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Acid modification of starch granules in alcohols: effects of temperature, acid concentration, and starch concentration

John F. Robyt *, Jun-yong Choe, Richard S. Hahn, Erica B. Fuchs

Laboratory of Carbohydrate Chemistry and Enzymology, Department of Biochemistry and Biophysics, Iowa State University, Ames, IA 50011, USA

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Abstract

The acid modifications of potato, amylomaize-7, and waxy maize starches were conducted in methanol, ethanol, 2-propanol, and 1-butanol using a wide range of acid concentrations (0.36 to 5.0%, w/v) and temperatures of 5 to 65 °C. It was found that limiting degrees of polymerization (dp) values were obtained in 72 h of reaction. The resulting products were modified starch granules that contained new types of limit dextrins. It also was found that the dp values of the limit dextrins were dependent on the concentration of the starch in the alcohol suspension. At a constant concentration of acid, the degree of hydrolysis was inversely proportional to the concentration of the starch suspended in the alcohol. This is explained as due to a decrease in the concentration of acid inside the starch granules as the number of starch granules are increased. We further explore the scope of preparing modified starches with different limiting dp values by studying the reaction of potato starch in the four alcohols with 0.36 to 5% w/v HCl at different temperatures from 5 to 65 °C in 10 °C intervals. The study shows that by the selection of the alcohol, the acid concentration, the temperature, and the starch concentration, a wide range of limit dextrins, with selected dp values, can be produced.

Keywords: Starch granules; Acid modification

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* Corresponding author.

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1. Introduction

Acid hydrolysis has been used to modify starch and produce "soluble starch" for over 150 y. Nägeli [1] reported the treatment of native starch in water with 15% sulfuric acid for one month at ~ 20 °C. He obtained an acid-resistant fraction that was readily soluble in hot water. This fraction has become known as *Nägeli amylodextrin* and has been shown to be a mixture of low molecular weight, linear and branched dextrins, with an average degree of polymerization (dp) of 25–30 [2]. Lintner [3] described an acid modification of starch in which starch granules were treated in an aqueous suspension with 7.5% (w/v) hydrochloric acid for approximately 7 days. The product was a relatively heterogeneous, high molecular weight starch. This still is the method used for preparing commercial "soluble starch".

Small [4] reported a study of the preparation of soluble starch by refluxing starch granules in 95% ethanol containing 0.2–1.6% (w/v) hydrochloric acid for 6–15 min. The procedure gave a product that was readily soluble in water and had a minimum amount of low molecular weight dextrins. Ma and Robyt [5] reported the acid modification of potato and waxy maize starch granules in four different anhydrous alcohols (methanol, ethanol, 2-propanol, and 1-butanol) containing 0.36% hydrochloric acid at 65 °C for 60 min. The acid modification in the different alcohols produced starches with different average dp values, with the highest value being obtained in methanol and the lowest value in 1-butanol. The size distribution of the starch chains was narrower and more homogeneous than that of the native starch. It also was shown that a minimum amount of alcoholysis had occurred, that the size and shape of the granules were maintained, that the amylose component was completely absent when potato starch was modified in 2-propanol and 1-butanol, and that the different alcohols produced different concentrations of acid *inside* the granules. Hydrolysis of the α-(1 → 4) glycosidic linkage was taking place exclusively inside the granule with the 10–12% (w/v) water in the granule.

Fox and Robyt [6] reported a kinetic study of the acid hydrolysis of potato starch granules in the four alcohols with two concentrations of hydrochloric acid (0.36 and 6.0% w/v) at 25 °C. They observed that the dp values rapidly dropped and unexpectedly became constant after 24–72 h of reaction. It had been expected that the dp values would continuously drop with the time of the reaction to a very low value. The different alcohols and acid concentrations gave different, constant dp values, with methanol giving the highest dp value and 1-butanol giving the lowest value, and the higher acid concentration giving lower dp values. Because the dp values became constant in the different treatments, the products from these modifications represented the formation of new types of limit dextrins that had different average dp values, chain length distributions, and proportions of amylose and amylopectin.

In the present communication the acid modification of starch granules in the four alcohols has been extended to include three types of starches from potato, amylomaize-7, and waxy maize. The time course of the modification of these starches has been studied using a wide range of acid concentrations and temperatures. The effect of the starch concentration is reported for 25, 50, and 100 g/100 mL of alcohol. Acid/alcohol limit dextrins were formed in 72 h of reaction. In an extensive study, the dp of the resulting limit dextrins from the modification of potato starch granules in the four alcohols is reported for various combinations of hydrochloric acid concentrations (0.36 to 5.0%, w/v) and temperatures of 5 to 65 °C in 10 °C intervals.

2. Experimental

Starches.—Potato starch and amylomaize-7 starch (Hylon VII, a maize starch with 70% amylose) were obtained from National Starch and Chemical Co., Bridgewater, NJ. Waxy maize starch (a 100% amylopectin starch) was obtained from American Maize Co., Hammond, IN. The starches contained 10–15% (w/w) water and the usual components found in commercial starch preparations.

Kinetic studies.—The different starches (25 g) were suspended in 100 mL of anhydrous alcohol. The suspensions were placed at ambient room temperature, 20–21 °C, or into a constant temperature bath at the desired temperature for 2 h. The reaction was started by manually shaking the suspension of starch in the alcohol and adding concentrated HCl in the desired amount (1.0–16.0 mL). At regular time intervals over 5–7 days, the suspensions were shaken and 2–5 mL aliquots were removed for analysis. The samples were filtered and the starches were washed with 70% EtOH until the washings were neutral to litmus. The starches were then air-dried. Modified potato starches and waxy maize starches were dissolved (10 mg/mL) by heating in water. Modified amylomaize-7 starches (50 mg) were dissolved in 0.5 mL dimethyl sulfoxide (Me_2SO) by stirring and mild heating to 50–60 °C. The Me_2SO–starch solution was then slowly diluted to 5 mL with distilled water. The reducing value was determined in triplicate using the micro copper–bicinchoninate procedure [7], and the amount of total carbohydrate was determined in triplicate by the micro phenol–H_2SO_4 procedure [7]. The dp was computed using these determinations [8].

