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PREPARATION AND CHARACTERIZATION OF SOLUBLE STARCHES HAVING DIFFERENT MOLECULAR SIZES AND COMPOSITION, BY ACID HYDROLYSIS IN DIFFERENT ALCOHOLS*

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ABSTRACT

Potato and waxy-maize starches were separately modified for 1 h at 65° with 0.36% hydrochloric acid in methanol, ethanol, 2-propanol, and 1-butanol. All of the modified starches were readily soluble in hot water, to give crystal-clear solutions up to a concentration of at least 20% (w/v). The modified granules were studied by light-microscopy and iodine–iodide staining. All of the modified starches retained their granule appearance, although with various degrees of damage that progressively increased from methanol to 1-butanol. Both hydrolysis and alcoholysis occurred, but to different extents in the different alcohols. The highest proportion of alcoholysis occurred in methanol where 50% of the resulting molecules were glycosides, the lowest in 1-butanol where 6% were glycosides. The number-average molecular weights of the modified starches also progressively decreased from 126,670 for the methanol-modified waxy-maize starch to 4,750 for the 1-butanol-modified potato starch. The methanol- and ethanol-modified potato starches were fractionated into amylose and amylopectin components. The 2-propanol- and 1-butanol-modified potato starches gave only an amylopectin component. The amylose components were characterized by gel-permeation chromatography on Bio-Gel A-5m, and the amylopectin components, on Bio-Gels A-150m and A-0.5m. The molecular sizes of the amylose and amylopectin components progressively decreased from methanol- to 1-butanol-modified starches. Furthermore, the polymodal composition of the amylopectin component was decreased to give a more homogeneous product. Waxy-maize starch was modified in methanol and 2-propanol and gave products that were of lower molecular size and more homogeneous than the polymodal native starch. It is shown that the differential effect of the different alcohols on the modification of the starch granules is produced by effecting different concentrations of acid inside the granule, where hydrolysis occurs in the 10–12% of water contained in the granule. It is postulated that 2-propanol and

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1-butanol dissolve the double-helical, crystalline regions in the starch granule to give different types of products under otherwise identical conditions of modification.

INTRODUCTION

The usual method for preparing soluble starch is the method of Lintner[1], in which raw starch is treated with 7.5% hydrochloric acid for approximately one week at 22–24°. The mixture is stirred several times each day, and after one week the liquid is decanted, and the starch washed and dried. The product is heterogeneous and has a relatively high reducing value.

In 1919, Small[2] reported a study on preparing soluble starch by refluxing raw starch for 6 to 15 min in 95% ethanol containing 0.2–1.6% of hydrochloric acid, to obtain the maximum conversion of the raw starch into soluble starch with minimal production of low-molecular-weight dextrins.

In the present investigation we have extended Small's study and now report the production of soluble starch by acid hydrolysis in four different alcohols at 65° and the characterization of the products by microscopy, iodine–iodide absorbance, molecular weight, fractionation into linear and branched components, and gel-permeation chromatography of the components on Bio-Gel A-0.5m, A-5m, A-50m, and A-150m. Both potato starch and waxy-maize starch were modified, and the characteristics of the linear and branched components of the soluble, modified starches are compared with those of unmodified starch and Lintner soluble starch.

EXPERIMENTAL

Materials. — Potato starch was obtained from Stein and Hall (New York, NY), and had 11.9% (w/w) of water. Waxy-maize starch was from American Maize Products (Roby, IN), and had 10.5% (w/w) of water. Lintner's soluble starch was from Fisher Scientific. Bio-Gels A-0.5m, A-5m, A-50m, and A-150m were obtained from Bio-Rad Laboratories, Richmond, CA.

Modification of starch granules, and the preparation of soluble starch. — Potato starch or waxy-maize starch (25 g) was suspended in 100 mL of one of the four alcohols [methanol (0.05% water), ethanol (0.01% water), 2-propanol (0.04% water), or 1-butanol (0.1% water)] in a 250-mL round-bottomed flask fitted with a reflux condenser. The suspension was stirred and heated to 65°. Reaction was started by adding 1 mL of concentrated (36% by weight) hydrochloric acid, and allowed to proceed for 60 min at 65°. The reaction was stopped by adding 14 mL of M $NaHCO_3$, and cooling in an ice-bath. The starch was filtered off and washed twice with 1:1 alcohol–water (200 mL).

Fractionation of starch and a modified starch. — A solution of the modified starch (10 g) in hot water (100 mL) was cooled; 1-butanol (10 mL) was added, and the solution was stirred for ~15 h at room temperature. The resulting butanol com-

plex of the amylose component[3] was centrifuged off. Ethanol (2 vol.) was added to the supernatant liquor to precipitate the amylopectin, which was collected by centrifugation. The two precipitates were triturated four or five times with dry acetone and once with dry ethanol and then dried under vacuum at 50°. Unmodified starch was fractionated in the same way, except that 10 g was first dissolved in 9:1 (v/v) Me_2SO–water (200 mL) by stirring for ~15 h. The Me_2SO–starch solution was then carefully diluted to 1 L with water. The amylose component was precipitated by adding 1-butanol (100 mL). After removal of the amylose–butanol complex, the amylopectin component was precipitated by adding ethanol (2 vol.).

