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RESEARCH

Acid modification of starch granules in alcohols: reactions in mixtures of two alcohols combined in different ratios

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Abstract

The acid modification of starch granules has been studied in different ratios of mixtures of the following combination of alcohols, methanol and 2-propanol, methanol and ethanol, methanol and 1-butanol, ethanol and 2-propanol, and ethanol and 1-butanol, from 100/0 to 0/100 in 10% (v/v) intervals. The time-course of the reaction of potato starch with 0.36% (w/v) HCl in methanol and 2-propanol at 25 °C showed that a series of limit dextrins were formed in 48 to 72 h. The dp of the limit dextrins decreased as the content of the higher alcohol increased. Limit dextrins were formed from the reaction of potato, waxy maize, and amylomaize-7 starches with 0.36% (w/v) HCl in various combinations and ratios of the alcohols. Plots of the dp of the limit dextrins vs. the ratio of each alcohol mixture gave curves that resembled titration curves with different plateau regions in which the dp of the limit dextrins changed very slowly or not at all. These plateau regions were characteristic and dependent on the types of alcohols, the volume ratios of the two alcohols in the mixture, the temperature, and the type of starch. It is proposed that the formation of limit dextrins and the formation of plateaus in the plots of dp of the limit dextrins vs. the alcohol ratios are due to the presence of physically different "kinds" of α-(1 $\rightarrow$ 4) glycosidic linkages in the starch granules. It is further proposed that the mechanism by which the alcohols or combination of alcohols are producing the different kinds of linkages to become more susceptible to acid hydrolysis involves the conversion of crystalline regions in the granule into amorphous regions.

Keywords: Starch granules; Acid/alcohol modification; Limit dextrins

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1. Introduction

We previously have shown that starch granules could be acid hydrolyzed in four alcohols (methanol, ethanol, 2-propanol, and 1-butanol) to give different degree of polymerization (dp) values that were dependent on the type of alcohol [1]. It also was found that the acid hydrolysis was occurring inside the granule with the granule bound water. A time-course of the reaction showed that the hydrolysis did not proceed indefinitely but that the reaction levelled-off in 72 h and gave new types of limit dextrins, the so-called acid/alcohol-limit dextrins [2]. These limit dextrins were shown to be produced over a wide range of acid concentrations and temperatures [3]. The reaction was found to be dependent on the concentration of the starch in the alcohol. At constant acid and constant temperature, the amount of hydrolysis was inversely proportional to the concentration of the starch in the alcohol suspension [3]. Limit dextrins were produced from three widely differing types of starches: potato starch, a B-type of starch with a "normal" distribution of 25% amylose and 75% amylopectin; a high amylose A-type starch, amylomaize-7 starch with 70% amylose and 30% amylopectin; and waxy maize starch, an A-type starch with 100% amylopectin.

In the present communication, we present a study of the formation of limit dextrins from the three kinds of starches reacting with 0.36% (w/v) HCl in 10% (v/v) ratios of mixtures of the alcohols.

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.

Reactions.—The starches (25 g) were suspended in 100 mL of various mixtures of anhydrous alcohols (methanol–2-propanol, methanol–ethanol, methanol–1-butanol, ethanol–2-propanol, and ethanol–1-butanol) that differed by 10% (v/v) intervals. The reactions were initiated by the addition of 1.0 mL of concentrated HCl to 100 mL of alcohol–starch mixture to give a concentration of 0.36% (w/v) HCl. The reactions were conducted at various temperatures, 25 °C, 65 °C, and ambient room temperature (20–21 °C).

Samples (5 mL) were taken at various times over 5 days or after 72 h of reaction (100 mL), filtered, and the starch washed 3–4 times with 70% (v/v) ethanol to remove the acid and a final wash with 100% ethanol. The starch samples were air dried and dissolved in water (10 mg/mL). The reducing value was determined in triplicate by the micro copper–bicinchoninate method [4] and the total carbohydrate was determined in triplicate by the micro phenol–sulfuric acid method [4]. The mean values and the standard deviations were computed for each determination. The average dp values and standard deviations were computed as the quotient of the average reducing value divided by the average total carbohydrate value [5].

