UNIVERSITI PUTRA MALAYSIA

ACID MODIFICATION OF SAGO STARCH IN ALCOHOL

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By

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A Project Report Submitted in Partial Fulfillment of the Requirement
for the Degree of Bachelor of Bioindustry Science in the
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This thesis is specially dedicated to

my father and mother,

brother and sisters,

with love and gratitude

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 present of methanol, ethanol and propanol at 45^0C for 1 hour. The granule size, granular structure and solubility of the modified starch were investigated. Results showed that recovery yields of the modified starch were higher than 57% and the average granule size of the starch ranged from 24.8 to 30.1 μm. Images under light microscope revealed that starch granules suffered some internal fissures after acid-alcohol treatment. The degree of polymerization progressively decreased as the carbon number of the alcohol increased. Results also showed that acid concentration had a pronounced effect on the degree of polymerization. Hence, sago starch can be modified through hydrolysis and alcoholysis to give various limit dextrins with high solubility.

ABSTRAK

Modifikasi kanji sagu dengan menggunakan kaedah kimia biasanya dijalankan untuk mengatasi dan memperbaiki ciri-ciri asli kanji sagu yang kurang stabil dan kurang memuaskan semasa pemprosesan. Dalam kajian ini, kanji sagu asli telah dirawat dengan asid hidroklorik dengan kehadiran metanol, etanol dan propanol pada suhu 45^0C selama 1 jam. Saiz butiran, struktur butiran dan keterlarutan kanji telah dikaji. Keputusan kajian menunjukkan bahawa lebih daripada 57% hasilan kanji berjaya diperolehi semula, manakala purata saiz butiran kanji berada dalam lingkungan 24.8 ke 30.1 μm. Gambar-gambar mikroskop cahaya menunjukkan peretakan pada butiran-butiran kanji selepas rawatan asid dan alkohol. Nilai darjah pempolimeran (D.P) berkurangan apabila bilangan karbon alkohol meningkat. Keputusan ini juga menunjukkan bahawa kepekatan asid telah menyumbangkan kepada penyusutan tersebut. Dengan ini, kanji sagu boleh dirawat secara hidrolisis dan alkoholisis untuk memberikan berbagai produk dekstrin yang mempunyai keterlarutan yang tinggi.

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APPROVAL

I certify that this research project report entitled "Acid Modification of Sago Starch in Alcohol" has been examined and approved as a partial fulfillment of the requirement for the degree of Bachelor of Bioindustry Science in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus.

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D.P	-	Degree of polymerization
DMSO	-	Dimethyl sulfoxide
g	-	Gram
h	-	Hour
HCl	-	Hydrochloric acid
KI	-	Potassium iodine
M	-	Mol
mg	-	Miligram
ml	-	Mililiter
nm	-	Nanometer
rpm	-	Revolutions per minute
µm	-	Micrometer
µl	-	Microliter
x g	-	Relative centrifugal force (RCF)
α	-	Alpha
^{0}C	-	Degree Celcius
%	-	Percentage

1.0 INTRODUCTION

Starch is the second most abundant, important and digestible food carbohydrate in the biosphere next to cellulose and is therefore a major source of energy for many organisms. Native starches which are unmodified show considerable retrogradation at low temperature, insolubility in cold water, loss of viscosity, thickening power after cooking, texture and stability limitations (Xie, 2005). Consequently, some food products produce undesirable characteristics. Hence, the idea of modification could solve some of these characteristics. Modified starch can provide a wide range of functions. It can extend products shelf-life and the range of it functionalities by producing a short, stringy, cuttable texture and perform as stabilizer for an emulsion.

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 many ways such as in glue, coating, chemicals and building materials (Guilbot *et al.*, 1985). Some chemical methods were used to produce simple carbohydrates such as hydrolysis, cross linking or oxidation.

Acid hydrolysis has been used to modify starch for over 150 years (Robyt *et al.*, 1996). This process involves suspending starch in an aqueous solution of hydrochloric acid or sulfuric acid at certain temperature. In the presence of a strong acid and heat, the glycosidic bond between monosaccharide residues in a polysaccharide is cleaved.

The reaction condition for preparing modified starches with acid-alcohol modification in several starch bases such as potato, wheat, rice, maize and tapioca has been studied (Ma, 1987; Fox, 1992; Robyt, 1996; Chang, 2006; Chung, 2006), but the preparing of acid-alcohol modified sago starch has not reported.

In this study sago starch was acid modified in various types of alcohols. An attempt has also been made to evaluate some physical properties and degree of polymerization of the products.