Analysis of the starch fractions. — The modified starches and the amylopectin components were dissolved in hot water to give a concentration of 25 mg/mL, and the amylose components were dissolved in Me_2SO and diluted to 5 mg/mL with water. Columns of Bio-Gel A-0.5m (8 × 280 mm), A-5m (8 × 280 mm), Bio-Gel A-50m (8 × 280 mm), and Bio-Gel A-150m (8 × 470 mm) were used. Samples (1–2 mL) were added to the columns and eluted with water, with the collection of 1-mL fractions. The fractions were analyzed by addition of 50 μL of iodine–iodide reagent (2 mg of I_2 and 20 mg of KI/mL) to 0.1 mL of the fraction, followed by addition of 2 mL of water, and the absorbance maximum was determined.

Determination of the number-average molecular weight. — Solutions containing 10.0 mg of starch/mL were prepared and their reducing values were determined by the Somogyi–Nelson, alkaline-copper procedure[4], using maltose as the standard. The number-average molecular weight was computed from the weight (μg) of the sample and its reducing value in μg of maltose[5].

Iodine–iodide complex of starch and its components. — Complex-formation was obtained by adding 50 μL of iodine–iodide reagent (2 mg of I_2 and 20 mg of KI/mL) to 100 μL of starch solution (5 mg/mL), followed by dilution with 3.0 mL of water.

Solubility test. — The modified starches were prepared in concentrations of 10 to 200 mg/mL by addition of the solid to hot water with stirring.

Microscopy. — The unmodified and modified starches were observed with the light-microscope, using the high-dry objective (675X magnification). A small amount of starch was suspended in one drop of water, and a cover slip was placed on top of the drop. After observation, a drop of iodine–iodide reagent was added at the edge of the cover slip, and the iodine-stained starch was observed.

Determination of the extent of alcoholysis. — The reactions were conducted as already described, but with starch (500 mg), alcohol (2 mL), and 370 kBq (10 μCi) of ^{14}C-methanol (as a tracer). After reaction, the starch was thoroughly washed several times with alcohol to remove nonbonded label, and the starch was then suspended in 15 mL of a toluene cocktail containing 4% of Cab-O-Sil, and counted by liquid scintillation spectrometry.

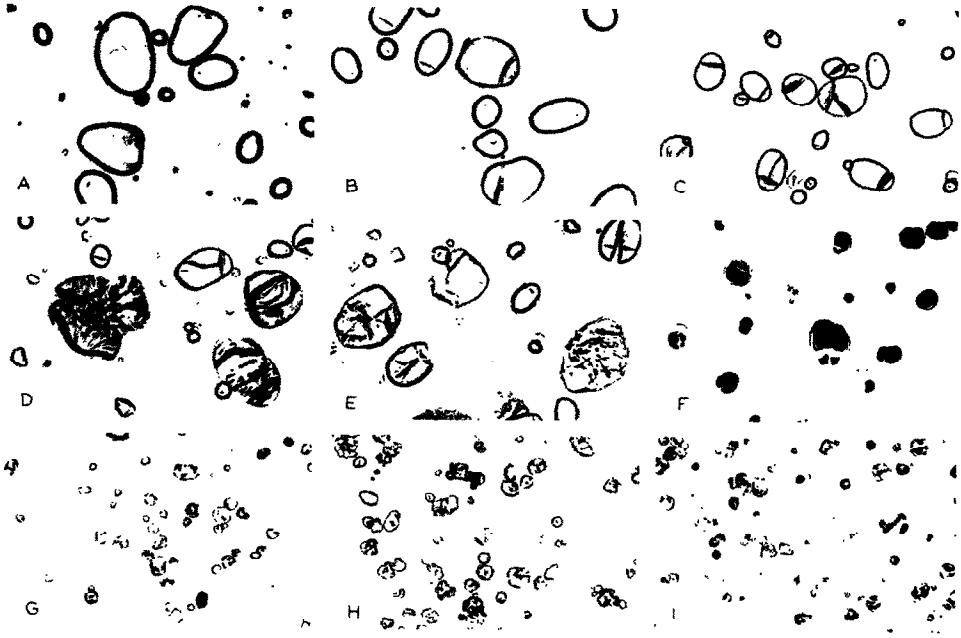

Fig. 1. Light-microscopy of unmodified and modified starches. Key: A, unmodified potato starch; B, methanol-modified potato starch; C, ethanol-modified potato starch; D, 2-propanol-modified potato starch; E, 1-butanol-modified potato starch; F, iodine stain of 2-propanol-modified potato starch; G, unmodified waxy-maize starch; H, methanol-modified waxy-maize starch; and I, 2-propanol-modified waxy-maize starch.

RESULTS

Light-microscopy revealed that all of the modified starches retained their granule appearance, although various degrees of granule damage were observed (see Fig. 1). This damage increased with the size of the alcohol, methanol giving the least apparent damage and 1-butanol the most. Methanol-treated potato starch had, on one end of several of the granules, a small cut or crack that appeared to be located uniformly near the center of the hilum. The iodine stain of the methanol-treated potato-starch granules appeared dark purple, very similar to that of the unmodified starch. Modification in ethanol, 2-propanol, and 1-butanol gave progressively increased granule damage, as indicated by the number of cracks and fissures (see Fig. 1). The 1-butanol-modified potato-starch granules appeared to be intact but highly fractured. Methanol-modified waxy-maize starch showed a few damaged granules that were intact. After staining with iodine, however, ~50% of the granules appeared damaged. 2-Propanol-modified waxy-maize starch showed intact, but damaged and fractured, granules.