The amylose and amylopectin components for the various methanol–2-propanol reactions were fractionated by dissolving 10 g of the samples in water (10 mg/mL) and adding 10% (v/v) 1-butanol to form the amylose–1-butanol complex after 15 h at 20–21 °C. The complex was removed by centrifugation and the amylopectin component was precipitated from the supernatant by the addition of 2 volumes of ethanol. The amylose complex and the amylopectin precipitate were triturated several times (6–7) with anhydrous acetone, followed by anhydrous ethanol and drying at 40 °C under vacuum. The percent by weight of each component was obtained.

3. Results and discussion

Fig. 1 shows the time-course of eleven reactions of potato starch granules with 0.36% (w/v) HCl in various combinations of 10% (v/v) intervals of methanol and 2-propanol at 25 °C. In each reaction, the dp of the starch dropped relatively rapidly, becoming constant after 48 to 72 h. As was previously found for reactions of starch in different alcohols with different acid concentrations at different temperatures [3], reactions of starch in mixtures of two alcohols, each with different volume ratios, also formed limit dextrins with different dp values. In studies with the different ratios of alcohols, the reactions were usually allowed to go 72 h to ensure that a limit dextrin had been formed.

The reactions of potato starch with 0.36% (w/v) HCl in various proportions of methanol and 2-propanol also were performed at 25 °C for 72 h and the dp of the resulting limit dextrins plotted against the ratios of the alcohols (Fig. 2A). A plot of dp

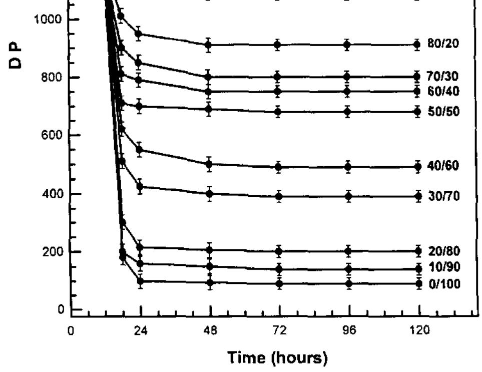

Fig. 1. Time-course of the reactions of potato starch with 0.36% (w/v) HCl at 25 °C in different ratios of methanol and 2-propanol. Each data point is the average of three measurements and the error bar is the standard deviation.