2.0 LITERATURE REVIEW

2.1 Role of Starch

Starch is the dominant carbohydrate reserve material by nature in the form of granules in higher plants of polysaccharides. This polysaccharide is composed of a mixture of amylose and amylopectin. Amylose is essentially found to be a long linear, most simple and smallest macromolecule consisting of α-D-glucopyranose residues linked together by (1→4) bonds (Guilbot *et al.*, 1985). As reported by Meyer (1940) and supported by other investigators (1943), amylose has one reducing end, one non-reducing end and slightly branched (Radley, 1968). Meanwhile, amylopectin has D-glucopyranose residues linked together mainly by (1→4)-α-D and (1→6)-α-D bonds at the branch points (Guilbot *et al.*, 1985). Hence, it provides greater solution stability as compared to amylose (Young, 1984).

In recent years, a number of authors have reported the importance of starch in various applications (Anderson, 2001; Atichokudomchai, 2003; Thirathumthavorn, 2005). It can be used to improve functional properties of food products and used in many ways such as in glues, coating, sizing and flocculating agents, chemicals and building materials (Guilbot *et al.*, 1985). Since the native starch can't contribute to such properties, the idea of starch modification was developed to enhance and extend the application of starch so that its behavior under specific conditions is predictable.

2.2 Starch Modification

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 various methods such as chemical degradation, physical alteration, genetic modification or enzymatic transformation.

Recently, Wong (2007) has reported an enzymatic method in producing long chain dextrin from sago starch, which has the ability to impede migration of air between the environment and food. Thus, it is suitable for use as coating for fresh fruits and vegetables to prevent browning. In 1987, Ma and Robyt successfully prepared soluble starch from potato and waxy-maize starches by acid hydrolysis in different alcohols. They found that 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. This method can avoid gelatinization and removes lipids and pigments from the starch (French, 1984).

2.3 Chemical Degradation

The chemical modification of starches continues to attract the attention of researchers for applications as diverse as biodegradable packaging materials to components of oil drilling mud (Fang *et al.*, 2004). Starch and its subsequent refinement is candidate for use as a raw material since it has a huge potential as a versatile renewable resource for various material applications (Blennow, 2004; Moore *et al.*, 1984).

Acid hydrolysis has been used to modify starch since 150 years ago. Much research has been carried out to determine the potential of starches after various degrees of modification. Fox and Robyt (1992) continued their study on starches and investigated how acid concentration influences the hydrolysis inside the granule. The results of the study showed lower concentration of acid yield products with higher degree of polymerization (D.P), with D.P values dropping with time. It is possible to obtain starch granules with wide ranges of D.P. values by selecting the alcohol and the acid concentration. These 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 (Robyt *et al.*, 1996). Thus, new types of limit dextrin can be produced by appropriate choice of alcohol and concentration of acid.

The physiochemical properties of starches treated in hydrochloric acid with different alcohols showed high starch recovery (Lin, 2003; Chang, 2006). In their study, the internal fissures and cavities of the starches were found after the modification. Besides, the solubility profoundly increased with time and temperature.

3.0 METHODOLOGY

3.1 Materials

All reagents used in this study were analytical grade chemicals while food grade sago sample was obtained from Nit Sei Sago Industries, Mukah, Sarawak.

3.2 Procedure

3.2.1 Treatment of Sago Starch

Sago starch was acid-alcohol treated according to the procedure described by Chang (2006) with minor modification. Samples of 25 g each were suspended in a 500 ml conical flask containing 100 ml of alcohol either methanol, 99.5% ethanol or 2-propanol. The reaction was initiated by adding 36% hydrochloric acid and allowed to proceed for 1 hour at 45^0C in a shaking water bath (Memmert, WB14). The reaction was stopped by neutralizing the solution media with 1 M NaOH. The samples were then filled into 50 ml centrifuge tube and centrifuged (Heittich Zentrifugen, Rotina 38R) at 3,500 x g for 5 minutes. The supernatant was collected and the precipitate was washed with 50% ethanol until it showed neutral to litmus. The starch samples were filtered using whatman filter paper No. 1 and dried in an oven at 40^0C. The percentage of starch recovery was expressed as recovery yield based on weight.

3.2.2 Determination of Degree of Polymerization

Degree of polymerization was calculated by divided the total carbohydrates with reducing sugars. The total carbohydrates and reducing sugars were determined according to the procedures described in sections 3.2.2.1 and 3.2.2.2 respectively.

3.2.2.1 Total carbohydrate determination

Acid modified starch (0.1 g) was dissolved in a solution of dimethyl sulfoxide, DMSO (0.425 ml) and distilled water (0.075 ml) in a conical flask. The mixture was shake for 15 minutes in a water bath at 60^0C. The solution was then slowly diluted to 10 ml by using distilled water.

Phenol sulfuric acid procedure (Dubois *et al.*, 1956) was used in this study to determine total carbohydrate. A volume of acid modified solution (50 µl) was made up to 100 µl by adding distilled water. The solution was mixed with 50 µl of 80% aqueous phenol and 2.0 ml of concentrated sulfuric acid in a glass tube. The tube was allowed to stand for 10 minutes at room temperature. The solution was vortex before the final volume was recorded. The absorbance of the solution was determined at 490 nm using UV-visible spectrophotometer (CARY 50 Conc). Total carbohydrate of the acid modified sample was determined by comparing the readings with the absorbance of standard glucose (10 mg/ml).

