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Effects of β-Amylolysis on the Resistant Starch Formation of Debranched Corn Starches

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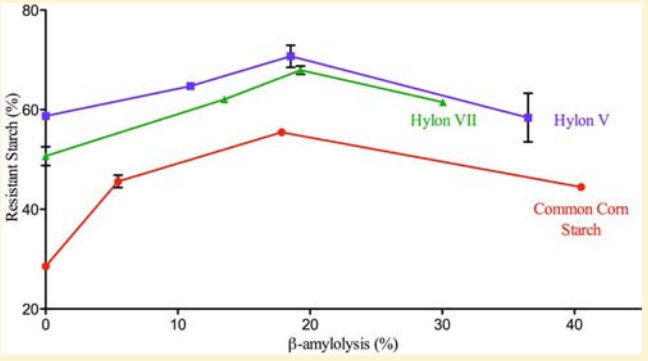
ABSTRACT: Retrograded amylose is resistant to digestion by amylolytic enzymes, which is known as resistant starch type III (RS3). In this study we investigated the effect of β-amylase hydrolysis on the formation and physicochemical properties of RS3 from debranched corn starches. Three types of corn starch (Hylon VII, Hylon V, and common corn) were first gelatinized and then hydrolyzed using β-amylase to varying degrees. The resultant hydrolyzed starch was debranched with isoamylase and then exposed to temperature cycling to promote RS formation. A broad endotherm from approximately 45 to 120 °C and a small endotherm above 150 °C were noted for all retrograded starches. All three corn starches had increased RS contents after moderate β-amylolysis, with Hylon V having the highest RS content at 70.7% after 4 h of β-amylolysis. The results suggest that RS3 formation is affected by the starch composition as well as the starch structure and can be increased by moderate β-amylolysis.



KEYWORDS: *resistant starch, debranching, β-amylase, retrogradation, temperature cycling*

INTRODUCTION

Resistant starch is defined as the fraction of starch that escapes digestion in the small intestine of healthy people.[1] This portion of starch is nutritionally relevant because it has been shown to contribute to colonic health in animal models and humans.[2−5] Resistant starch is classified into four major categories by the mechanism in which they are resistant to digestion. Resistant starch type I (RS1) is physically inaccessible starch, type II (RS2) is native granular starch, type III (RS3) is retrograded starch, and type IV (RS4) is chemically modified starch.

RS3 is primarily composed of retrograded amylose because of its strong tendency to reassociate.[3,6−8] Therefore, amylose content is the main factor governing the formation of RS3.[6,8] There are several ways to further increase RS3 formation in starch. The most common one is enzymatic debranching, which results in all linear glucans that are more ready to reassociate.[6,9,10] In addition, autoclaving[6,8,9] and temperature cycling[8,11] have been shown to increase RS3 production. Temperature cycling refers to the process of autoclaving a starch slurry to a temperature >120 °C and then storing it at a specified temperature to promote retrogradation.

Although amylose content is the predominant factor, amylose degree of polymerization (DP) has also been shown to affect RS3 formation. Eerlingen et al.[12] reported that resistant starch increased with increasing amylose DP up to 100 glucose units and then leveled off.[13] Gidley et al.[14] corroborated this by showing the minimum DP for double helix formation was 10 glucose units. However, these results were obtained using purified amylose instead of debranched starch that contains linear glucans with a broader DP range. Modifying the chain length of native starch to promote RS3 formation has shown mixed results. Hasjim and Jane[15] found that a mild acid hydrolysis increased the RS content of extruded common corn starch. They hypothesized that the shorter, acid-modified starch molecules had more mobility and therefore reassociated more easily. Ozturk and others[16] reported that the RS content of acid-treated high-amylose starch did not increase under various storage conditions to promote retrogradation; however, when immediately dried, a slight increase in RS content was observed. Zhang and Jin[17] found that chain-length reduction could improve the RS content using a combination of α-amylase and pullanase on maize starch.

Both acid and α-amylase hydrolyses create random cleavage of starch in contrast to more controlled cleavage by β-amylase, which is an exoenzyme that cleaves the α-(1−4) linkages from the nonreducing ends. There has been no report on using a combination of isoamylase and β-amylase modification to promote RS3 formation. In this study, corn starches of varying amylose contents were modified by the controlled hydrolysis of β-amylase to various degrees and then debranched by isoamylase. The effects of β-amylase hydrolysis on the formation of RS and the physiochemical properties of the resultant starches were investigated.