Effects of starch concentration.—Various amounts of starch (25, 50, and 100 g) were suspended in 100 mL anhyd EtOH, and varying amounts of concd HCl (1.0–16.0 mL) were added and the reaction allowed to proceed at 20–21 °C for 72 h. After the reaction was complete, the starches were filtered and washed with 70% EtOH until the washings were neutral to litmus. The starches were air-dried and the average dp was determined as described above.

Effects of acid concentration and temperature.—The native starches were suspended in the anhydrous alcohols (25 g/100 mL) and placed at ambient room temperature, 20–21 °C, or placed into a water bath at a specified temperature from 5 to 65 °C. Reaction was started by the addition of the desired amount of concd HCl from 1.0 to 16.0 mL. The reaction was allowed to proceed for 72 h. The starches were then filtered and washed with 70% EtOH until the washings were neutral to litmus. The starches were air-dried and the average dp determined as described above.

3. Results and discussion

It was previously shown [6] that acid modification of potato starch in methanol, ethanol, 2-propanol, and 1-butanol with 0.36 and 6.0% w/v HCl at 25 °C gave a rapid

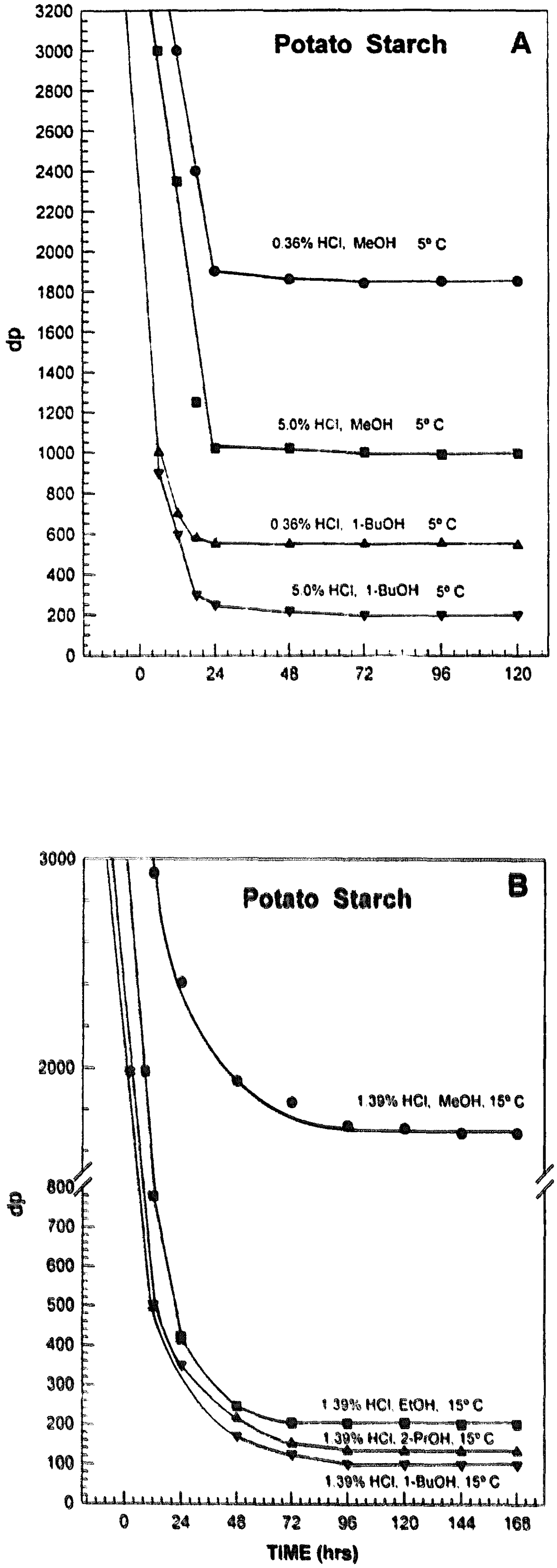

Potato Starch
A
dp
0.36% HCl, MeOH 5° C
5.0% HCl, MeOH 5° C
0.36% HCl, 1-BuOH 5° C
5.0% HCl, 1-BuOH 5° C
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
24
48
72
96
120
Potato Starch
B
dp
1.39% HCl, MeOH, 15° C
1.39% HCl, EtOH, 15° C
1.39% HCl, 2-PrOH, 15° C
1.39% HCl, 1-BuOH, 15° C
3000
2000
800
700
600
500
400
300
200
100
0
0
24
48
72
96
120
144
168
TIME (hrs)

Fig. 1.

