solution of hydrochloric acid or sulfuric acid at certain temperatures. In the presence of a strong acid and heat, the glycosidic bond between monosaccharides in a polysaccharide is cleaved.

Modification of several starch bases such as potato, wheat, rice, maize and tapioca with acid-alcohol treatment has been studied. Ma and Robyt [2] successfully prepared soluble starch from potato and waxy-maize starches by acid hydrolysis in different alcohols. All of the modified starches were readily

soluble in hot water and their molecular weights decreased progressively from methanol modified starches to 1-butanol modified starch. The modified starches showed uniform granular appearance. Fox and Robyt [3] continued their study on starches and investigated how acid concentration influences the hydrolysis inside the granule. Their results confirm that the mechanism of hydrolysis of starch granules suspended in alcohol involves the hydrolysis of glycosidic bonds with the water inside the granules [4]. Meanwhile, the molecular and granular characteristics of corn starches [5] and physicochemical properties of maize and potato starches [6] modified by acid-alcohol treatments have been investigated. In the present study, we investigated the effect of acid hydrolysis in alcohol on some physical properties and degree of polymerization of sago starch (*Metroxylon sago*).

MATERIALS AND METHODS

All reagents used in this study were analytical grade chemicals while food grade sago samples were obtained from a local sago starch manufacturer in Mukah, Sarawak.

Preparation of acid-alcohol treated sago starch: Sago starch was acid-alcohol treated according to the

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procedure described by Chang *et al.* [7] with minor modification. Samples of 25 g each were suspended in 100 ml of methanol, ethanol or 2-propanol in a 500 ml conical flask. The hydrolysis reaction was initiated by adding 36% hydrochloric acid (20, 30, 40 or 50ml) and allowed to proceed for 1 hour at 45°C in a shaking water bath (Memmert, WB14). The reaction was stopped by neutralizing the solution media with 1 M NaOH. The samples were then transferred into 50 ml centrifuge tubes and centrifuged (Heittich Zentrifugen, Rotina 38R) at RCF 3,500 \times g for 5 minutes. The supernatant was collected and the precipitate was washed with 50% ethanol until neutral to litmus. The starch samples were filtered using Whatman No.1 filter paper and dried in an oven at 40°C. They were weighed at room temperature.

Recovery yield: Starch recoveries are expressed as percentage yields based on weight.

Determination of degree of polymerization: Degree of polymerization (d.p.) was calculated by divided the total carbohydrate with reducing sugar. The total carbohydrate was determined according to the phenol sulfuric acid procedure [8] and, the reducing sugar in the starch solution was assessed by copper sulfate procedure according to Lane and Eynon [9] using glucose as the standard.

Morphology and size of starch granules: One drop of iodine-KI reagent was added onto starch samples suspended in distilled water on microscope slides. The iodine stained granules were observed under a light microscope (Leica CME). The morphology and size of starch granules were recorded.

Solubility: Starch samples were suspended in 40 ml of distilled water. They were heated to the desired temperature (60, 70 or 80°C) for 30 minutes with continuous shaking. The mixture was centrifuged at RCF 4,000 \times g for 15 minutes. An aliquot of supernatant (5 ml) was evaporated at 130°C and weighed. The solubility of the starch was the ratio in mass (g) of the dried supernatant to the initial mass (g) of dry starch.

Statistical analysis: The data obtained from the study were analyzed using the Statistical Analysis System (SAS) software and the means were separated by Tukey's test.

RESULTS AND DISCUSSION

Recovery yield: The recovery yields of sago starches after acid-alcohol treatments were higher than 57 % as shown in Fig 1. Alcohols with longer carbon chains exhibited lower yields as compared to the shorter chain methanol. Methanol showed the most alcoholity activity followed by ethanol and 2-propanol. The different alcohols had differential effects on the hydrolysis of starch granules by producing widely different amounts of acid inside the granules, where hydrolysis occurs preferentially over alcoholysis [12].

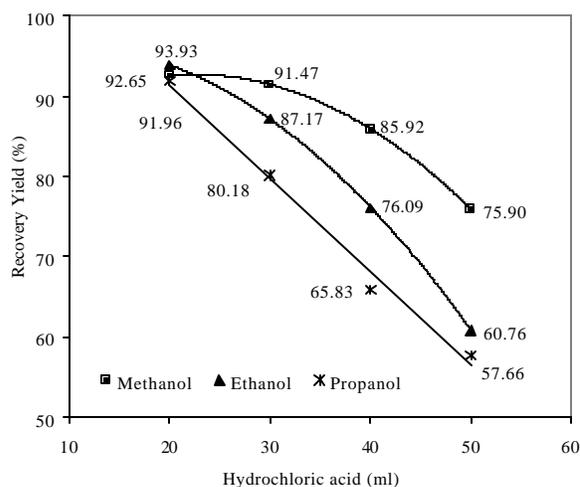


Fig. 1: Recovery yields of sago starch after acid-alcohol treatment at 45°C for 1 hour

Determination of degree of polymerization: The degree of polymerization of sago starch after acid-alcohol treatment is shown in Fig 2. Results showed that type of alcohol and acid concentration influence the degree of polymerization. Methanol gave the highest degree of polymerization compared to ethanol and 2-propanol, where high acid concentration resulted in low degree of polymerization. In each reaction, the d.p dropped rapidly with increase in acid concentration. This shows that the level of the degradation was high. Decrease in the degree of polymerization indicates that the starch is mainly singly branched material, with the branch located close to or at the reducing end of the molecule [10].