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MATERIALS AND METHODS

Materials. Corn starches with different amylose contents were used in this study. Hylon VII (70% amylose) and Hylon V (50% amylose) were provided by National Starch LLC (Bridgewater, NJ). Common corn starch (~25% amylose) was obtained from Cargill Inc. (Hammond, IN). β-Amylase from *Bacillus cereus* (activity 2484 U/mg of protein) and a D-glucose assay kit were purchased from Megazyme International (Wickow, Ireland). Isoamylase from *Pseudomonas amylodermosa* (activity 63 500 U/mg of protein) was purchased from Hayashibara Biochemical Laboratories Inc. (Okyama, Japan). Porcine pancreatin (pancrease activity 200 U) and amyloglucosidase from *Aspergillus niger* (≥300 U/mL) were purchased from Sigma-Aldrich (St. Louis, MO). All enzymes were used as received without further purification.

Enzymatic Treatments. A 20 g sample of starch was suspended in 400 mL of 100 mM acetate buffer (pH 6.5) in a boiling water bath for 30 min and then autoclaved at 135 °C for 30 min to become fully gelatinized. The starch solution was equilibrated in a 40 °C water bath, added to β-amylase (400 U/g of starch), and then incubated for 0, 2.5, 4, or 14 h to achieve varying degrees of hydrolysis. After the incubation, the starch was precipitated with 2 L of ethanol and then centrifuged at 7000*g* for 20 min. The supernatant was collected for the determination of the hydrolysis degree by measuring the total carbohydrate content using the phenol–sulfuric method.[18] The sediment was rinsed with a small amount of water and kept at 50 °C for 30 min to evaporate any residual ethanol. The samples were freeze-dried to allow for easier dispersion in buffer for the subsequent debranching treatment.

For the debranching treatment, the previously freeze-dried β-amylase-treated starch was dispersed in 200 mL and autoclaved at 135 °C for 30 min to ensure complete dissolution. The dispersion was then added to 200 mL of 200 mM acetate buffer (pH 3.5) and isoamylase (300 U/g of starch) and incubated at 45 °C with agitation for 48 h to achieve complete debranching.

Temperature Cycling. The enzyme-treated starch solution was autoclaved at 135 °C for 30 min, equilibrated in an oven at 95 °C for 24 h, and then autoclaved again at 133 °C for 30 min and equilibrated at 95 °C for 24 h. The second autoclaving temperature was 133 °C to avoid melting any already formed RS.[11] The autoclaving–oven cycle was repeated one more time to result in a total of three temperature cycles. After the third cycle the starch was transferred to watch glasses and dried in a forced-air oven at 50 °C for 48 h. The dried sample was ground with a cyclone mill (UDY Corp., Ft. Collins, CO) to fit through a 0.25 mm screen.

Structure of Enzyme-Treated Starch. The enzyme-treated starch (12 mg) was dissolved in 3 mL of 100% DMSO under boiling and stirring for 3 h and allowed to cool overnight with stirring. The sample was filtered through a 0.45 μm membrane, and 200 μL of the filtrate was injected into an HPLC system consisting of a Waters 515 HPLC pump, Waters 2410 refractive index detector, and two size-exclusion columns (Shodex OH-804 and OH-802 Showa Denko K.K. Kawasaki, Japan) used in tandem with 0.1 M ammonium acetate at 0.5 mL/min as the eluant. Dextran standards of 4400, 9900, 21 400, 43 500, 196 000, and 277 000 Da and glucose were used to establish the calibration curve.

Physiochemical Properties of Enzyme-Treated Starch. The X-ray powder diffraction was measured using a Phillips analytical diffractometer (Phillips, Almelo, The Netherlands). The samples were scanned from 4° to 35° (2θ) at 45 kV and 40 mA with a step size of 0.02°. The background was subtracted from the diffractogram by drawing a straight baseline tangentially to the curve at 5° (2θ). A relative crystallinity can be determined by comparing the area under the crystalline peaks with the area of the amorphous region.