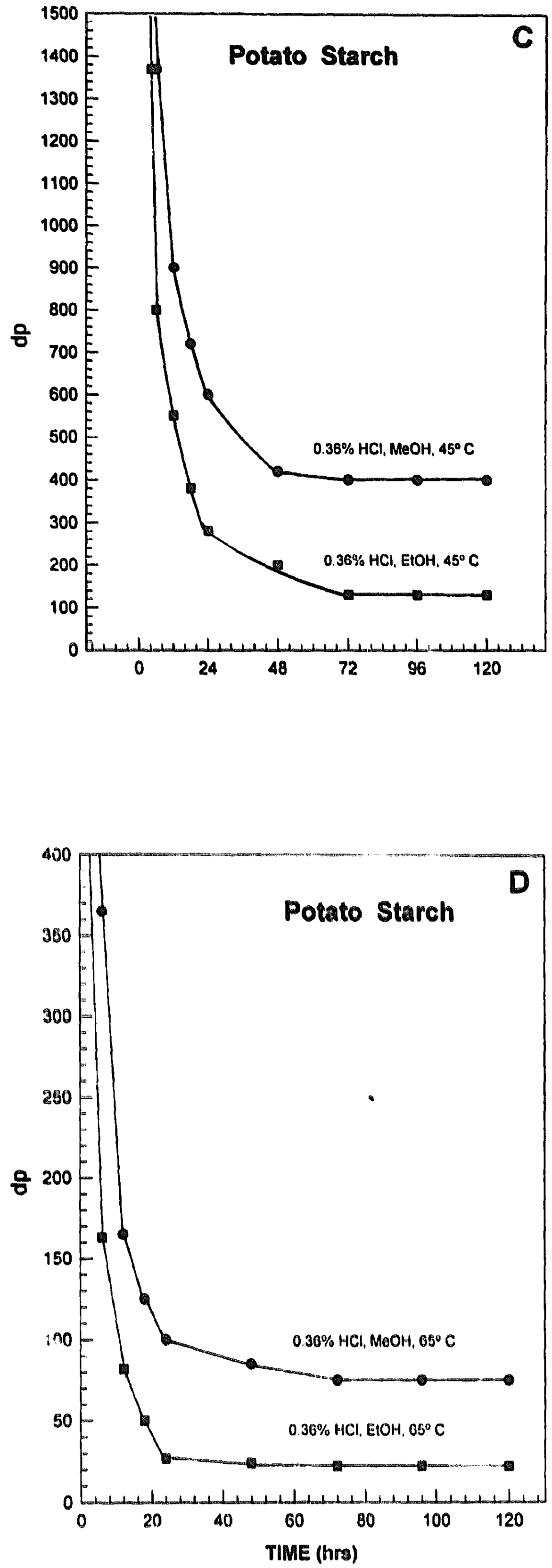


Fig. 1. (continued).

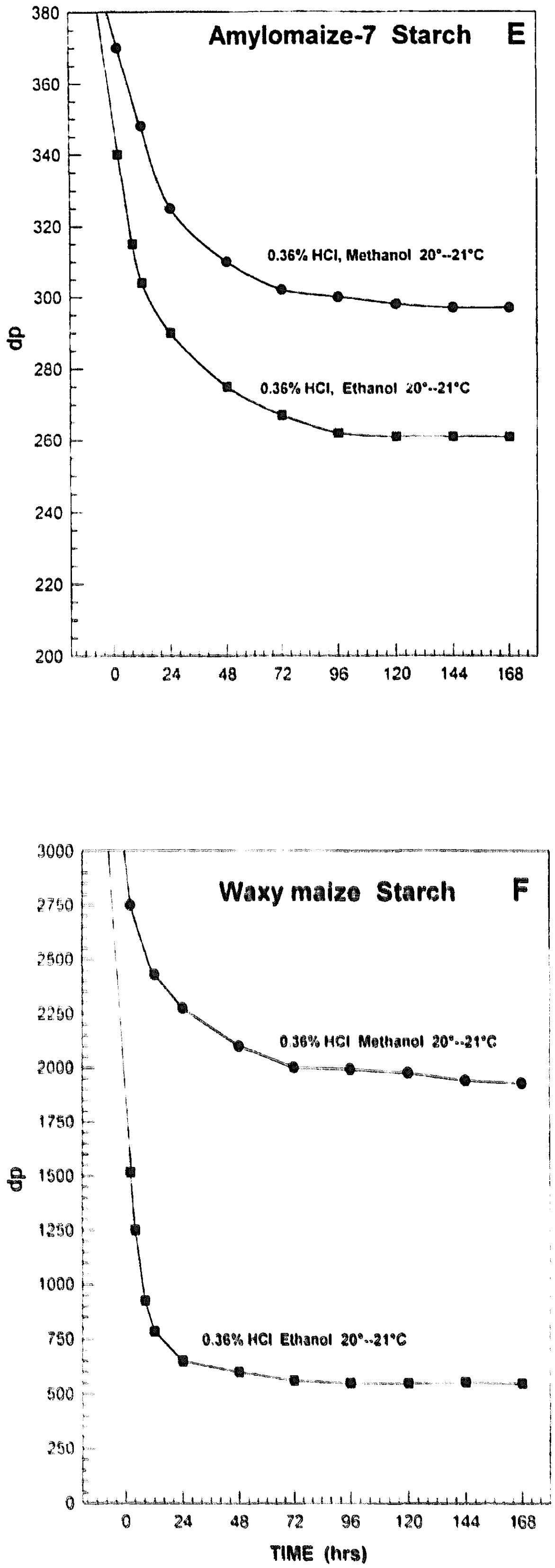


Fig. 1. (continued).

decrease in the dp value that reached a constant, limiting value within 24–72 h. We have extended the study of the conditions of the modification to include potato starch modified in methanol and 1-butanol at 5 °C with two concentrations of acid, 0.36 and 5% w/v HCl, and find that these conditions give a limiting dp value in 72 h (see Fig. 1A). Modification of potato starch in the four alcohols at 15 °C with 1.39% HCl gave limiting dp values in 72 h (see Fig. 1B). Modification of potato starch in the four alcohols at 45 °C using 0.36% HCl gave limiting dp values in 72 h (data for methanol and ethanol are given in Fig. 1C). Modification of potato starch in methanol and ethanol at 65 °C using 0.36% HCl gave limiting dp values in 72 h (see Fig. 1D). Modification of two other starches, amylomaize-7 starch, and waxy maize starch, in methanol and ethanol at 20–21 °C, using 0.36% HCl, also gave limiting dp values in 72 h (see Figs. 1E and 1F).