3.2.2.2 Reducing sugar determination

Acid modified starch (10 g) was dissolved in 42.5 ml of dimethyl sulfoxide (DMSO) and 7.5 ml of distilled water in a 250 ml conical flask. Flasks were shake for 15 minutes in a water bath at 60^0C. The solution was then slowly diluted to 100 ml with distilled water. The reducing sugar in the starch solution was determined by Copper sulfate procedure according to Lane *et al.*, (1923), using glucose as the standard.

In this study, Fehling stock solution A was prepared by dissolving 34.64 g of Copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$) in 500 ml of distilled water while Fehling stock solution B was prepared with 173 g of Potassium sodium tartrate ($KNaC_4H_4O_6.4H_2O$) and 50 g of Sodium hydroxide (NaOH) in 500 ml of distilled water. Mixed Fehling's solution was prepared by first transferring 12.5 ml of Fehling stock solution A followed by 12.5 ml of Fehling stock solution B to a 500 ml conical flask and through mixing. An aliquot of the starch solution (15 ml) was pipetted into the conical flask. The solution was boiled for 2 minutes. Three drops of methylene blue indicator (1% aqueous solution) was added to the boiling solution. It was titrated until the blue color disappeared completely. The experiment was repeated using standard glucose solution. Total reducing sugar was calculated by comparing the end point between standard glucose with acid modified starch.

3.2.3 Morphology of Starch Granule

A drop of iodine-KI reagent was added onto a microscope slide which contains a small amount of starch sample suspended in distilled water. The iodine stain was observed under a light microscope (Leica CME). The morphology and size of starch granules were recorded.

3.2.4 Solubility

Starch samples were suspended in 40 ml of distilled water. They were heated to the desired temperature (60^0C, 70^0C, and 80^0C) for 30 minutes with continuous shaking. The mixture was centrifuged at 4,000 x g for 15 minutes. An aliquot of supernatant (5 ml) was evaporated at 130^0C 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.

3.3 Statistical Analysis

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

4.0 RESULTS AND DISCUSSION

4.1 Recovery Yield and Average Granule Size

The recovery yields of sago starches after acid-alcohol treatments were higher than 57 % as shown in Figure 1. Alcohols with longer chains exhibit lower yields as compared to shorter carbon chain alcohols like methanol. Methanol showed the most alcoholytical activity followed by ethanol and propanol. The different alcohols have differential effects on the hydrolysis of starch granules by producing widely different amounts of acid inside the granules, where hydrolysis occurs preferentially over alcoholysis (Ma, 1987). However, there were no significant differences in average granule size of the starch products for the different 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 might be due to the slight swelling of the granules during modification.

4.2 Granule Structure

The images of sago starch granules observed under light microscopy are shown in Figures 2 to 5. Native sago starch granules exhibit oval shapes with smooth surface and with no obvious fissures or cavities (Figure 2). The granule surface of acid modified sago starch in different alcohols (Figure 3 to Figure 5) showed defects or signs of damages on the surface of granules. Light microscopy revealed that all of the modified starches retained their granular appearance, although various degrees of granule damage were observed. After staining with iodine, however, almost 50% of the granules appeared damaged.