Different types of alcohols demonstrate various modification patterns of the starch granules since acid concentrations inside the granules are different [3]. This is due to differential susceptibility to hydrolysis of the

various glycosidic bonds in the granule. The susceptibility depends on the alcohol used and the concentration of the acid. The difference in glycosidic bond susceptibility is indicated by the variation in d.p of the modified starch (Fig.2). Thus, modified starch of any desired d.p value can be obtained by varying the choice of alcohol and acid concentration.

Morphology and size of starch granules: The images of sago starch granules observed under light microscopy are shown in Fig 3. Native sago starch granules exhibit oval shapes with smooth surface and with no obvious fissures or cavities as shown in Fig 3 (A). The granule surface of acid modified sago starch in different alcohols (50 ml hydrochloric acid added) showed signs of disintegration of granule surfaces. Light microscopy revealed that all of the modified starches retained their granular appearance, although various degrees of disintegration were observed. Iodine stained samples showed that less than 50% of the granules were disintegrated. However, there were no significant differences in average granule size of the starch products for all concentrations of hydrochloric acid in the three types of alcohols (Table 1). The average granule size ranged from 24.8 μm to 30.1 μm . The increase in granule size of acid-alcohol treated starch is attributed to slight swelling of the granules during modification.

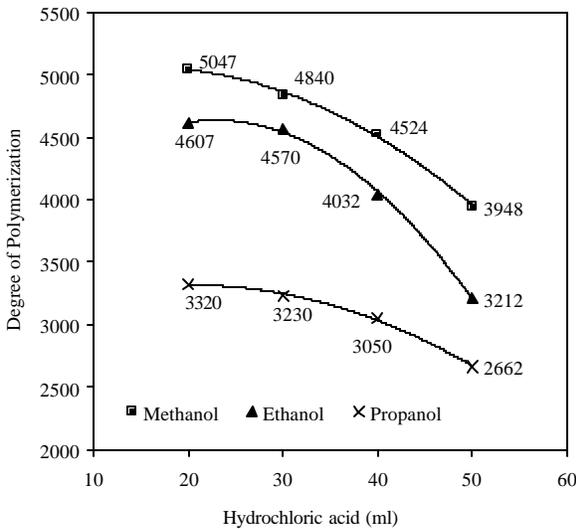
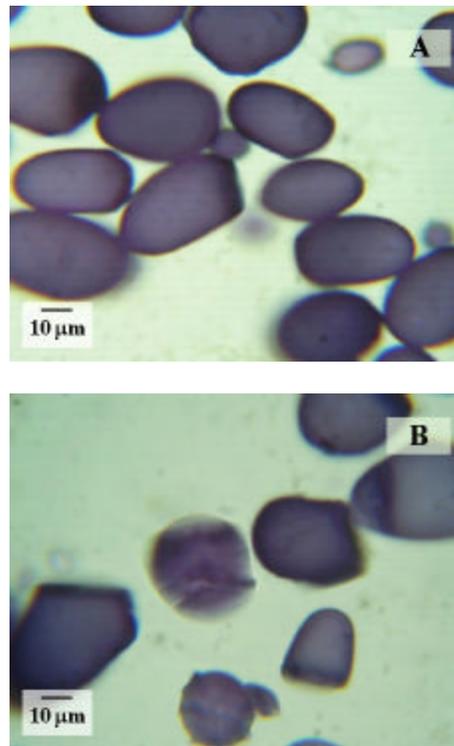


Fig. 2: Degree of polymerization of sago starch after acid-alcohol treatment at 45°C for 1 hour.

Table 1: Average granule size of sago starch after acid-alcohol treatment at 45°C for 1 hour.

Sago starch	Acid-alcohol Treatment	HCl (ml)	Average granule size (μm)
Native*	-	-	24.8 ^a
Treatment 1	Methanol	20.00	28.5 ^a
		30.00	28.5 ^a
		40.00	28.0 ^a
		50.00	28.1 ^a
Treatment 2	Ethanol	20.00	30.1 ^a
		30.00	29.1 ^a
		40.00	26.1 ^a
		50.00	25.8 ^a
Treatment 3	2-Propanol	20.00	28.5 ^a
		30.00	26.4 ^a
		40.00	25.4 ^a
		50.00	25.3 ^a

Note: Means with the same superscripts are not significantly different at $p < 0.05$ (Tukey's Test).
*Starch without treatment.