The time course of the acid modification of starch granules in the four alcohols at widely different conditions of acid concentration, temperature, and type of starch shows that the formation of modified starch granules with limiting dp values in 72 h is a general phenomenon that is applicable over a wide range of conditions. The limiting dp values of the resulting modified starches depend on the conditions of the reaction. This study shows that relatively simple procedures can be used to produce an extremely broad range of acid-modified starch granules with different dp values.

We have studied the effect of the concentration of starch by suspending 25, 50, and 100 g of potato starch and amylomaize-7 starch in 100 mL of ethanol with various concentrations of HCl. The reaction was allowed to proceed for 72 h at 20–21 °C, followed by the determination of the dp values of the modified starches. The results (Fig. 2) show that the concentration of the starch had an effect in that as the concentration was increased, the limiting dp value was increased for a specific concentration of acid. For example, to obtain a dp value of 240 for amylomaize-7 starch with 25 g/100 mL, an acid concentration of 1.0 mL/100 mL of alcohol was required; a concentration of 3.6 mL of HCl/100 mL of alcohol was required if the amylomaize-7 starch was 50 g/100 mL; and a concentration of 9.2 mL of HCl/100 mL of alcohol was required if the amylomaize-7 starch was 100 g/100 mL (see Fig. 2B). A similar observation was made for potato starch, although with different dp values due to the differences in the dp values of the two types of native starch granules (see Fig. 2A).

It was previously shown [5] that the different alcohols produce different concentrations of acid inside the granule where acid hydrolysis occurs. It was also shown [6] that higher acid concentrations gave lower, limiting dp values. The increase in the dp values as the concentration of the starch granules is increased reflects this mechanism for the

Fig. 1. Time course of the hydrolysis of three types of starches in different alcohols at different concentrations of acid and at different temperatures. (A) Hydrolysis of 25 g of potato starch in 100 mL methanol and 1-butanol with 0.36 and 5.0% w/v HCl at 5 °C. (B) Hydrolysis of 25 g of potato starch in 100 mL methanol, ethanol, 2-propanol, and 1-butanol with 1.39% w/v HCl at 15 °C. (C) Hydrolysis of 25 g of potato starch in 100 mL methanol and ethanol with 0.36% w/v HCl at 45 °C. (D) Hydrolysis of 25 g of potato starch in 100 mL methanol and ethanol with 0.36% w/v HCl at 65 °C. (E) Hydrolysis of 25 g of amylomaize-7 starch in 100 mL methanol and ethanol with 0.36% w/v HCl at 20–21 °C. (F) Hydrolysis of 25 g of waxy maize starch in 100 mL methanol and ethanol with 0.36% w/v HCl at 20–21 °C.

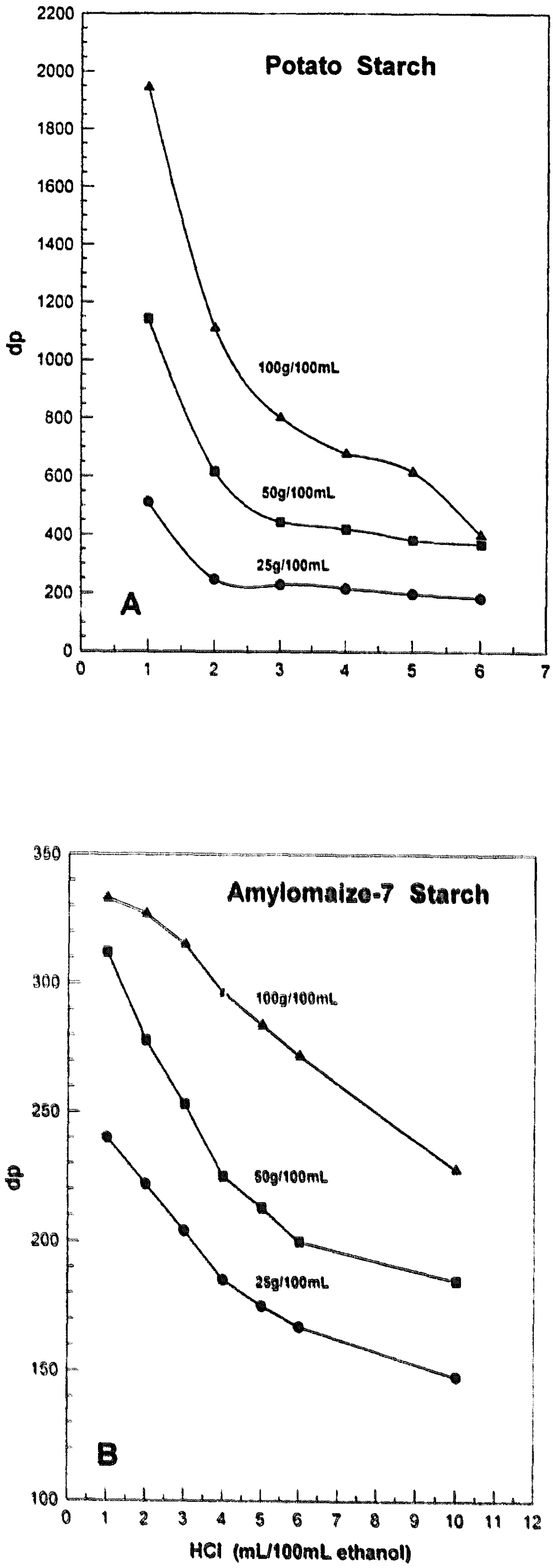

Potato Starch
dp
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
1
2
3
4
5
6
7
100g/100mL
50g/100mL
25g/100mL
A
Amylomaize-7 Starch
dp
350
300
250
200
150
100
0
1
2
3
4
5
6
7
8
9
10
11
12
100g/100mL
50g/100mL
25g/100mL
B
HCl (mL/100mL ethanol)

Fig. 2.