Thermal properties were assessed by a differential scanning calorimeter (Perkin-Elmer Co., Norwalk, CT). Approximately 10 mg (db) of starch was weighed into a stainless steel differential scannning calorimeter pan, and 20 μL of deionized water was added by a microsyringe. The mixture was hermetically sealed and equilibrated at room temperature for at least 24 h prior to heating from 25 to 180 °C at 10 °C/min. An empty pan was used as the reference. The onset, peak, and end gelatinization temperatures and enthalpy were calculated from the endotherms.

The proportions of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) were determined by following the method of Englyst et al.,[1] except that invertase was not used because of the absence of sucrose in starch.

Structure of Resistant Starch. The chain-length distribution of the RS residue from the method of Englyst et al.[1] was analyzed with HPLC. A 3 mL aliquot of the sample after 120 min of hydrolysis was added to 30 mL of ethanol and then centrifuged at 4000*g* for 10 min. The supernatant was discarded, and the precipitate was freeze-dried. A 10 mg sample of the freeze-dried hydrolysate was added to 3 mL of water, heated in a boiling water bath for 1 h, and injected into an HPLC system equipped with a guard column, a Carbopac PA1 analytical column, a pulsed amperometic detector, and an AS40 autosampler (Dionex-ICS-3000, Dionex Corp., Sunnyvale, CA). The eluants and eluant gradient were set up according to the method of Kasemsuwan et al.[19]

Statistical Analysis. The data were analyzed by using JMP software (SAS Institute Inc., Cary, NC). Comparison of means was executed using Tukey's test, and bivariate analysis was performed using the Pearson-product moment approach.

RESULTS AND DISCUSSION

β-Amylase Hydrolysis. Figure 1 displays the hydrolysis profiles of the three corn starches during the β-amylase treatment over 14 h. Common corn starch had the lowest hydrolysis degree among the starches at 2.5 h, which was not expected because of its higher proportion of amylopectin than those of Hylon V and VII and amylopectin consisting of more nonreducing ends for β-amylase to hydrolyze. The slow initial rate of hydrolysis for common corn starch was attributed to its higher viscosity after gelatinization compared with those of Hylon V and Hylon VII, which hindered the enzyme diffusion. All three starches exhibited a similar degree of hydrolysis after 4 h. At the end of the 14 h, the hydrolysis degrees of common corn, Hylon V, and Hylon VII were 40.5%, 35.5%, and 30.1%, respectively, which correlated with their amylopectin contents.

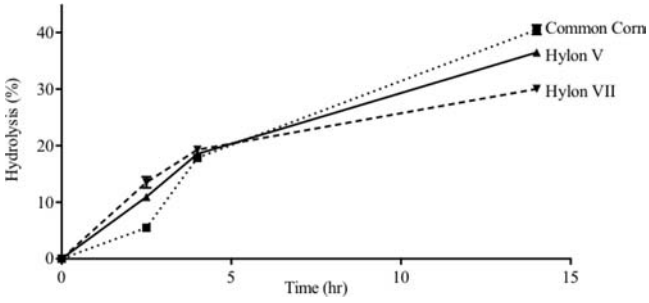

Figure 1. Hydrolysis of corn starches with varying amylose contents by β-amylase over time.

Structural Characteristics of Enzyme-Treated Starches. The normalized, molecular-size distributions of the enzyme-treated starches are displayed in Figure 2, and the proportions and corresponding peak DPs are listed in Table 1. Amylose was eluted first before 27 min (fraction I), followed by amylopectin long chains (fraction II) and then amylopectin short chains (fractions III and IV).

There were notable changes in the proportions of each fraction for all starches during β-amylolysis. Fraction I of all starches displayed a similar trend of an initial increase after 2.5 h, but a decrease after 14 h of β-amylolysis. The extent of