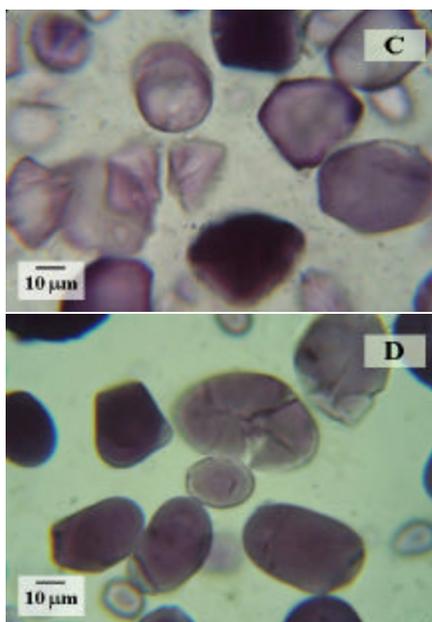


Fig. 3: Images of sago starch granules with 1000x magnification. (Photomicrographs B, C and D illustrate morphological changes in starch granules after acid-methanol, acid-ethanol and acid-2-propanol treatments respectively at 45⁰C for 1 hour)

Solubility: Native sago starch showed less than 50 % solubility at temperatures between 60⁰C and 80⁰C. The solubility of modified starch profoundly increased with increasing temperature (Table 2). At temperatures above 70⁰C, more than 100% of the modified sago starches were dissolved. This is due to gelatinization and swelling at 70⁰C. With gelatinization, water molecules are entrapped inside their structure. Results of the investigation on starch solubilisation indicated that the individual starch granules were altered, and were heavier than initial ungelatinized granules.

Table 2: Solubility (%) of sago starches after acid-alcohol treatment at 45⁰C for 1 hour

	60 ⁰ C	70 ⁰ C	80 ⁰ C
Native *	5.9 ^c	38.1 ^b	50.1 ^b
<u>Alcohol</u>			
Methanol	89.9 ^{ab}	107.2 ^a	120.3 ^a
Ethanol	66.7 ^b	100.8 ^a	113.1 ^a
2-Propanol	99.2 ^a	109.1 ^a	113.6 ^a

Note: Means within columns with the same superscripts are not significantly different at p<0.05 (Tukey's Test). A total of 50 ml of hydrochloric acid was added during the acid-alcohol treatments.

*Starch without treatment.

CONCLUSION

The present study shows that modified sago starch of any desired d.p value can be prepared by suspending hydrated starch granules in acidic alcohols for 1 hour at 45⁰C. Both hydrolysis and alcoholysis occurred, but to different degrees, depending on the type of alcohol. The highest amount of alcoholysis occurred in methanol and the lowest in 2-propanol. Sago starches showed recovery yields higher than 57%. Starch granules modified in all treatments were oval in shape with internal fissures and cavities. The acid modification of native starch in different alcohols gives highly soluble starches. Thus, it is possible to obtain various types of limit dextrins from sago starch through acid-alcohol treatment.

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REFERENCES

1. Guilbot, A. and C. Mercier. 1985. Starch. In: *The polysaccharides*. Edited by G.O. Aspinall, Academic Press, Inc., London, pp: 210-244.
2. Ma, W.P. and J.F. Robyt, 1987. Preparation and characterization of soluble starches having different molecular sizes and composition, by acid hydrolysis in different alcohols. *Carbohydr. Res.* 166: 283-297
3. Fox, J.D. and Robyt, J.F. 1992. Modification of starch granules by hydrolysis with hydrochloric acid in various alcohols, and the formation of new kinds of limit dextrins. *Carbohydr. Res.* 227: 163-170.
4. Robyt, J.F., J.Y. Choe, R.S. Hahn and E.B. Fuchs, 1996. Acid modification of starch granules in alcohols: Effects of temperature, acid concentration, and starch concentration. *Carbohydr. Res.* 281: 203-218.
5. Chung, Y.L. and H.M. Lai, 2006. Molecular and granular characteristics of corn starch modified by HCl-methanol at different temperatures. *Carbohydr. polym.* 63: 527-534.
6. Lin, J.H., S.Y. Lee, and Y.H. Chang, 2003. Effect of acid-alcohol treatment on the molecular structure and physicochemical properties of maize and potato starches. *Carbohydr. Polym.* 53: 475-482

7. Chang, Y.H., J.H. Lin and S.Y. Chang, 2006. Physiochemical properties of waxy and normal corn starches treated in different anhydrous alcohols with hydrochloric acid. *Food Hydrocolloids* 20: 332-339.
8. Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28: 350-356.
9. Lane, J.H. and L. Eynon, 1923. Determination of reducing sugars by means of Fehling's solution with methylene blue as internal indicator. *J. Soc. Chem. Ind. Trans.* 32-36.
10. French, D. 1984. Organization of starch granules. In *Starch: Chemistry and technology*. Edited by R.L. Whistler, J.N. Bemiller and E.F. Paschall, Academic Press, Inc., London, pp: 184-242.