Table 1
Average limiting dp values for potato starch granules modified for 72 h in methanol with different concentrations of HCl at different temperatures

mL HCl [a]	%HCl (w/v)	Temperature (°C)						
		5	15	25	35	45	55	65
1.0	0.36	1811±306 [b]	1784±110	1210±68	471±6	419±45	90±5	72±7
2.0	0.71	1518±267	1567±147	780±18	440±16	145±9	55±3	45±4
4.0	1.39	1233±264	1202±71	543±8	212±2	71±3	49±4	40±2
8.0	2.67	1118±104	789±15	395±14	149±8	56±3	50±4	33±1
16.0	5.00	947±79	437±11	195±3	109±9	50±3	42±2	31±3

[a] mL of concentrated HCl/100 mL of alcohol.

[b] The dp of the limit dextrins were determined in triplicate; the means and standard deviations were calculated from these determinations.

acid–alcohol hydrolysis of starch granules. For any one concentration of acid, the concentration of acid inside the granule is decreased as the number of granules is increased, thus giving less hydrolysis and a higher limiting dp value. When the concentration of starch granules was increased, more acid was required to obtain an equivalent dp value (see Fig. 2).

We have also studied the effect of different concentrations of HCl on the formation of modified starch granules with limiting dp values. We report the modification of potato starch, amylomaize-7 starch, and waxy maize starch in the four alcohols at 20–21 °C (ambient room temperature) and at 40 °C for amylomaize-7 starch. Each starch was allowed to react at the indicated temperature and acid concentration for 72 h and then the dp value was determined. From the curves of Fig. 3, it can be seen that a modified starch of almost any desired dp value can be obtained. For example, if a potato starch with an average dp value of 500 was desired, 250 g of starch would be suspended in 1 L of methanol and 53 mL of concentrated HCl would be added, and the reaction allowed to proceed for 72 h at 20–21 °C; if a waxy maize starch with an average dp of 500 was desired, 250 g of starch would be suspended in 1 L of ethanol and 32 mL of concentrated HCl would be added, and the reaction allowed to proceed for 72 h at 20–21 °C; and if an amylomaize-7 starch with an average dp of 200 was desired, 250 g of starch would be suspended in 1 L of ethanol and 32 mL of concentrated HCl would be added, and the reaction allowed to proceed for 72 h at 20–21 °C. An alternative to the last example would be the addition of 500 g of amylomaize-7 starch to 1 L of ethanol with 60 mL of concentrated HCl (see Fig. 2B) and the reaction allowed to proceed for 72 h at 20–21 °C to obtain a modified amylomaize-7 starch with an average dp of 200.

Fig. 2. Effect of the concentration of starch on the limiting dp value obtained from acid hydrolysis in ethanol at 20–21 °C. (A) Hydrolysis of three concentrations of potato starch (25, 50, and 100 g/100 mL of ethanol) with 0.36% w/v HCl at 20–21 °C for 72 h. (B) Hydrolysis of three concentrations of amylomaize-7 starch (25, 50, and 100 g/100 mL of ethanol) with 0.36% w/v HCl at 20–21 °C for 72 h.