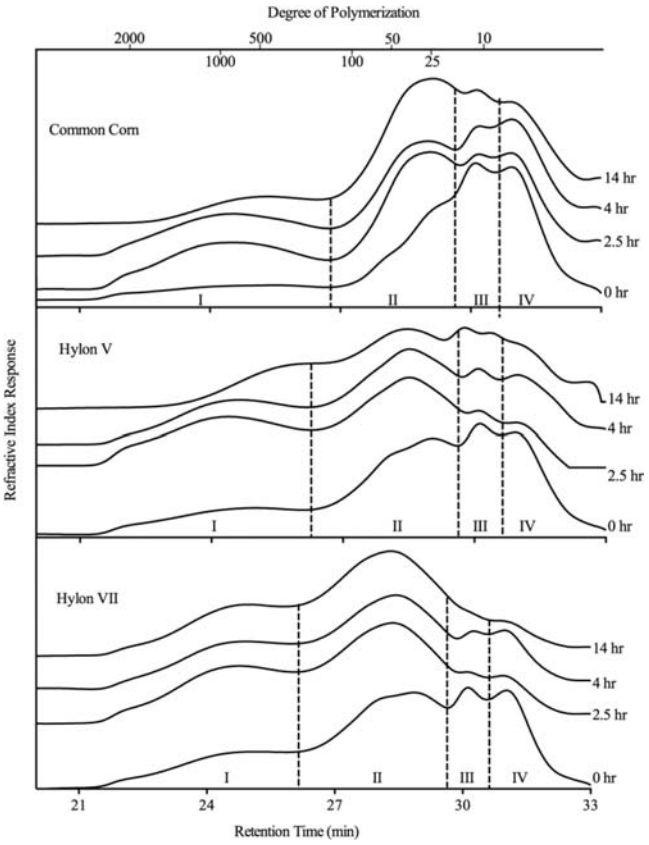

Figure 2. Molecular-size distributions of common corn starch, Hylon V, and Hylon VII after β-amylase hydrolysis for varying times and then debranching by isoamylase.

changes, however, varied with the starch type. Hylon V had the most increase in fraction I after 2.5 h (18%), whereas Hylon VII had the least (4.6%). Overall, fraction I was least affected by β-amylase hydrolysis in Hylon VII and most affected in Hylon V.

Fraction II, mostly from amylopectin long chains with DP of approximately 20−210, increased with increasing β-amylolysis in both common corn starch and Hylon VII, but decreased in Hylon V from 45.6% to 37.6% from 4 to 14 h of β-amylolysis. For fraction III, amylopectin short chains with DP of approximately 9−20, Hylon VII displayed a continual decrease as β-amylolysis increased. Common corn starch and Hylon V exhibited an initial decrease in fraction III, followed by an increase at higher β-amylase hydrolysis levels. Fraction IV, which consisted of amylopectin short chains of DP < 9, decreased with increasing hydrolysis for common corn starch and Hylon VII, but decreased initially and then increased at 4 and 14 h hydrolysis times for Hylon V.

The fraction I peak DP decreased from 856 to 475 for common corn starch, from 895 to 275 for Hylon V, and from 832 to 520 for Hylon VII after β-amylolysis for 14 h. As noted above fractions II, III, and IV did not show significant shifts in peak DP, but were observed to change in proportion. This was expected due to the current cluster model for amylopectin, coupled with the inability of β-amylase to traverse α-(1,6) bonds. The lack of change in fraction DP is due to the complete degradation of many of the amylopectin branch chains versus only partial shortening by β-amylase. As expected amylopectin branch chains were affected most by the β-amylase treatment, due the high amount of reducing ends which they contain.

X-ray Diffraction. The X-ray diffraction patterns and relative cyrstallinities of retrograded enzyme-treated starches are presented in Figure 3. Most starches with the exception of debranched-only common corn starch showed a B-type crystalline polymorph. Debranched-only common corn starch

Table 1. Distribution of the Chain Lengths of Enzyme-Treated Starch

sample	β-amylase hydrolysis duration (h)	property	fraction I	fraction II	fraction III	fraction IV
common corn starch	0	peak area (%)	11.6	28.2	24.6	35.6
		DP_{peak}	856	58, 26	11	6
	2.5	peak area (%)	22.3	32.9	15.8	29.1
		DP_{peak}	824	27	11	6
	4	peak area (%)	22.9	40.0	9.9	27.3
		DP_{peak}	818	26	11	7
	14	peak area (%)	11.1	41.2	17.3	25.5
		DP_{peak}	475	25	12	7
Hylon V	0	peak area (%)	16.4	39.6	20.1	23.9
		DP_{peak}	895	56, 26	12	6
	2.5	peak area (%)	34.4	45.8	9.9	9.8
		DP_{peak}	878	39	12	6
	4	peak area (%)	25.8	45.6	10.0	19.2
		DP_{peak}	669	39	12	6
	14	peak area (%)	22.9	37.6	12.6	26.8
		DP_{peak}	275	40	15, 12	6
Hylon VII	0	peak area (%)	22.1	43.2	16.4	20.4
		DP_{peak}	832	59, 30	12	6
	2.5	peak area (%)	26.7	51.5	6.8	14.5
		DP_{peak}	654	42	11	6
	4	peak area (%)	31.0	52.1	7.0	9.9
		DP_{peak}	646	43	12	6
	14	peak area (%)	27.2	58.8	6.4	7.5
		DP_{peak}	520	44	11	6