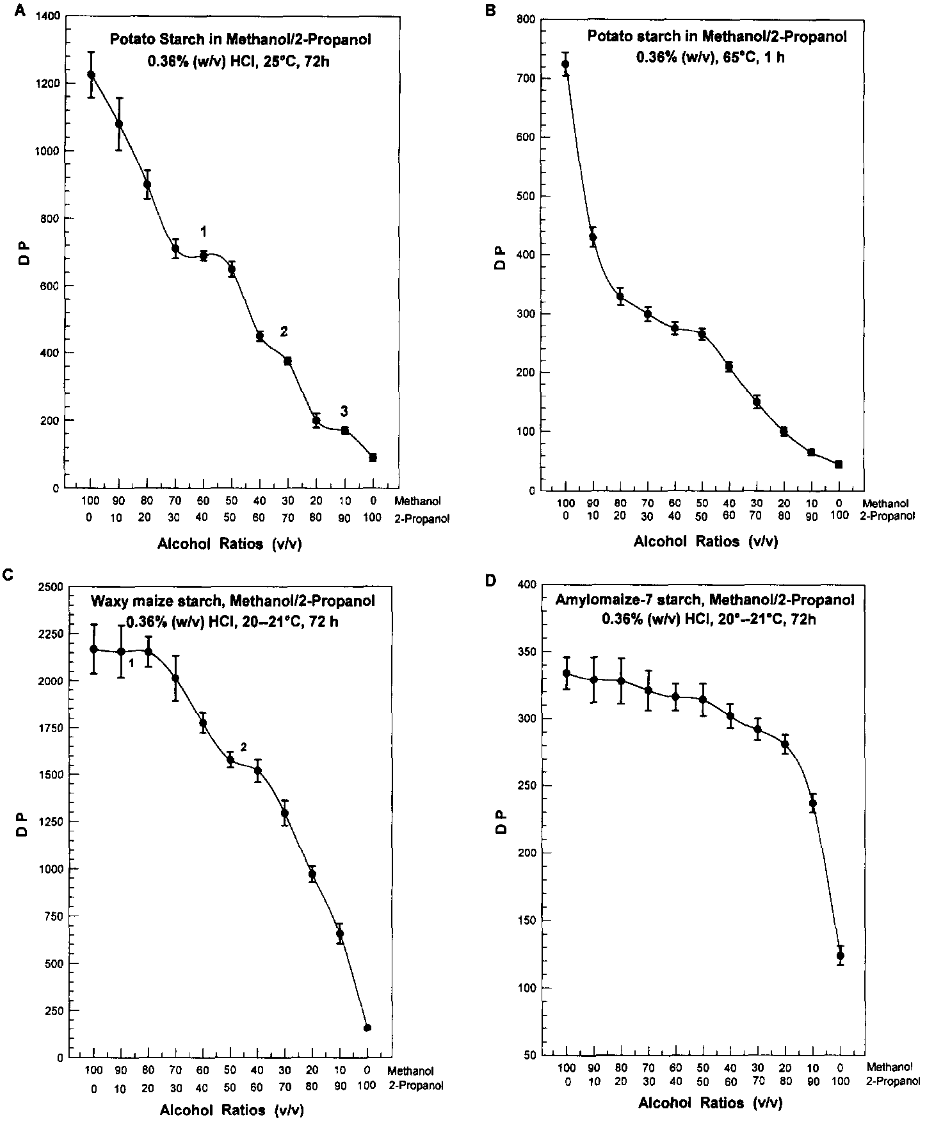

Fig. 2. Dp of the limit dextrins resulting from the 72-h reaction of potato, waxy maize, and amylomaize-7 starches with 0.36% (w/v) HCl in different ratios of methanol and 2-propanol. (A) Reaction of potato starch at 25 °C; (B) reaction of potato starch at 65 °C; (C) reaction of waxy maize starch at 20–21 °C; (D) reaction of amylomaize-7 starch at 20–21 °C. Each data point is the average of three measurements and the error bar is the standard deviation.

vs. alcohol composition (Fig. 2A) indicated that the dp changed in a non-linear manner that resembled a titration curve with three plateaus. The first plateau occurred between the ratios of 70:30 and 50:50; the second occurred between 40:60 and 30:70; and the third between 20:80 and 10:90. Reaction of potato starch in the same mixtures of

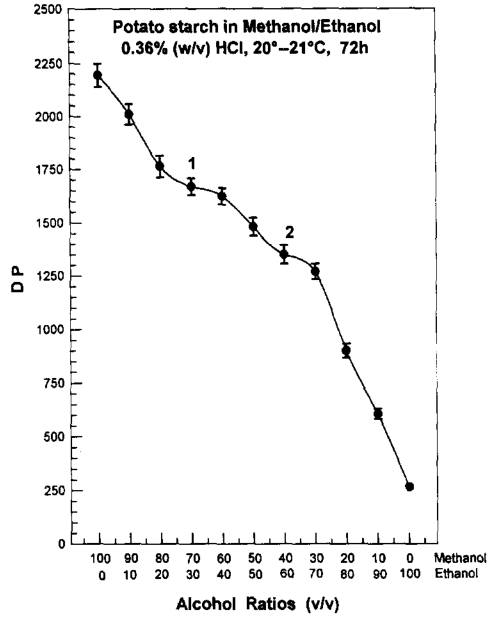

Fig. 3. Dp of the limit dextrins resulting from the 72-h reaction of potato starch with 0.36% (w/v) HCl at 20–21 °C in different ratios of methanol and ethanol. Each data point is the average of three measurements and the error bar is the standard deviation.