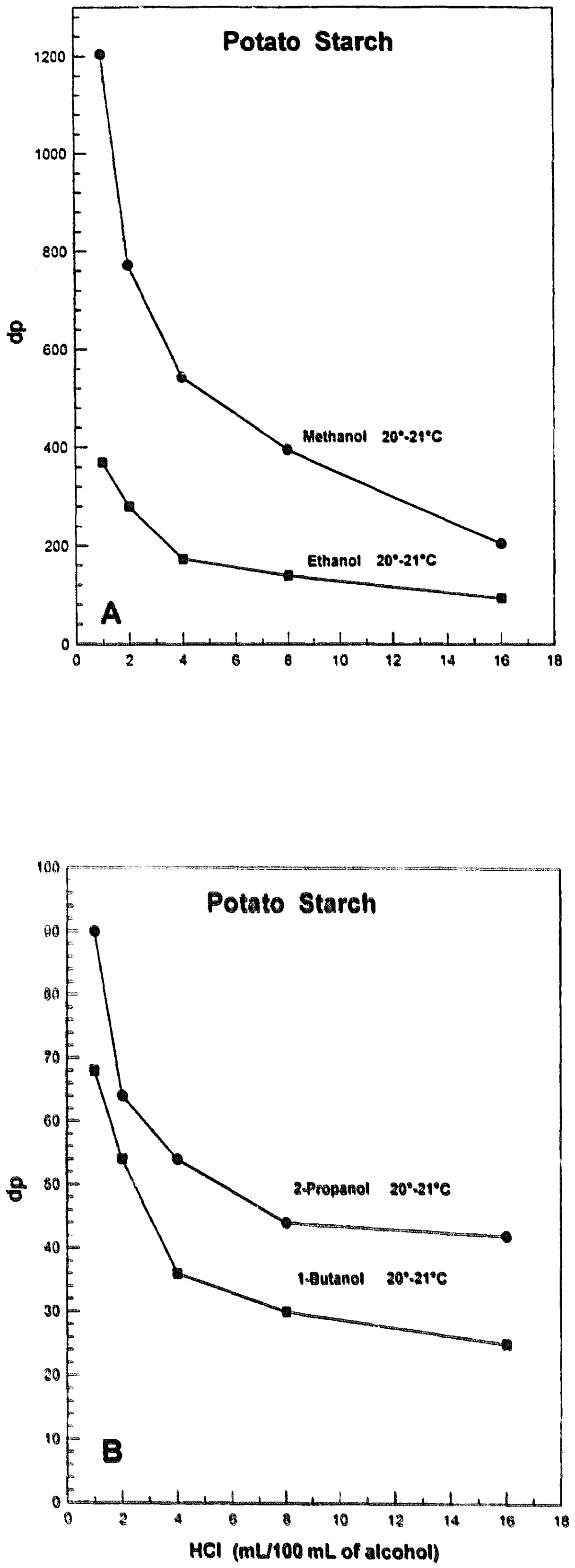

Potato Starch
dp
1200
1000
800
600
400
200
0
Methanol 20°-21°C
Ethanol 20°-21°C
A
0
2
4
6
8
10
12
14
16
18
Potato Starch
dp
100
90
80
70
60
50
40
30
20
10
0
2-Propanol 20°-21°C
1-Butanol 20°-21°C
B
0
2
4
6
8
10
12
14
16
18
HCl (mL/100 mL of alcohol)

Fig. 3.

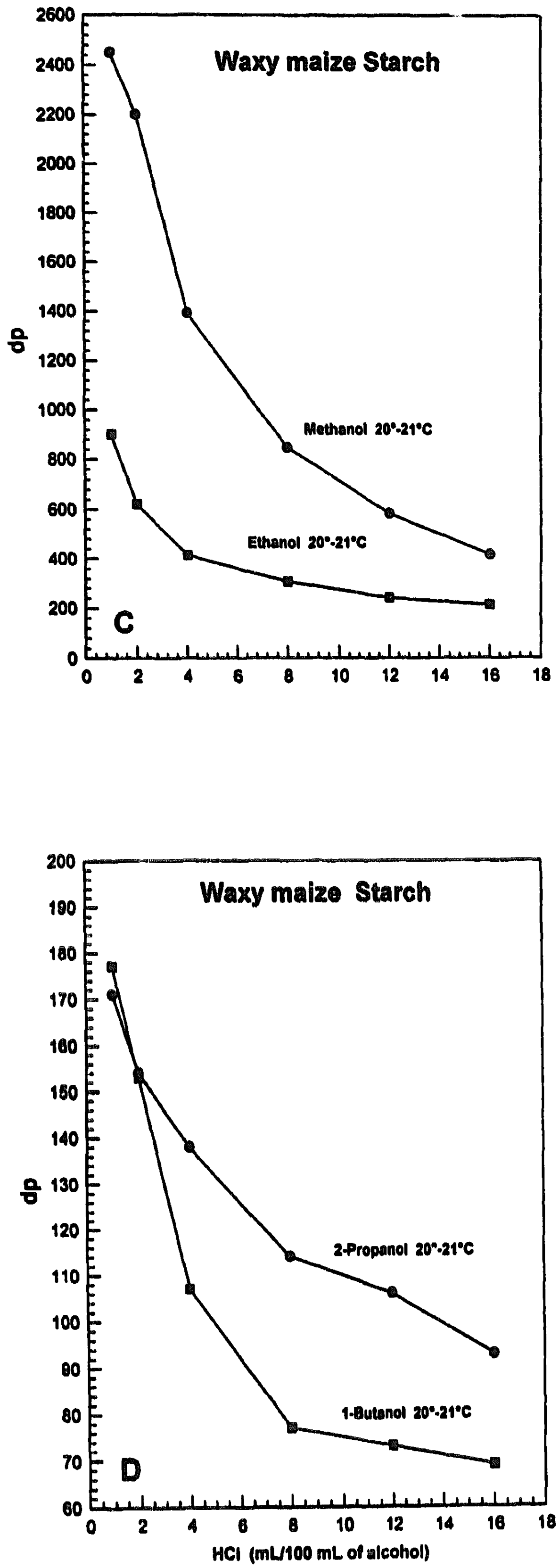
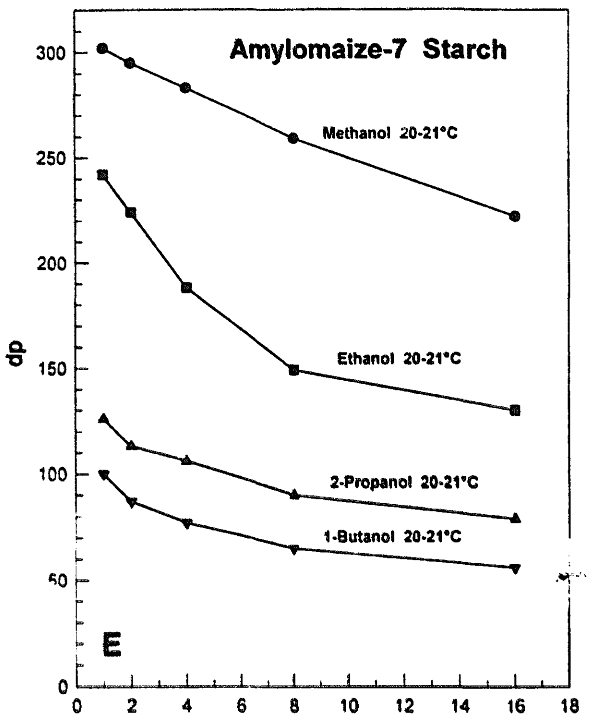


Fig. 3. (continued).