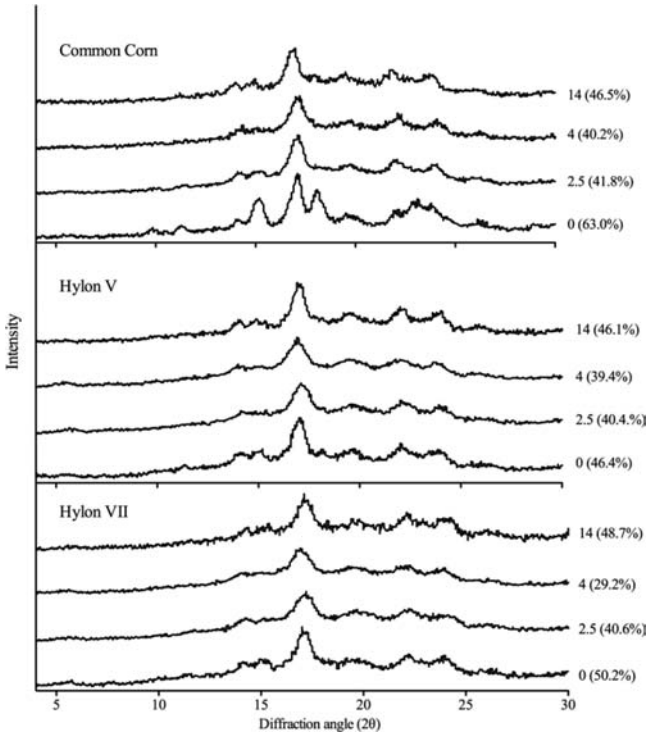

Figure 3. X-ray diffraction patterns of retrograded debranched corn starches with varying durations (h) of β-amylolysis. The relative crystallinity values are shown in parentheses.

showed an A-type characteristic with major peaks at 2θ = 15.3°, 17.2°, 18.2°, and 23.2° and minor peaks at 10°, 11.5°, 14.2°, 19.5°, 22.3°, and 26.3°, but changed to a B-type pattern with major peaks at 17.2°, 22.2°, and 24.3° and minor peaks at 14.2°, 15.3°, and 19.5° when the β-amylase treatement was applied. The β-amylase treatment had little impact on the crystalline structure of high-amylose corn starches. The intenisty of the peaks at 14.2° and 19.5° remained relatively unchanged for all starches, but the peak at 15.3° seemed to be strongly affected by the β-amylase treatment. In high-amylose starches the peak at 15.3° was reduced at 2.5 and 4 h of hydrolysis, but increased with 14 h of hydrolysis. For common corn starch this peak was significantly decreased after 2.5 h of hydrolysis and reduced further after 4 h of hydrolysis, but reappeared after 14 h of β-amylase treatment. All three starches shared a similar X-ray pattern and relative crystallinity after 14 h of β-amylolysis.