methanol and 2-propanol at 65 °C for 1 h also gave a titration-type curve (Fig. 2B) with one plateau between 80:20 and 50:50 volume proportions in which the dp values only changed to a small degree. Reaction of waxy maize starch in various ratios of methanol and 2-propanol at 20°–21 °C gave a plot (Fig. 2C) with 2 plateaus, one at the beginning and the other halfway through the mixtures (50:50–40:60, methanol–2-propanol). Reaction of amylomaize-7 starch in various ratios of methanol and 2-propanol at 20–21 °C gave a plot (Fig. 2D) in which there was a slow drop in dp between the ratios of 100:0 to 20:80. Thereafter the dp dropped more rapidly. While a smooth curve could be drawn, the curve through the average data points suggest three slight inflection areas as marked on Fig. 2D.

Reactions of potato starch in different ratios of methanol and ethanol were run at 20–21 °C for 72 h (Fig. 3). These reactions gave a titration-type curve with two plateaus, one at 70:30–60:40 and the other at 40:60–30:70. These plateaus occurred at nearly identical alcohol ratios as those obtained with methanol–2-propanol at 25 °C, except that the first plateau was narrower. The dp values of these plateaus were different for the two types of alcohol mixtures, those obtained in the methanol–ethanol mixtures were significantly higher than those obtained in the methanol–2-propanol mixtures.

Reactions of potato starch in different ratios of methanol and 1-butanol at 20–21 °C gave a titration-type curve (Fig. 4) with three plateaus. The first occurred between 90:10 and 70:30, the second between 60:40 and 50:50, and the third, although not too distinct, occurred between 30:70 and 20:80. The first plateau occurred at a higher ratio of

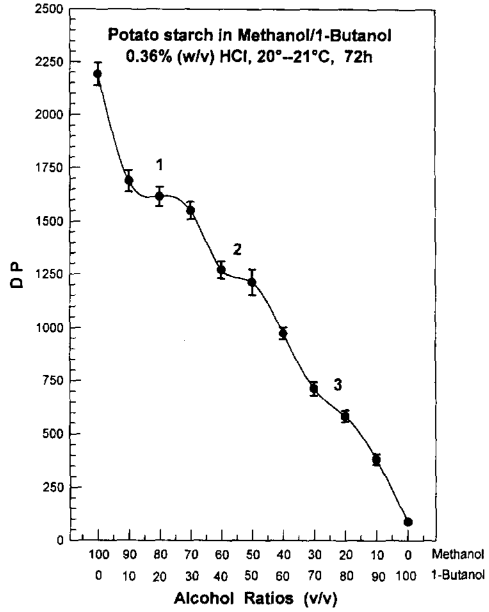

Fig. 4. Dp of the limit dextrins resulting from the 72-h reaction of potato starch with 0.36% (w/v) HCl at 20–21 °C in different ratios of methanol and 1-butanol. Each data point is the average of three measurements and the error bar is the standard deviation.

methanol than the other three methanol reactions, although the dp values were about the same as those measured for the first plateau of the methanol–ethanol reaction (Fig. 3).

Reactions of potato starch in different ratios of ethanol and 2-propanol gave a titration-type curve with three plateaus (Fig. 5), the first at 80:20–70:30, the second at 30:70–20:80, and the third at 10:90–0:100. The first plateau occurred at the lowest dp values (245–235) than any of the previous reactions. Plateaus 2 and 3 occurred one after the other with low dp values of 100 and 90, respectively.