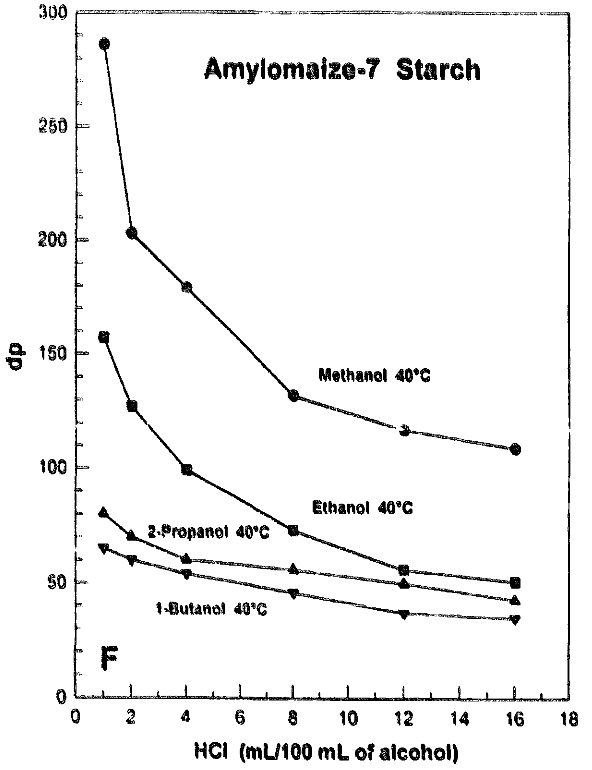


Fig. 3. (continued).

Table 2
Average limiting dp values for potato starch granules modified for 72 h in ethanol with different concentrations of HCl at different temperatures

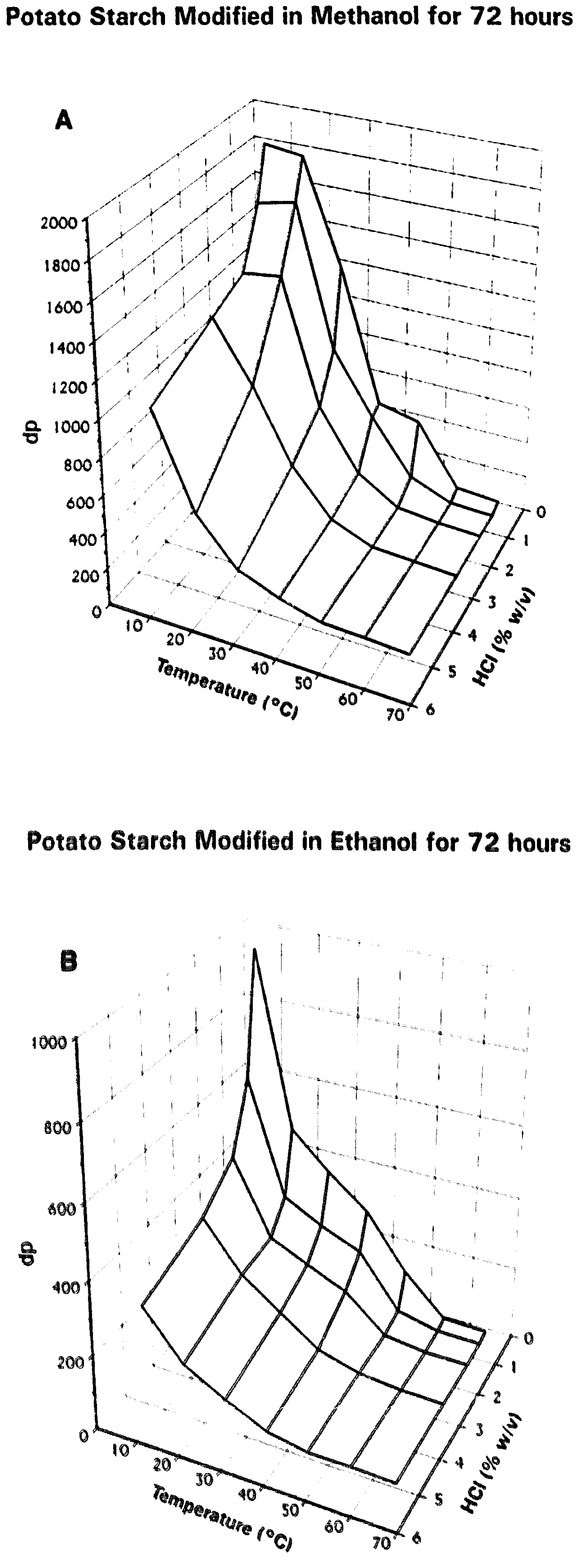
mL HCl [a]	%HCl (w/v)	Temperature (°C)						
		5	15	25	35	45	55	65
1.0	0.36	938±113 [b]	466±21	370±10	283±5	137±6	30±3	26±4
2.0	0.71	604±48	292±12	237±9	191±4	48±3	20±1	18±1
4.0	1.39	423±21	220±21	173±2	126±3	30±3	14±1	14±2
8.0	2.67	339±18	208±22	132±8	59±1	27±1	15±1	13±1
16.0	5.00	270±23	143±11	83±3	34±2	15±1	16±1	12±1

[a] mL of concentrated HCl/100 mL of alcohol.
[b] The dp of the limit dextrins were determined in triplicate; the means and standard deviations were calculated from these determinations.

The three types of starches gave different limiting dp values for the same concentrations of acid. For example, 25 g of potato starch in methanol at 20–21 °C required 8.0 mL of HCl/100 mL of methanol to obtain a dp of 400; 25 g of waxy maize starch under the same conditions required 16.0 mL of HCl to obtain a dp of 400; 25 g of amylomaize-7 starch in 100 mL of methanol with 8.0 mL of HCl at 20–21 °C gave a dp of 135 and with 16.0 mL gave a dp of 115; and with 1.0 mL of HCl under the same conditions of alcohol and temperature, potato starch, waxy maize starch, and amylomaize-7 starch gave limiting dp values of 1200, 2460, and 310, respectively. Compare Figs. 3A, 3C, and 3E.