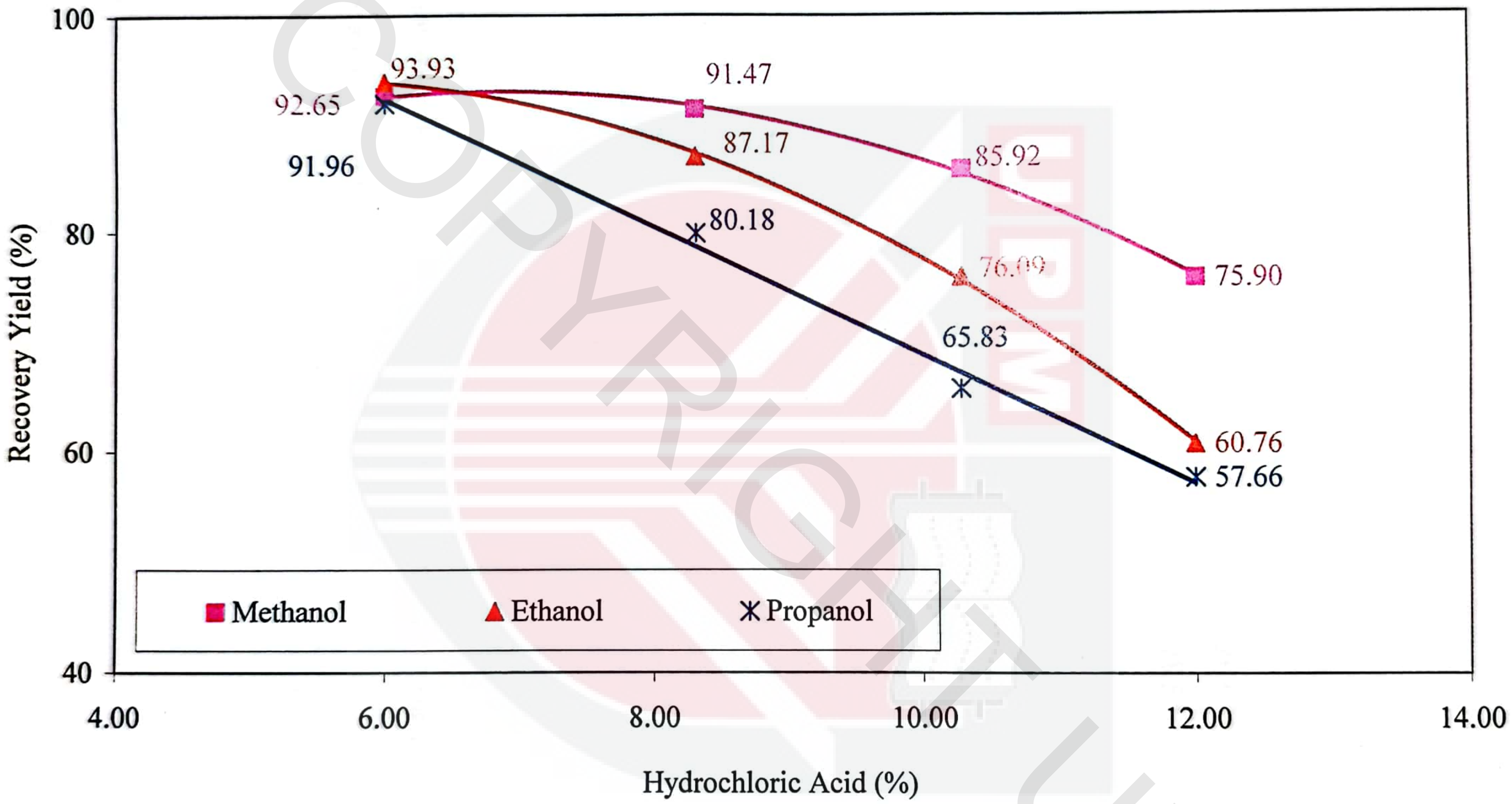


Figure 1: Recovery yields of sago starch after acid-alcohol treatment at 45^0C for 1 hour.

Table 1: Average granule size of sago starch after acid-alcohol treatment at 45^0C for 1 hour.

Treatment	Average granule size (µm)
Native *	24.8 ^{a}
Methanol	
6.00 % HCl	28.5 ^{a}
8.31 % HCl	28.5 ^{a}
10.29 % HCl	28.0 ^{a}
12.00 % HCl	28.1 ^{a}
Ethanol	
6.00 % HCl	30.1 ^{a}
8.31 % HCl	29.1 ^{a}
10.29 % HCl	26.1 ^{a}
12.00 % HCl	25.8 ^{a}
Propanol	
6.00 % HCl	28.5 ^{a}
8.31 % HCl	26.4 ^{a}
10.29 % HCl	25.4 ^{a}
12.00 % HCl	25.3 ^{a}