The iodine stain of the 2-propanol- and 1-butanol-modified potato-starch was

red-purple and showed a differential staining in which the outside of the granule appeared to have a lighter iodine-staining sheath or capsule surrounding a darker-staining interior (see Fig. 1F). The interior of these granules stained a dark purple to reveal four parts that were distributed to give a cross similar to the polarization cross observed by plane-polarized light. Iodine staining of the modified waxy-maize granules did not show the sheath-like capsule that the 2-propanol- and 1-butanol-modified potato-starch granules did.

Plane-polarized light showed the characteristic "Maltese cross" for potato starch and the modified potato-starches. The polarized cross was progressively intensified for methanol- to 1-butanol-modified starch. Waxy-maize starch and its alcohol modifications did not show the polarization cross.

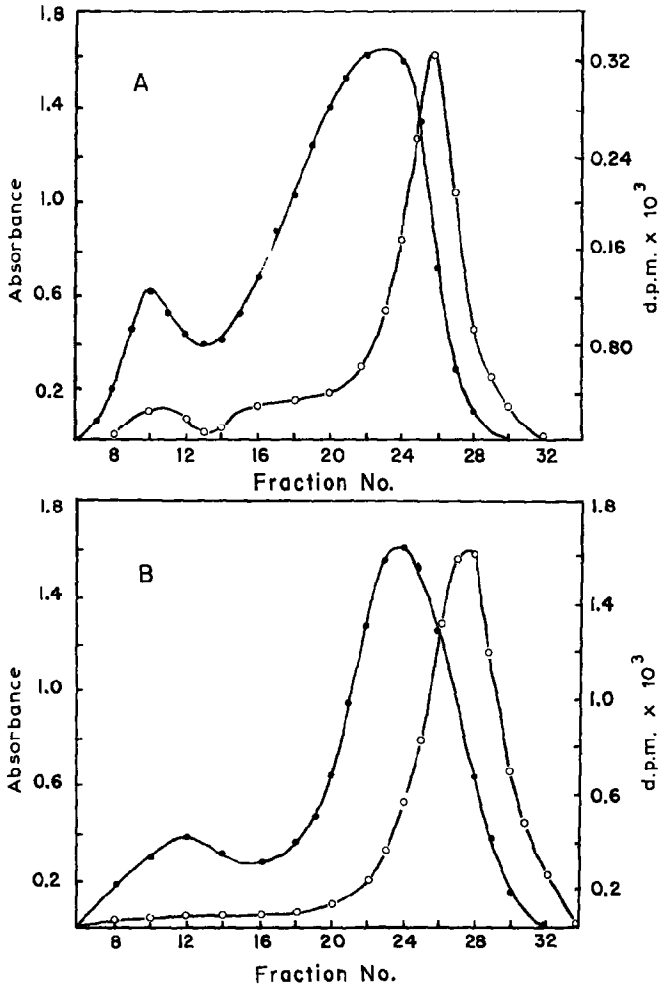

Fig. 2. Gel-permeation chromatography of (A) methanol- and (B) ethanol-modified potato starch on Bio-Gel A-50m. Key: —●—, absorbance (A_{585} for A; A_{575} for B) of iodine–iodide color; —○—, d.p.m. ($\times 10^3$) of ^{14}C incorporated into the starches. The radioactivity represents glycosides formed by alcoholysis.