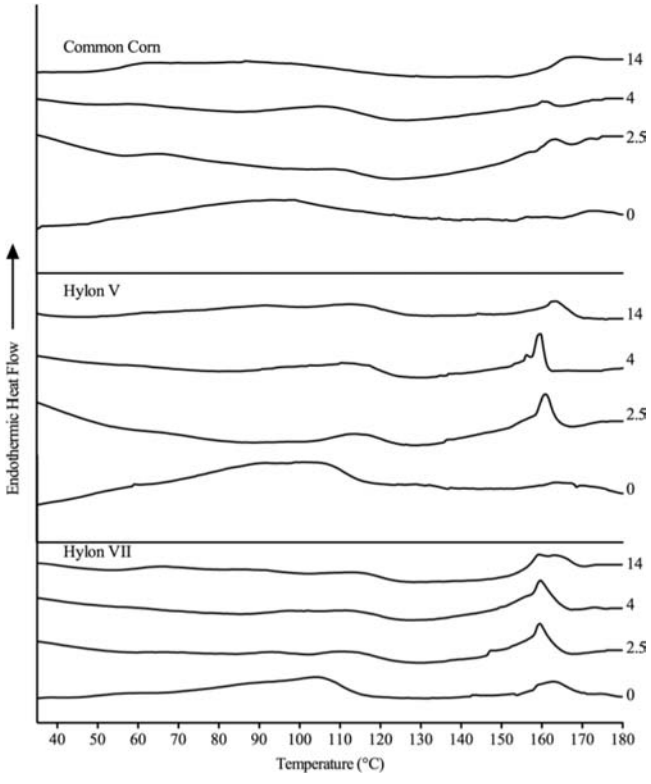

Figure 4. Differential scanning calorimetry thermograms of different enzymatically treated corn starches. The numbers correspond to the duration (h) of β-amylase treatment.

The formation of the A-type crystalline polymorph has been shown to be favored with shorter chain amylose and higher temperatures.[20,21] The A-type pattern displayed by debranched-only common corn starch was attributed to the high proportion of amylopectin short chains. The β-amylase treatment decreased the proportion of short chains. Therefore, the transition to a B-type polymorph appeared to be associated with an increase in long chains after the degradation of short chains. In addition, when the β-amylase hydrolysis time increases from 4 to 14 h, the increase in intensity at 15.3° in all starches indicates the increase of A-type polymorph with an increase in amylopectin short chains (fractions III and IV) as reported in Table 1.

A trend was noted among all starches that the crystallinity initially decreased with 2.5 and 4 h of hydrolysis but then increased after 14 h of hydrolysis. Common corn starch had the highest crystallinity after debranching alone, most likely because of its A-type polymorph as a result of a large proportion of short chains (fractions III and IV) (Table 1). After 2.5−4 h of β-amylase hydrolysis, a significant proportion of short chains in common corn starch were degraded, thus resulting in decreased crystallinity. However, after 14 h of hydrolysis, long chains were degraded into short chains, which contributed to the increase in crystallinity.

Thermal Properties. All three retrograded debranched-only starches showed a broad endotherm from approximately 45 to 120 °C and a small endotherm above 150 °C (Figure 4). The β-amylase treatment shifted the end temperature of the broad endotherm to a higher temperature and sometimes resulted in multiple small peaks. Moates et al. [22] reported that the melting temperature of crystals from short chains, such as those from retrograded debranched amylopectin, increased from 57 to 119 °C as the chain length increased from 12 to 55 glucose units. It is assumed that the multiple small peaks in the temperature range between 50 and 120 °C correspond to the three distinct populations of amylopectin chains (fractions II, III, and IV in Figure 2). The small differences in the temperature range and size of these small peaks might be due to the differences in their chain length distributions of the amylopectin chains (Figure 2).

The endotherm above 150 °C, which is associated with the melting of amylose double helices,[8] was most pronounced for debranched-only Hylon VII, presumably because of its high amylose content. When β-amylase was applied, the high-temperature endotherm became larger. Two possible factors could contribute to the increase of this endotherm with β-amylolysis. One is that the degradation of amylopectin short chains (fractions III and IV) might allow improved amylose reassociation because amylopectin short chains could interfere

with amylose reassociation. The other possibility is that the long amylose chains might not be optimum but become more suited for reassociation after the β-amylase treatment.

Digestibility. Table 2 summarizes the fractions of RDS, SDS, and RS of the enzyme-treated starches. The procedure for producing RS3 was modified from Ozturk et al.,[11] in which their RS contents ranged from 41% to 58% as measured by American Association of Cereal Chemists (AACC) Approved Method 32-40.[23] In this study, increased RS3 production was anticipated because of the incorporation of enzymatic treatments and temperature cycling. For the debranching-only treatment, Hylon V had a higher amount (58.7%) of RS than Hylon VII (50.7%), which was also reported by Ozturk et al.[11] All starches showed significantly higher RS contents after the β-amylase treatment for 2.5 and 4 h, but their RS contents decreased after 14 h of β-amylase treatment. Zhang and Jin[17] observed a similar trend in resistant starch formation, i.e., an initial increase followed by a decrease with increasing enzyme hydrolysis. The β-amylase-treated (4 h) common corn starch produced more RS (55.5%) than debranched-only Hylon VII (50.7%). Although amylose content is one of the most important factors for starch to form RS3, these results indicate that other aspects of starch structure appear to be as important in RS3 formation. Hylon V and Hylon VII had the highest RS content after 4 h of β-amylolysis. Within each starch type, the highest RS level was found at 4 h of β-amylolysis. The present results support the findings of Hasjim and Jane[15] and Ozturk et al.,[16] and their different conclusions were attributed to their uses of different extents of hydrolysis and starch types.