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

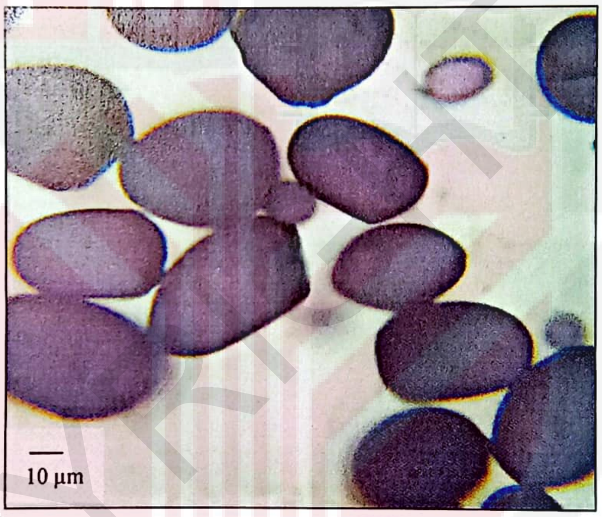
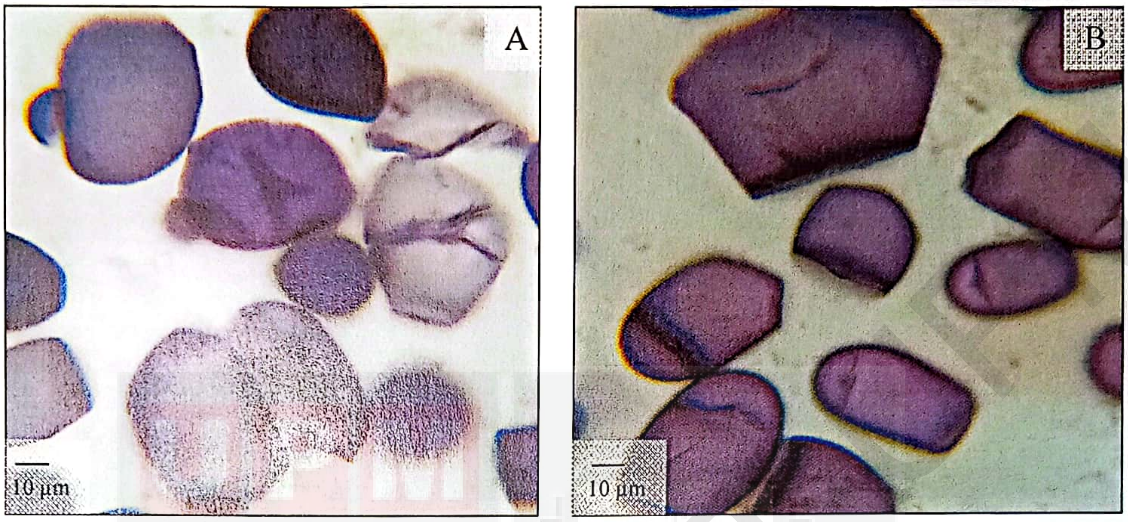
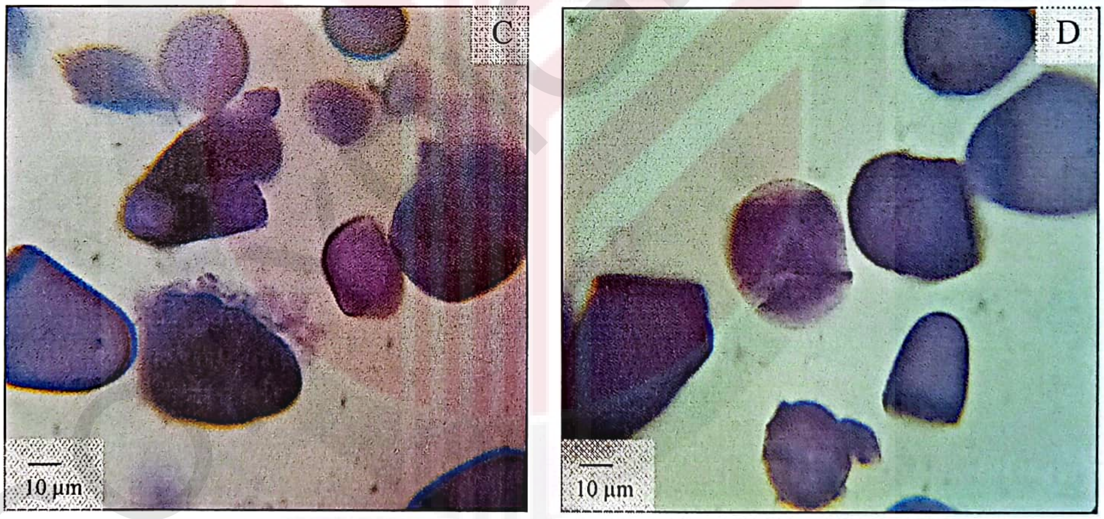


Figure 2: Image of native sago starch granules (magnificent 1000 X).