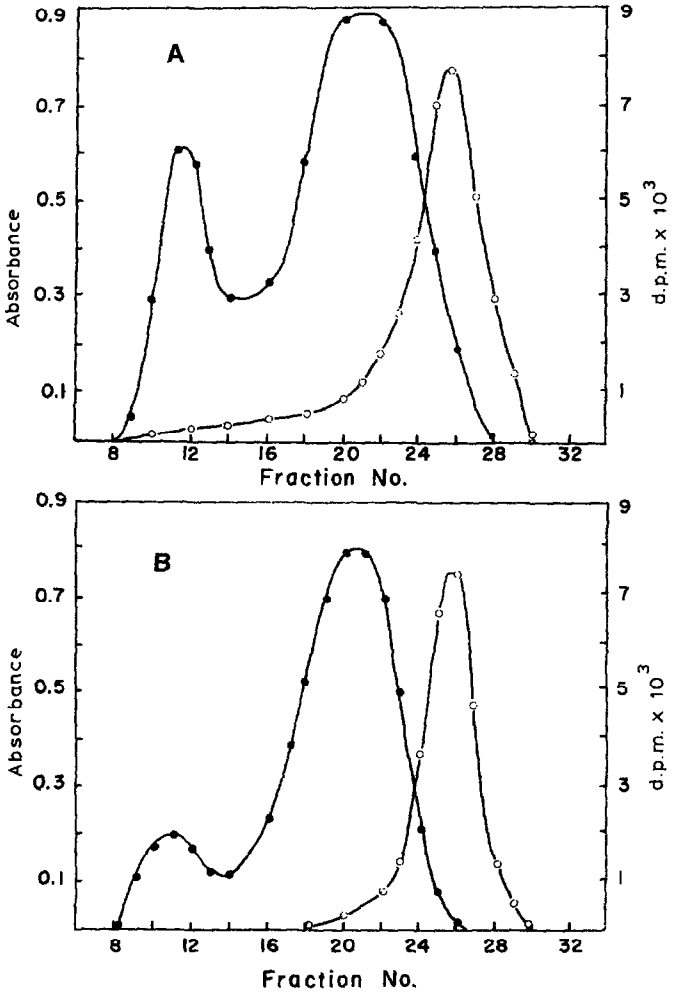

Fig. 3. Gel-permeation chromatography of (A) 2-propanol- and (B) 1-butanol-modified potato starch on Bio-Gel A-0.5m. key: —●—, absorbance (A_{545} for A; A_{540} for B) of iodine–iodide color; —○—, d.p.m. ($\times 10^3$) of ^{14}C incorporated into the starches. The radioactivity represents glycosides formed by alcoholysis.

Various extents of alcoholysis occurred, depending on the alcohol. The methanol-modified starch had ~50% of the molecules as glycosides; the ethanol-modified starch, ~20% glycosides; the 2-propanol-modified starch, 12% glycosides; and the 1-butanol-modified starch, 6% glycosides. The distribution of the glycosides was asymmetrical, being skewed toward the lower molecular weights (see Figs. 2 and 3). Each of the modified starches had a significant proportion (25% to 75%) of glycosides that did not give an iodine color. In particular, the 2-propanol- and 1-butanol-modified starches had a high percentage of the glycosides that did not give an iodine color (see Fig. 3).

TABLE I

PROPERTIES OF MODIFIED STARCHES

Type of starch[a]	λ_{max} *(nm)*[b]	*d.p.*	*Mol. wt*	*Yield (%)*
MeOH-p.s.	585	728	117,930	99
EtOH-p.s.	575	133	21,510	95
2-PrOH-p.s.	545	47	7,600	88
1-BuOH-p.s.	540	29	4,750	88
L.s.s.	595	502	81,430	—
MeOH-w.m.s.	535	781	126,670	100
2-PrOH-w.m.s.	510	49	7,860	99

[a]MeOH-p.s. = methanol-modified potato starch, EtOH = ethanol, 2-PrOH = 2-propanol, 1-BuOH = 1-butanol, L.s.s. = Lintner soluble starch, and w.m.s. = waxy-maize starch. [b]Maximum wavelength of the iodine–iodide color.

The yields of the modified starches were high, ranging from 100 to 88% (see Table I). The number-average molecular weights were decreased significantly from that of the native starch and ranged from 126,670 for the methanol-modified waxy-maize starch to 4,750 for the 1-butanol-modified potato starch. The number-average molecular weights for the two starches (potato and waxy-maize) were very close when modified in the same alcohol (see Table I).

The soluble starches prepared in methanol and ethanol at 65° gave two fractions, an amylose fraction that could be precipitated with 1-butanol, and an amylopectin fraction that was precipitated with two volumes of ethanol after the removal of the 1-butanol–amylose complex. Gel-permeation chromatography of the methanol- and ethanol-modified amylose fractions on Bio-Gel A-5m gave peaks that came at the same elution position (see Figs. 4B and 4C). The elution pattern indicated that both of these modified amyloses were of lower average molecular weight than the native, potato amylose fraction (see Fig. 4A). Furthermore, the ethanol-modified amylose had a narrower distribution than the methanol-modified amylose. The native potato amylose gave two distinct peaks on elution from the Bio-Gel A-5m column (Fig. 4A), one of which came at the void volume of the column; this is in contrast to the single peaks of the modified amyloses (see Figs. 4B and 4C). The iodine–iodide maximum absorbance of the native potato amylose, methanol-modified amylose, and ethanol-modified amylose was at 630, 600, and 595 nm, respectively, and these had number-average molecular weights of 82,260, 65,770, and 16,210, respectively (see Table II).

Gel-permeation chromatography of the native potato amylopectin, the methanol-modified amylopectin, and the ethanol-modified amylopectin on Bio-Gel A-150m each gave a wide distribution having two distinct peaks (see Fig. 5). The native, potato amylopectin fraction had a relatively sharp peak that came at the void volume of the column (see Fig. 5A). This material had a very high molecular weight ($\geq 1.5 \times 10^8$). The first peak of the methanol-modified amylopectin fraction had a broad distribution with two shoulders and constituted a much greater fraction