The minimum and optimum chain lengths on amylose reassociation have been studied.[12,20,24] Pfannemuller and Bauer-Carnap[24] reported that the maximum rate of aggregation took place in amylose molecules of DP 80. Gidley and Bulpin[20] demonstrated that the minimum DP for amylose double helix formation was 10 and later reported that amylose molecules with DP values above 100 were likely to form networks that were more sensitive to enzyme digestion. Eerlingen et al.[12] showed that the amylose chain length had no significant effect on RS formation once it reached DP 100. Gidley et al.[14] reported that RS3 was comprised of amylose chains of DP 10−100.

The RDS and SDS contents of common corn starch decreased with 2.5 and 4 h of β-amylolysis, but then increased with further treatment of 14 h. During β-amylolysis, the RDS levels in Hylon V only slightly changed, while the SDS level in Hylon VII stayed relatively constant. Amylopectin short chains after debranching have been associated with SDS formation.[25−27] Robin and others[27] reported that SDS formation was highest for chains with a peak DP of 32. However, recrystallization is also greatly affected by the retrogradation conditions, particularly temperature. In the present study, most Hylon V had a higher SDS level than common corn starch for the same hydrolysis conditions, suggesting that amylopectin structure may have a greater impact than amylopectin quantity in SDS formation. For common corn starch and Hylon V, their SDS contents significantly decreased with the 2.5 and 4 h of β-amylolysis, but then increased with further hydrolysis. The increase in SDS content was attributed to the degradation of amylopectin long chains to short chains. For Hylon VII the SDS level did not significantly change with the β-amylase treatment, probably because its high amylose content compensated for the degradation of amylopectin.

In an effort to better understand how starch chain length correlated with its digestibility, a bivariate analysis was performed on starch structures (fractions I−IV in Table 1) and digestibility (Table 2), and the results are displayed in Table 3. The RS content was positively correlated with fractions I and II and negatively correlated with fraction IV, and the RDS content was negatively correlated with fractions I and II and positively correlated with fraction IV. The SDS content did not show any significant correlation with any starch fraction, which might be attributed to the high storage temperatures used in this study. The conditions to promote SDS formation often involve storage at lower temperatures.[10,28,29]

RS Residue Structure. Table 4 presents the average chain length and chain-length distribution of the recovered RS from the digestibility study. The average DP of recovered RS residue ranged from 21.3 to 26.4 with DP 13−24 as the predominant fraction. In general, the 14 h samples had smaller proportions of DP 36+ fraction. Small changes were noted in the average DP between the RS residue from common corn starch and those from the two high-amylose corn starches. It has been reported that the RS chain length is independent of the starting chain length prior to starch retrogradation.[12] The present results of slight differences in average RS chain length with respect to starch source and β-amylase treatment might be due to differences in the method of recovering the RS. Eerlingen et al.[12] recovered the RS residue after subjecting RS to a modified version of the dietary fiber method,[30] whereas in this study we recovered the RS residue after the Englyst method.[1] Never-

Table 2. Nutritional Properties of Retrograded Enzyme-Treated Corn Starches[a]

sample	β-amylolysis duration (h)	[RDS] (%)	[SDS] (%)	[RS] (%)
common corn starch	0	62.1 a	9.4 b	28.6 h
	2.5	49.9 b	4.5 bc	45.6 g
	4	41.8 c	2.7 c	55.5 ef
	14	51.0 b	4.6 bc	44.5 g
Hylon V	0	25.4 ef	20.6 a	58.7 de
	2.5	27.4 def	7.8 bc	64.7 bc
	4	28.1 def	2.5 c	70.7 a
	14	24.6 f	17.0 a	58.4 e
Hylon VII	0	40.0 c	8.7 bc	50.7 f
	2.5	29.3 de	8.6 bc	62.1 cd
	4	25.0 ef	6.6 bc	67.9 ab
	14	30.2 d	8.3 bc	61.5 cd

[a]Means of three replications. Values with the same letter in the same column are not significantly different ($p < 0.05$). RDS = rapidly digestible starch, SDS = slowly digestible starch, and RS = resistant starch [[RS] = 100 − ([RDS] + [SDS])].