6.00 % HCl

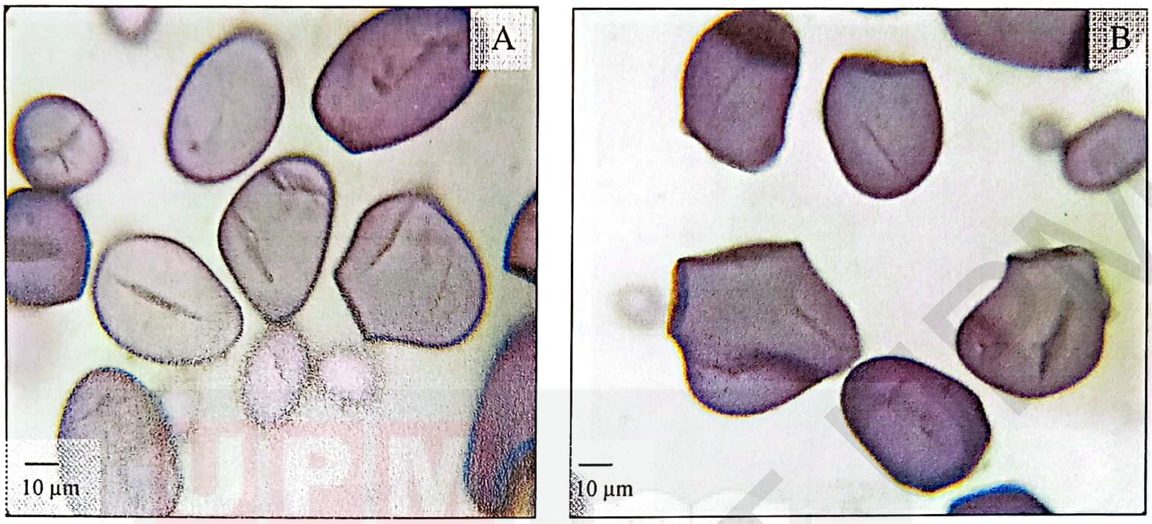
8.31 % HCl



10.29 % HCl

12.00 % HCl

Figure 3: Images of sago starch granules after acid-methanol treatment at 45^{0}C for 1 hour.(magnification 1000 X)



6.00 % HCl

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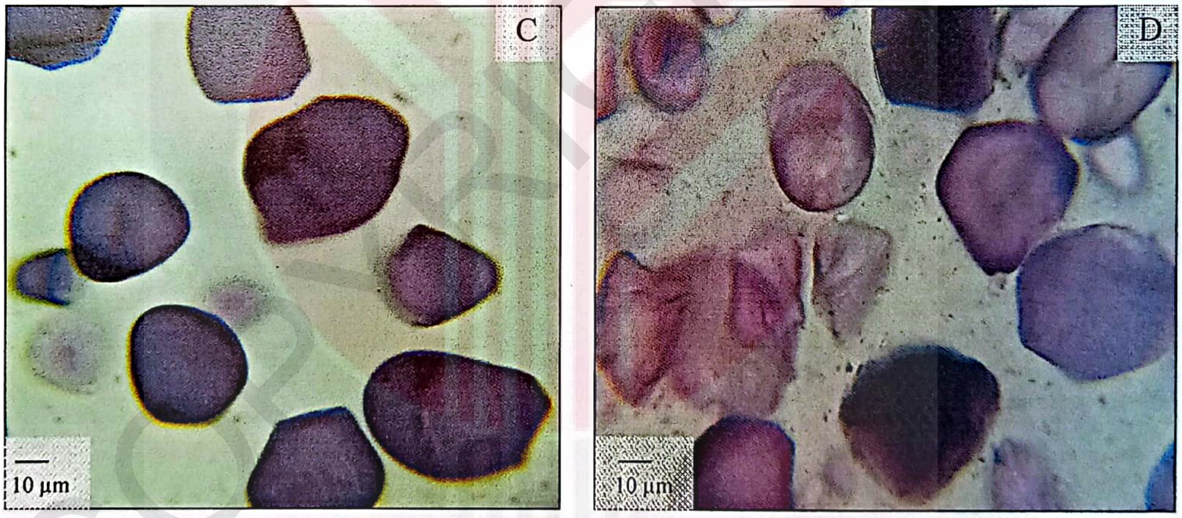
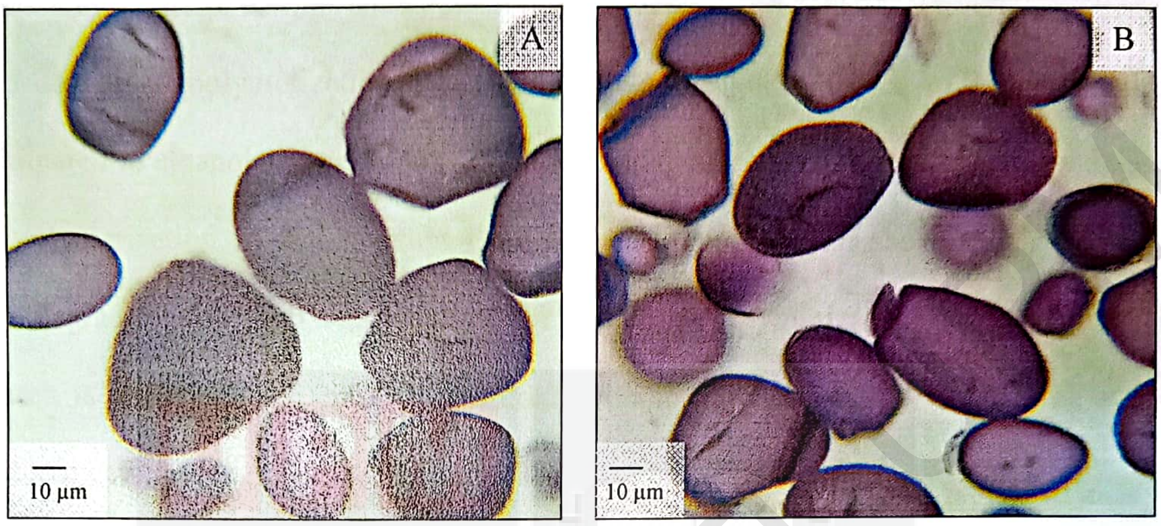