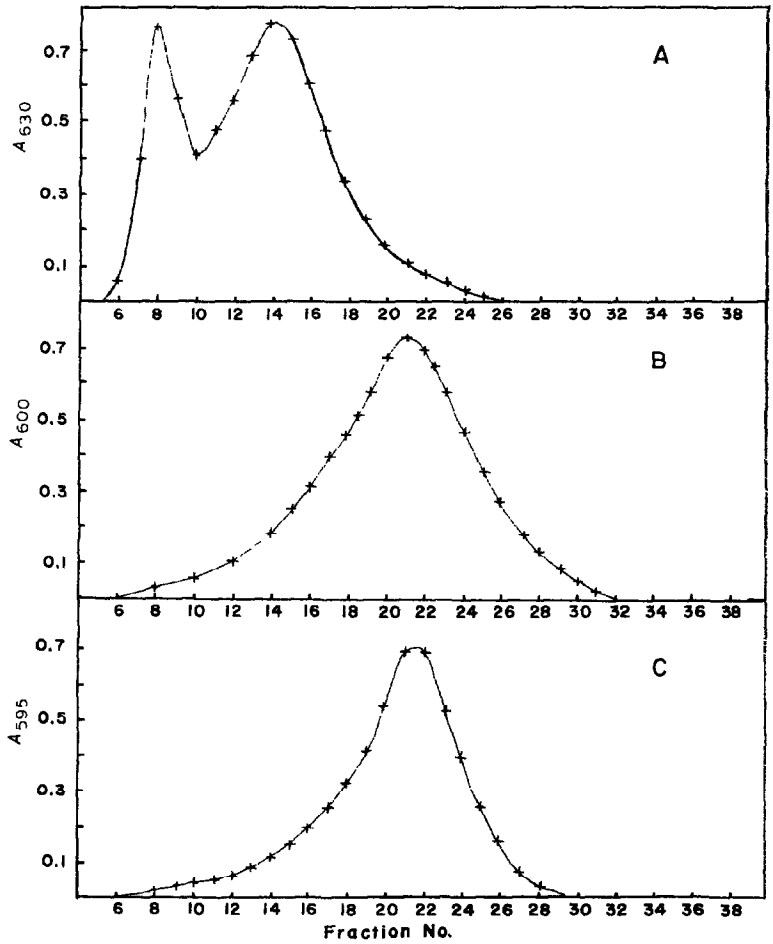

Fig. 4. Gel-permeation chromatography of amylose fractions (1 mL each) on Bio-Gel A-5m. A, Amylose from native potato starch; B, amylose from potato starch modified with methanol–HCl; and C, amylose from potato starch modified with ethanol–HCl.

of the total than the second peak (see Fig. 5B); this is in contrast to the ethanol-modified amylopectin fraction for which the second peak had a distinct shoulder and constituted a greater fraction of the total (see Fig. 5C). The average molecular weight of the ethanol-modified amylopectin was 25,710, a value considerably lower than that of the methanol-modified amylopectin (155,460). Both of these modified potato amylopectins were of lower average molecular weight than that (213,750) of native potato amylopectin. The second peak of the ethanol-modified amylopectin is of slightly higher molecular weight than the second peak of the methanol-

TABLE II

PROPERTIES OF STARCH FRACTIONS

Starch fraction	λ_{max} *(nm)*[a]	*d.p.*	*Mol. wt*	*Yield (%)*[b]
MeOH-amylose	600	406	65,770	25
EtOH-amylose	595	100	16,210	16
L.s.s.-amylose	595	340	55,161	26
Potato amylose	620	507	82,260	28
MeOH-amylopectin	560	959	155,460	75
EtOH-amylopectin	560	159	25,710	84
L.s.s.-amylopectin	560	603	97,710	74
Potato amylopectin	560	1319	213,750	72
Waxy-maize	540	1005	162,860	—

[a]Maximum wavelength of the iodine–iodide color. [b]Based on the yield from modified starch.

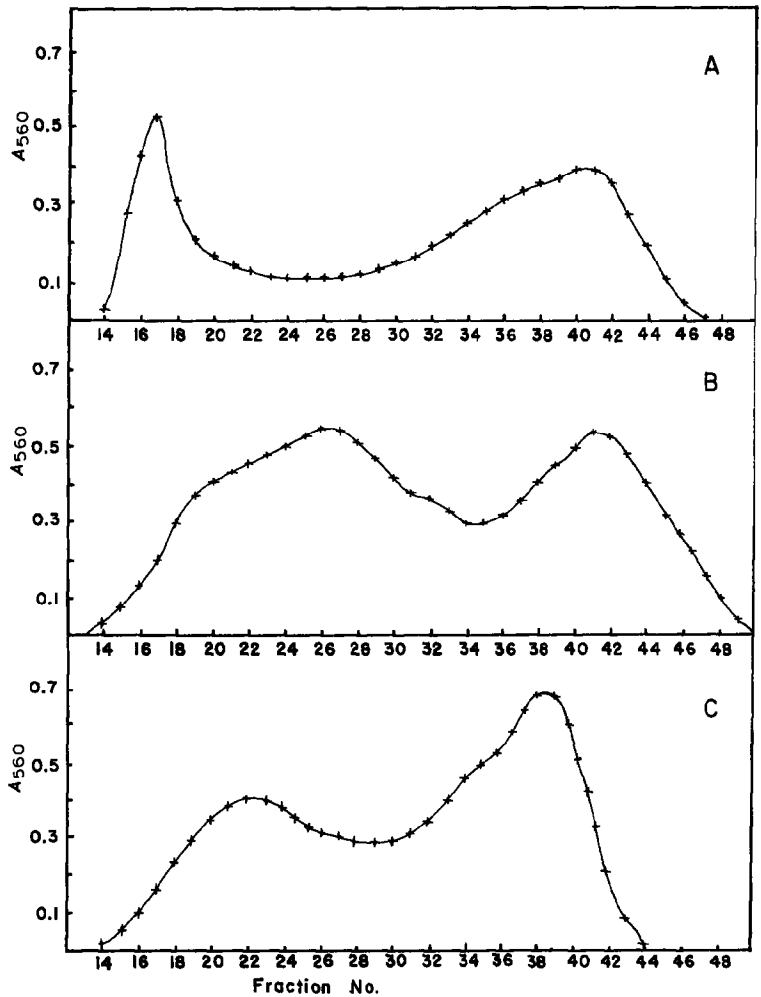

Fig. 5. Gel-permeation chromatography of amylopectin fractions (1 mL each) on Bio-Gel A-150m. A, Amylopectin from native potato starch; B, amylopectin from potato starch modified with methanol–HCl; and C, amylopec

modified amylopectin as judged by their elution positions on Bio-Gel A-150m (compare Fig. 5C with Fig. 5B).