Reactions of potato starch in ethanol and 1-butanol (Fig. 6A) gave a curve with three regions, none of which, except possibly region 3, could be considered plateaus. Region 1 gave a slow, non-linear drop in dp from 270 to 235. Between alcohol ratios of 70:30 and 60:40, there was a sharp drop in the dp from 235 to 158. Region 2, then gave a nearly linear drop from 158 to 100 between the alcohol ratios of 60:40 and 30:70. Region 3 gave a relatively small, linear drop in dp from 100 to 85 between the alcohol ratios of 30:70 and 0:100. Reactions of waxy maize starch in various ratios of ethanol and 1-butanol at 20–21 °C show a nearly linear decrease in the dp of the limit dextrins, although the curve gave three inflection points as shown in Fig. 6B. These inflections occurred in different regions than those that occurred for potato starch in ethanol and 1-butanol (Fig. 6A). The reaction of amylomaize-7 starch in various ratios of ethanol and 1-butanol at 20–21 °C gave a plot (Fig. 6C) that was quite different from the plots obtained with potato starch and waxy maize starch. There were 2 plateaus, one at the beginning and the other over a relatively broad range of 70:30 to 30:70.

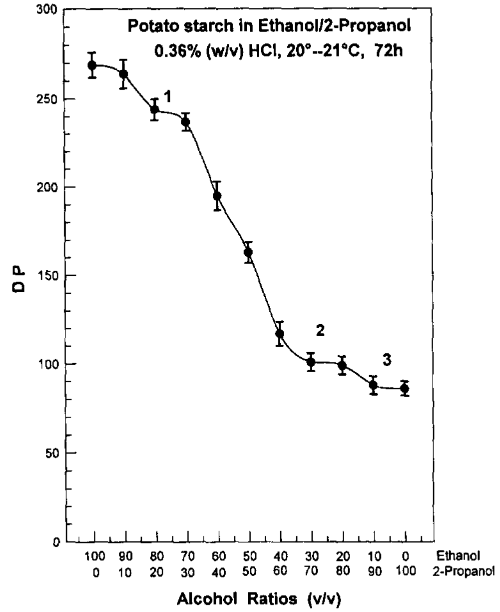

Fig. 5. Dp of the limit dextrins resulting from the 72-h reaction of potato starch with 0.36% (w/v) at 20–21 °C in different ratios of ethanol and 2-propanol. Each data point is the average of three measurements and the error bar is the standard deviation.

Fig. 7 shows the decrease in the amylose content as the 2-propanol concentration was increased in the reaction of potato starch in various ratios of methanol and 2-propanol with 0.36% (w/v) HCl at 65 °C for 1 h. This curve shows a broad plateau between the alcohol ratios of 80:20 and 20:80. The amount of amylose drops to a very low amount (1%) in the 10:90 ratio and to zero for the 0:100 ratio.

Reactions in 2-propanol and 1-butanol at 20–21 °C were run, but the data are not presented as the dp values were low and there were not large differences between the different dp values obtained in the different ratios of alcohols. The dp vs. alcohol ratio plot was relatively flat.

In a previous study [1], it was shown that the different alcohols were producing different concentrations of acid inside the granules. The acid concentration increased and the dp values decreased in going through the alcohol series, methanol, ethanol, 2-propanol, and 1-butanol. It also was shown [2,3] that the dp values decreased as the amount of acid added to the alcohol/granule mixture was increased. An additional, unusual factor in the acid/alcohol hydrolysis of the glycosidic linkages in the granule is the rapidity (see Fig. 1 and refs. [2,3]) with which the hydrolysis occurs under a low acid concentration of 0.36% (w/v) and at a low temperature of 20–21 °C.

In the present study, the dp values did decrease, as was expected, when the amount of 2-propanol was increased in the methanol–2-propanol mixtures, when ethanol was increased in the methanol–ethanol mixtures, when 1-butanol was increased in the methanol–1-butanol mixtures, when 2-propanol was increased in the ethanol–2-pro-