In an extended study to further explore the scope of preparing modified starches with different dp values, we reacted potato starch in the four alcohols with different concentrations of acid from 0.36 to 5% w/v HCl and at different temperatures from 5 to 65 °C in 10 °C intervals for 72 h. We determined the average dp for each of the resulting modified starches. The results are summarized in Tables 1–4 for the four alcohols. The data show that for a fixed concentration of acid (e.g., 0.36%), the dp decreases steadily with an increase in the temperature; and at a fixed temperature (e.g., 25 °C), the dp decreases with an increase in the concentration of acid. This correlation holds for different concentrations of acid and different temperatures. For 2-propanol and 1-butanol, the modification at higher temperatures (e.g., 35–65 °C) are only reported (Tables 3 and 4) for 0.36% HCl. At the higher acid concentrations and higher temperatures, the starch granules are destroyed in 2-propanol and 1-butanol and gels are formed. Modification of the starch granules, without destruction of the granule, at the higher acid concentrations

Fig. 3. Effect of acid concentration on the limiting dp of potato starch, amylomaize-7 starch, and waxy maize starch in methanol, ethanol, 2-propanol, and 1-butanol. (A) Acid hydrolysis of 25 g of potato starch in 100 mL methanol and ethanol at 20–21 °C for 72 h. (B) Acid hydrolysis of 25 g of potato starch in 100 mL 2-propanol and 1-butanol at 20–21 °C for 72 h. (C) Acid hydrolysis of 25 g of waxy maize starch in 100 mL methanol and ethanol at 20–21 °C for 72 h. (D) Acid hydrolysis of 25 g of waxy maize starch in 100 mL 2-propanol and 1-butanol at 20–21 °C for 72 h. (E) Acid hydrolysis of 25 g of amylomaize-7 starch in 100 mL methanol, ethanol, 2-propanol, and 1-butanol at 20–21 °C for 72 h. (F) Acid hydrolysis of 25 g of amylomaize-7 starch in 100 mL methanol, ethanol, 2-propanol, and 1-butanol at 40 °C for 72 h.



Potato Starch Modified in Methanol for 72 hours

Potato Starch Modified in Ethanol for 72 hours

Fig. 4.

Table 3
Average limiting dp values for potato starch granules modified for 72 h in 2-propanol with different concentrations of HCl at different temperatures

mL HCl [a]	%HCl (w/v)	Temperature (°C)						
		5	15	25	35	45	55	65
1.0	0.36	550±24 [b]	458±16	90±9	39±2	18±1	9±1	5±1
2.0	0.71	379±21	278±11	64±5	– [c]	–	–	–
4.0	1.39	335±10	234±6	54±4	–	–	–	–
8.0	2.67	245±11	201±7	44±2	–	–	–	–
16.0	5.00	223±10	147±10	41±2	–	–	–	–

[a] mL of concentrated HCl/100 mL of alcohol.

[b] The dp of the limit dextrins were determined in triplicate; the means and standard deviations were calculated from these determinations.

[c] Granule structure was destroyed at 35 °C and above and 0.71% HCl and above.

Table 4
Average limiting dp values for potato starch granules modified for 72 h in 1-butanol with different concentrations of HCl at different temperatures

mL HCl [a]	%HCl (w/v)	Temperature (°C)						
		5	15	25	35	45	55	65
1.0	0.36	538±40 [b]	448±32	68±4	32±1	18±2	9±1	4±1
2.0	0.71	424±45	277±19	60±2	– [c]	–	–	–
4.0	1.39	295±13	209±6	36±3	–	–	–	–
8.0	2.67	252±25	152±8	34±3	–	–	–	–
16.0	5.00	196±28	96±6	25±2	–	–	–	–

[a] mL of concentrated HCl/100 mL of alcohol.

[b] The dp of the limit dextrins were determined in triplicate; the means and standard deviations were calculated from these determinations.

[c] Granule structure was destroyed at 35 °C and above and 0.71% HCl and above.

and higher temperatures, however, could be obtained in methanol and ethanol (Tables 1 and 2). Fig. 4 summarizes the modification of potato starch granules, using different acid concentrations and different temperatures with methanol and ethanol. The three-dimensional graphs permit the determination of a specific concentration of acid and specific temperature to obtain any desired average dp from 1800 to 12.

In summary, we have shown that selective acid hydrolysis can be obtained by suspending starch granules in one of four alcohols: methanol, ethanol, 2-propanol, and 1-butanol. Modified starch granules with varied dp values can be produced by the selection of the alcohol, acid concentration, and temperature. The kinetics of the reaction showed that a limiting dp value is formed in 72 h of reaction over a wide range of acid

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Fig. 4. Three-dimensional plots (dp vs. temperature and concentration of HCl) of the acid hydrolysis of potato starch in methanol and ethanol. (A) Hydrolysis of 25 g of potato starch in 100 mL methanol. (B) Hydrolysis of 25 g of potato starch in 100 mL ethanol.

concentrations, temperatures, type of alcohol, and type of starch. The reaction is dependent on the concentration of the acid in the alcohol and on the temperature. The higher the acid concentration and the higher the temperature, the lower is the limiting dp The reaction is also dependent on the concentration of the starch in the alcohol suspension; as the concentration of starch increases, lower degrees of hydrolysis and a higher limiting dp value is obtained. This result confirms that the mechanism of hydrolysis of starch granules suspended in alcohol involves the hydrolysis of glycosidic bonds with the water contained inside the granules. The data show how a relatively simple procedure can be used to produce a wide range of modified starch granules, containing limit dextrins with different dp values.

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