The starches modified in 2-propanol and 1-butanol did not give an amylose fraction by precipitation with 1-butanol. The resulting products were amylopectins, as judged by their iodine–iodide maximum absorbance[6] at 545–540 nm. They gave bimodal elution profiles on Bio-Gel A-0.5m (see Figs. 3A and 3B). These products are in contrast to the native potato amylopectin and the methanol- and ethanol-modified amylopectins that gave polymodal elution profiles from Bio-Gel A-150m (compare Figs. 3A and 3B with Fig. 5). The 2-propanol and 1-butanol amylopectins were of low molecular weights (7,600 and 4,750, respectively; see Table I).

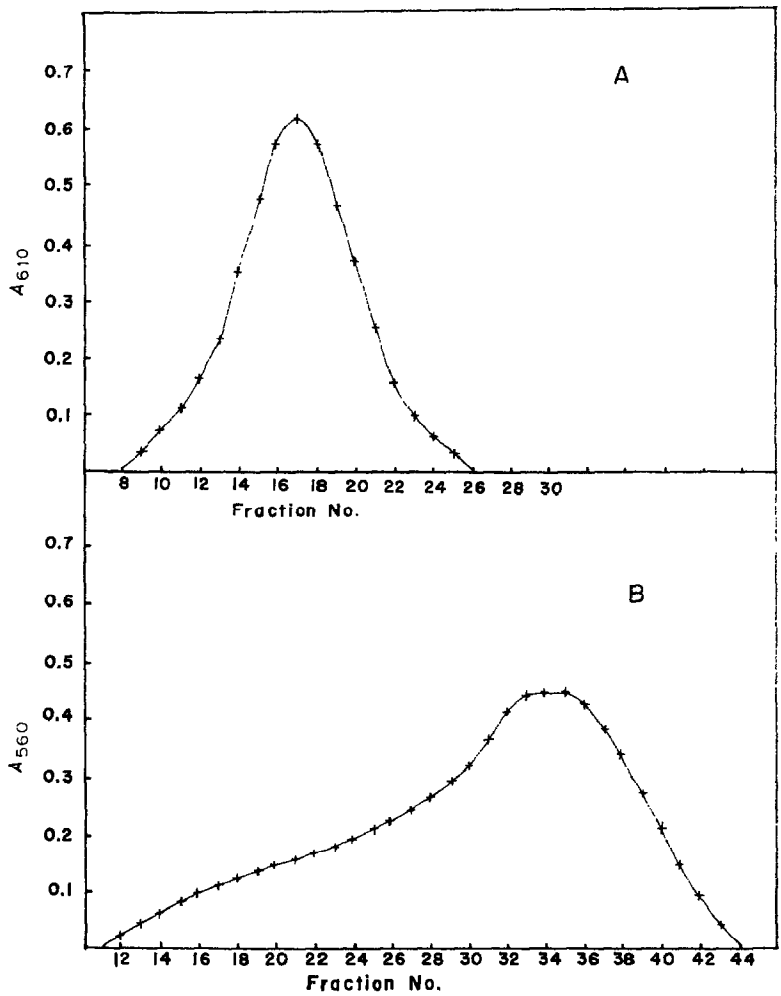

Fig. 6. Gel-permeation chromatography of the amylose and amylopectin fractions (1 mL each) of Lintner soluble starch. A, Chromatography of amylose on Bio-Gel A-5m; and B, chromatography of amylopectin on Bio-Gel A-150m.

The average molecular weights of the amylose and amylopectin fractions from Lintner soluble starch were 55,160 and 97,710, respectively. The elution profile of Lintner amylose and amylopectin fractions on Bio-Gels A-5m and A-150m are shown in Fig. 6. The amylopectin fractions from the methanol- and ethanol-modified starches have a much greater proportion of the high-molecular-weight fraction than the Lintner amylopectin fraction (compare Figs. 5B and 5C with Fig. 6B). The Lintner amylopectin fraction has a wide molecular-weight distribution, with the largest proportion of material in the lower-molecular-weight fraction. In contrast, both the amylopectin fractions from methanol- and ethanol-