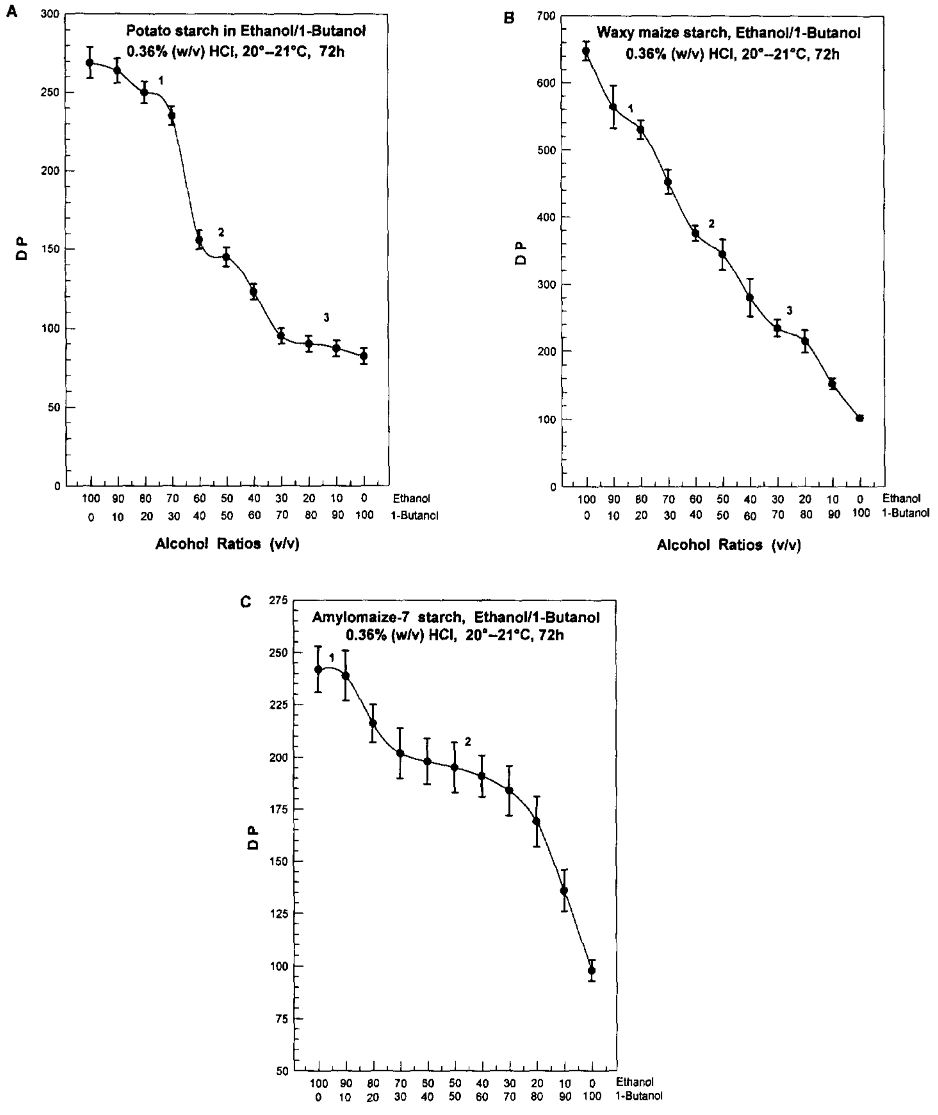

Fig. 6. Dp of the limit dextrins resulting from the 72-h reaction of starches with 0.36% (w/v) at 20–21 °C in different ratios of ethanol and 1-butanol. (A) Potato starch; (B) waxy maize starch; (C) amylomaize-7 starch. Each data point is the average of three measurements and the error bar is the standard deviation.

panol mixtures, and when 1-butanol was increased in the ethanol–1-butanol mixtures. It was expected, however, that the decrease in the dp values would be linear as the concentrations of the higher alcohols were increased, due to an increase in the acid concentrations inside the granules. The dp values vs. the increase in the higher alcohol ratios, however, were not linear for any of the alcohol mixtures. Instead the plots were non-linear and showed various plateaus or inflections (Figs. 2–6). The plateaus did not