Table 3. Bivariate Analysis of Starch Structure and Digestibility

fraction	RDS content	SDS content	RS content
I	−0.6916[a]	−0.3485	0.8409[a]
II	−0.5809[a]	−0.3049	0.6428[a]
III	0.0486	0.2739	−0.0873
IV	0.7017[a]	0.2780	−0.7848[a]

[a]Statistically significant ($p < 0.05$).

Table 4. Chain-Length Distribution of Recovered Resistant Starch[a]

sample	β-amylolysis duration (h)	average DP	DP 6−12	DP 13−24	DP 25−36	DP 36+
common corn	0	22.3 ± 0.3	17.6 ± 1.0	46.7 ± 0.2	25.1 ± 0.5	10.6 ± 0.3
	2.5	22.9 ± 0.6	17.8 ± 1.8	43.4 ± 0.7	26.7 ± 1.1	12.1 ± 1.4
	4	24.4 ± 0.9	14.6 ± 1.2	40.3 ± 1.4	30.4 ± 1.1	14.7 ± 1.5
	14	22.1 ± 0.9	17.2 ± 1.3	46.6 ± 1.6	27.4 ± 0.7	8.8 ± 0.2
Hylon V	0	21.3 ± 0.8	23.9 ± 1.3	42.2 ± 3.6	24.2 ± 0.7	9.7 ± 4.2
	2.5	26.5 ± 0.1	11.1 ± 3.1	37.4 ± 3.3	30.9 ± 1.3	20.6 ± 1.4
	4	26.4 ± 0.0	10.0 ± 0.3	39.1 ± 0.5	31.2 ± 0.2	19.7 ± 0.4
	14	23.5 ± 0.2	15.8 ± 0.9	42.8 ± 0.1	28.7 ± 0.4	12.6 ± 0.2
Hylon VII	0	26.1 ± 0.1	10.7 ± 0.4	39.8 ± 0.6	30.4 ± 0.3	19.2 ± 0.5
	2.5	24.0 ± 0.1	15.6 ± 2.8	41.6 ± 2.8	28.6 ± 1.2	14.2 ± 1.1
	4	25.0 ± 1.7	13.3 ± 2.1	40.6 ± 4.2	29.3 ± 1.5	16.8 ± 4.9
	14	24.0 ± 0.4	17.6 ± 1.1	38.8 ± 0.3	28.4 ± 0.6	15.1 ± 0.5

[a]Means of duplicate measurements with standard deviations.

theless, the small DP range of RS residue between 21 and 27 supports the lamellar model[31] for polymer crystallization and the micelle model for starch reassociation by Jane and Robyt.[32] In these proposed models the molecules were proposed to align over short crystalline regions (~24 glucose units) interspersed with amorphous regions. Once these RS molecules were exposed to digestive enzymes, the amorphous regions were digested, and the recovered resistant starch had a narrow range of DP corresponding to the length of the helical portion of the molecule involved in RS formation.[12] The β-amylase treatment did not change the structure of the resultant RS3, only the quantity. The shorter amylose molecules after the β-amylase treatment might have more mobility, which allowed them to reassociate more easily.[15−17]

In summary, this study demonstrates that both the chemical composition and structure of starch affected RS3 formation in debranched starch. Debranched amylose-containing corn starches exhibited increased RS contents with the incorporation of a mild β-amylase treatment. The mild β-amylase treatment hydrolyzed amylopectin short chains, which interfere with reassociation of linear chains, and thus increased the proportions of amylose and amylopectin long chains, which contributed to RS formation. However, extensive β-amylolysis might create new amylopectin short chains and/or shorten amylose and amylopectin long chains to become not as optimal to form RS under the temperature cycling conditions in this study.

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Notes

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ABBREVIATIONS

DMSO, dimethyl sulfoxide; DP, degree of polymerization; XRD, X-ray diffraction

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