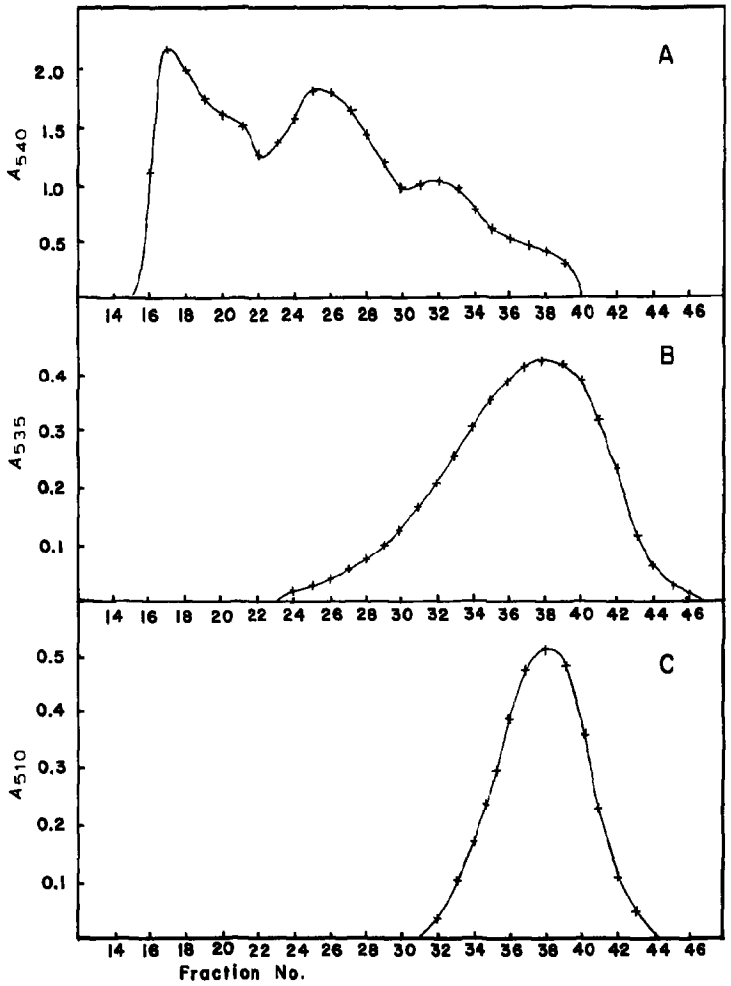

Fig. 7. Gel-permeation chromatography (1-mL fractions) of waxy-maize starch and modified waxy-maize starch on Bio-Gel A-150m. A, Chromatography of native waxy-maize starch; B, chromatography of waxy-maize starch modified with methanol–HCl; and C, chromatography of waxy-maize starch modified with 2-propanol–HCl.

modified starch have two distinct peaks, with a sizable proportion of material in the higher-molecular-weight fraction.

The 2-propanol- and 1-butanol-modified amylopectin fractions differ significantly from the methanol- and ethanol-modified amylopectin fractions and the Lintner amylopectin fraction by having (*a*) a bimodal elution profile from Bio-Gel A-0.5m and (*b*) low molecular weights.

Acid modification of waxy-maize starch, a starch that does not have an amylose component, in methanol–HCl and 2-propanol–HCl, converts the original polymodal waxy-maize starch (see Fig. 7A) into single peaks on elution from Bio-Gel A-150m (see Figs. 7B and 7C). Modification in 2-propanol gave a more homogeneous, lower-molecular-weight product than modification in methanol, as judged by Bio-Gel A-150m elution profiles and the number-average molecular weights given in Table I.

All of the soluble starches produced by hydrolysis in the various alcohols gave crystal-clear solutions at concentrations up to 20% (w/v). This is in contrast to the 10 and 20 percent Lintner soluble starch that had some incompletely dissolved material; furthermore, the 20% preparation set to a gel.

The number-average molecular weights, as determined by reducing values, of the soluble starches progressively decreased as the size of the alcohol increased (see Table I). The relatively high reducing values of the modified starches surprised us, as we had expected that the acid in the presence of an alcohol would produce

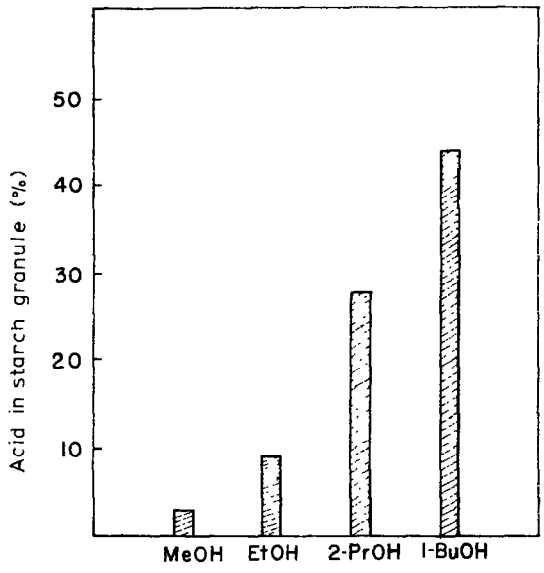

Fig. 8. Percentage of acid inside the starch granule when modified in different alcohols. The starch was stirred in the alcohol with acid for 1 h at 22°, filtered, washed with alcohol, stirred in an equal volume of water for 1 h at 22°, filtered, and the amount of acid in the water determined.

alcoholysis instead of hydrolysis, and hence, that the modified starches would have low reducing values. The high reducing value coupled with the progressive decrease in the molecular size of the starch when treated under identical conditions of acid concentration, temperature, and time, led us to postulate that the different alcohols produce different acid concentrations *inside* the starch granules where hydrolysis takes place with the water (10–12%, w/w) in the granules. To test this hypothesis, the starch granules were stirred in the different alcohols with acid for 60 min at 22°. The granules were then filtered off, washed with alcohol, and suspended in water (100 mL) for 60 min at 22°. The granules were filtered off, and the amount of acid in the filtrate was determined by titration. The results (see Fig. 8) show that the different alcohols do produce widely differing amounts of acid inside the granule. The granules modified in methanol had 2.5% of the amount of added acid; ethanol-modified granules had 8.3%; 2-propanol-modified granules had 27.5%; and 1-butanol-modified granules had 43.4%.