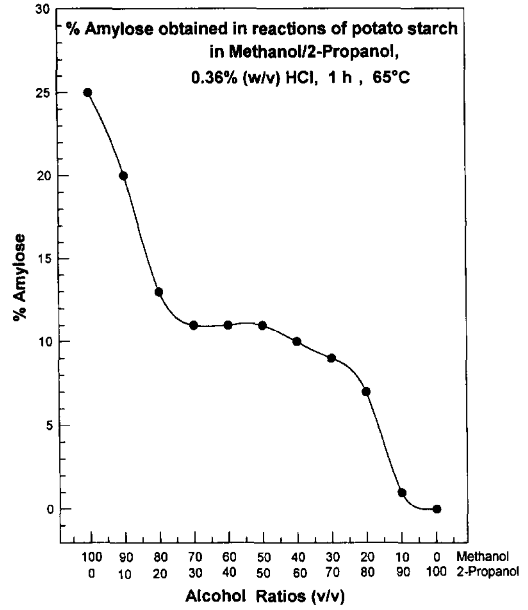

Fig. 7. Percent amylose in the limit dextrins obtained by reaction of potato starch granules with 0.36% (w/v) HCl in various ratios of methanol and 2-propanol for 1 h at 65 °C.

occur at the same regions for the different mixtures of alcohols and further varied for the different starches. These observations suggest that the alcohols are affecting something more than the concentration of acid inside the granules. The affect of different alcohols and different mixtures of alcohols on the dp value of the resulting limit dextrins indicates that they somehow are affecting the availability and/or the susceptibility of various α-(1 → 4) glycosidic linkages inside the granule. The differential loss of the amylose component when 2-propanol or 1-butanol were used as the alcohols [1] and when 2-propanol was increased in the methanol–2-propanol mixtures (Fig. 7), further indicates that the alcohols are having a selective effect on the hydrolysis of the α-(1 → 4) glycosidic linkage of amylose inside the granule.

It also was previously shown [5] that both acid and enzymes preferentially attack the non-crystalline, amorphous areas of non-solubilized starch molecules, such as occurs with the amylose–1-butanol and other organic molecule complexes and with retrograded amylose. From this we postulate that the different alcohols or mixtures of different alcohols are affecting the crystalline areas in the starch granule by making them more amorphous and therefore more susceptible to acid hydrolysis. The exact mechanism(s) by which the alcohols are affecting the structures in the starch granule are not apparent at this time. Perhaps the alcohols are breaking the hydrophobic and hydrogen bonds that occur between the double helical starch chains in the granule, thereby disrupting the crystalline micelles and unwinding the double helices so that acid can penetrate into the interior of the helix and hydrolyze the glycosidic linkages. The temperature effect (that is, as the temperature is increased there is an increase in the degree of hydrolysis [3]) supports this hypothesis. The increased temperature increases the breaking of hydrogen and hydrophobic bonds, giving the disruption of crystalline micelles and the unwinding of the double helicies. This then produces a decrease in the crystallinity and the increase in the amorphous regions. Further studies are underway to obtain additional insight into the mechanism(s) by which the alcohols are affecting the internal structure of the starch granule and facilitating acid hydrolysis.

The different plateaus in the dp vs. alcohol ratios that were observed with the mixtures of alcohols suggests that the formation, or lack thereof, of acid susceptible α-(1 → 4) glycosidic linkages in the granule occur at discrete alcohol compositions. Formation of these acid susceptible linkages in the granule are dependent on the kinds of alcohols in the mixture, on the relative ratios of the alcohols, the temperature, and the type of starch. This suggests, along with the formation of limit dextrins, that there are physically different ''kinds'' of α-(1 → 4) glycosidic linkages in the starch granules and that the alcohols or combination of alcohols are inducing the different kinds of linkages to become more chemically susceptible to acid hydrolysis. The data of this and previous studies [1–3] show that the use of the different alcohols or the use of different combinations of alcohols produce limit dextrins with different dp values. The studies further show that starch granules from different types of botanical sources also appear to have different ''kinds'' of α-(1 → 4) glycosidic linkages that can be made more susceptible to acid hydrolysis by using different alcohols. The differences that we have observed in the formation of acid susceptible α-(1 → 4) glycosidic linkages in starch granules as a function of the different kinds of alcohols, composition of alcohol mixtures, temperature, acid concentration, and type of starch may be reflecting differences in the degree and order of crystallinity in the various types of granules.

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