10.29 % HCl

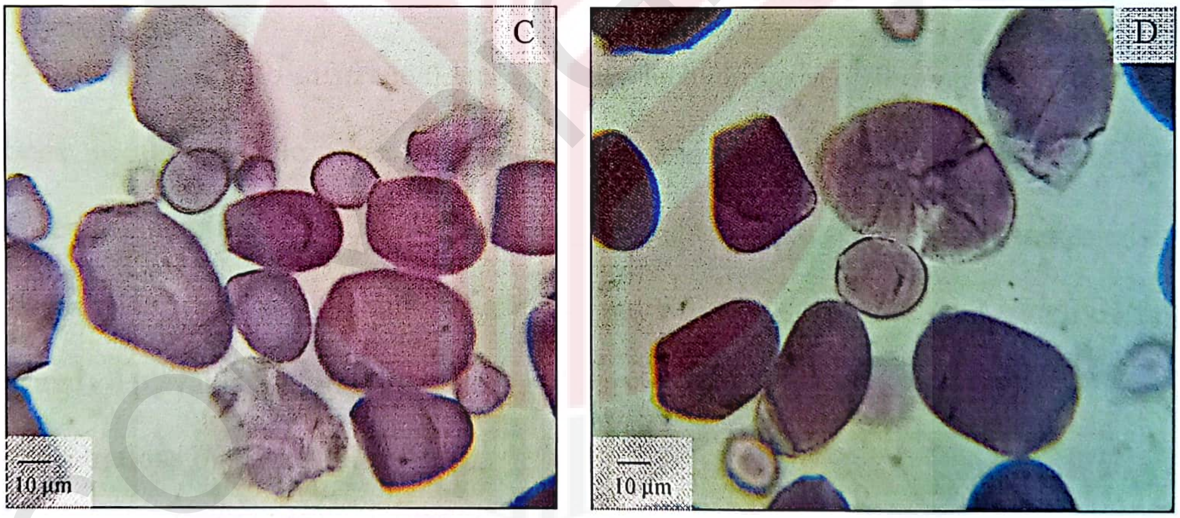
12.00 % HCl

Figure 4: Images of sago starch granules after acid-ethanol treatment at 45^0C for 1 hour. (magnification 1000 X)



6.00 % HCl

8.31 % HCl



10.29 % HCl

12.00 % HCl

Figure 5: Images of sago starch granules after acid-propanol treatment at 45^{0}C for 1 hour. (magnification 1000 X)

4.2 Degree of Polymerization

The degree of polymerization of sago starch after acid-alcohol treatment is shown in Figure 6. 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 propanol, where high acid concentration resulted in low degree of polymerization. In each reaction, the d.p dropped rapidly. This shows that the level of the degradation is 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 (French, 1984).

Different types of alcohols demonstrate various modification patterns of the starch granules since acid concentrations inside the granules are different (Fox *et al.*, 1992). 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. Figure 6 shows that modified starch of any desired d.p value can be obtained by varying the choice of alcohol and acid concentrations in the treatment basin.