DISCUSSION

Acid hydrolysis has been used to modify starch granules from the latter part of the Nineteenth century. Nägeli[7] reported that treatment of native starch with 15% sulfuric acid for one month at room temperature produced an acid-resistant fraction that was readily soluble in hot water. This material has been called *Nägeli amylodextrin* and has been shown to be of relatively low molecular weight with an average d.p.[8] of 25 to 30. Watanabe and French[8] showed that Nägeli amylodextrin is composed of three types of molecules—multi-branched, singly branched, and linear. Lintner[1] described an acid modification in which native starch was treated with 7.5% hydrochloric acid for one week. This is now the usual commercial method for the preparation of hot-water-soluble starch. Robin *et al.*[9] studied the progressive acid hydrolysis of potato starch under Lintner conditions and examined the structures of fractions that were produced at 15, 70, and 85% hydrolysis. They found the progressive appearance of major chain populations, of d.p. 25 and 15, that were singly branched and linear, respectively. Electron microscopy has shown that acid modification of starch granules occurs by the preferential hydrolysis of the amorphous regions of the granule[10,11].

The present study shows that soluble starches can be prepared by suspending hydrated (containing 10–12%, w/w, of water) starch granules in acidic alcohols for 1 h at 65°, and that different alcohols have different effects on the types of products that are produced. Both hydrolysis and alcoholysis occurred, but to differing degrees, depending on the alcohol. The highest amount of alcoholysis occurred in methanol and the lowest in 1-butanol. The glycosides that are formed are not uniformly distributed among the molecules.

Methanol–HCl and ethanol–HCl produce limited hydrolysis of both the amylose and amylopectin components of native potato starch and they produce soluble starches that have number-average molecular weights of 117,930 and

21,510, respectively (see Table I). Modification in ethanol–HCl at the reflux temperature (78°) completely destroyed the amylose component (data not shown). All subsequent modifications were conducted at 65°, the reflux temperature of methanol.

Differences between the methanol- and ethanol-modifications were also observed for the amylopectin fractions. Both of these amylopectin fractions were bimodal on elution from Bio-Gel A-150m, but the methanol-modified amylopectin contained more material in the higher-molecular-weight fraction than did the ethanol-modified amylopectin, which had more material in the lower-molecular-weight fraction. This lower-molecular-weight fraction was, however, of higher molecular weight than the lower-molecular-weight fraction of the methanol-modified amylopectin. Acid modifications of potato starch in 2-propanol and 1-butanol gave another type of product in which the amylose components were completely destroyed and the amylopectin components were converted into bimodal products of low molecular weight (7,600 and 4,750, respectively; see Fig. 3 and Table I).

Some of the amylose and amylopectin chains in starch granules may have a double helical structure[12,13]. Jane *et al.*[14] have postulated that the double helices in the starch granules "melt" or dissolve when heated in aqueous 1-propanol and then rapidly form single helices with 1-propanol complexed in the center cavity of the helix. The selective hydrolysis of the amylose component when the starch granules were heated in 2-propanol or 1-butanol, may thus be due to the dissolution of the amylose double helices by the alcohol, followed by acid-catalyzed hydrolysis. Jane *et al.*[14] also postulated that double helices may occur between amylopectin and amylose in the starch granule or between chains of amylopectin molecules in the granule. The dissolution of these complexes when heated in acidic alcohol could account for selectivity in cleaving parts of the amylopectin molecules as well.

Methanol–HCl and 2-propanol–HCl similarly converted the polymodal, native, waxy-maize starch into products that were eluted from Bio-Gel A-150m as single peaks with the 2-propanol-modified product having a relatively narrow distribution of lower molecular weight.

The different acidified alcohols thus modify potato and waxy-maize starch granules to give different kinds of products in terms of the amylose–amylopectin components, their molecular weights, and their distributions. The different alcohols have differential effects on the hydrolysis of the starch granules by producing widely different amounts of acid inside the granule were hydrolysis occurs preferentially over alcoholysis. It is postulated that 2-propanol and 1-butanol also affect the double helical, crystalline regions, to give different types of products under otherwise identical conditions of acid concentration, temperature, and time of reaction.

All of the acid–alcohol modifications retained the granule structure of the starch, although with various degrees of granule damage, and gave products that were soluble in hot water to give crystal-clear solutions. Acid modification of native starch in different alcohols thus gives soluble starches that are different types

of products whose number-average molecular weights and molecular distributions vary widely and are dependent on the type of alcohol used. Furthermore, the time required for the preparation of the soluble starches is decreased from one week for the Lintner procedure to 1 h for the alcohol procedure.

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