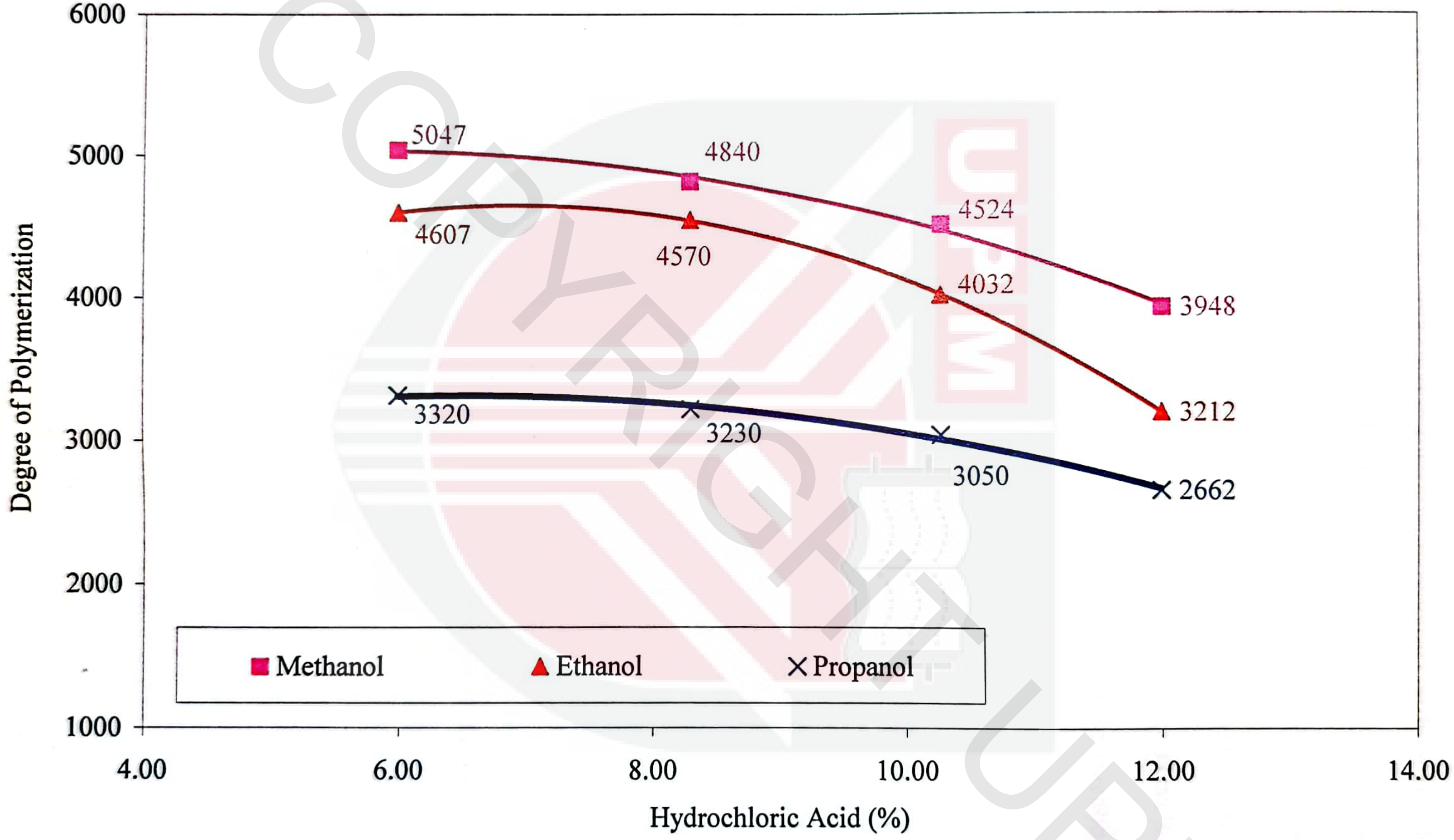


Figure 6: Degree of polymerization of sago starch after acid-alcohol treatment at 45^0C for 1 hour.

4.3 Solubility

Native sago starch showed less than 50 % solubility at temperatures between 60^{0}C and 80^{0}C. The solubility of modified starch profoundly increased with increasing temperature (Table 2). At temperatures above 70^{0}C, more than 100% of the modified sago starches were dissolved. This is due to gelatinization and swelling at 70^{0}C. With gelatinization, water molecules are entrapped inside their structure. Therefore, they are heavier than initial ungelatinized granules. Results of the investigation on starch solubilisation indicated that the individual starch granules are completely destructurised.

Table 2: Solubility (%) of sago starches after 12 % hydrochloric acid-alcohols treatment at 45^{0}C for 1 hour.

Treatment	60^{0}C	70^{0}C	80^{0}C
Native *	5.9^{c}	38.1^{b}	50.1^{b}
Methanol	89.9^{ab}	107.2^{a}	120.3^{a}
Ethanol	66.7^{b}	100.8^{a}	113.1^{a}
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).
* Starch without treatment.

5.0 CONCLUSION

The present study shows that modified starch of any desired d.p value can be prepared by suspending hydrated starch granules in acidic alcohols for 1 hour at 45^0C. 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 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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APPENDICES

Appendix 1 Average Granule Size Analysis

Source	dF	SS	Mean Square	F Value	Pr > F
Total	127	1664.867188			
Treatment	3	96.96093750	32.32031250	2.60	0.0556
Concentration	3	61.02343750	20.34114583	1.63	0.1852
Error	121	1506.882813	12.453577		

CV= 12.98382

Appendix 2 Solubility (60^{0}C) Analysis

Source	dF	SS	Mean Square	F Value	Pr > F
Total	14	17043.79733			
Treatment	4	16066.73067	4016.68267	41.11	<.0001
Error	10	977.06667	97.70667		

CV= 14.69766

Appendix 3 Solubility (70^{0}C) Analysis

Source	dF	SS	Mean Square	F Value	Pr > F
Total	14	11428.43733			
Treatment	4	10668.11733	2667.02933	35.08	<.0001
Error	10	760.32000	76.03200		

CV= 9.583418

Appendix 4 Solubility (80^{0}C) Analysis

Source	dF	SS	Mean Square	F Value	Pr > F
Total	14	11383.36000			
Treatment	4	11097.17333	2774.29333	96.94	<.0001
Error	10	286.18667	28. 61867		

CV= 5.143886

PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "Acid Modification of Sago Starch in Alcohol" by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus and published in the form approved by the Faculty.

Loh Siew Ling

Date: 1/5/2007