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Mechanism and Enzymatic Contribution to In Vitro Test Method of Digestion for Maize Starches Differing in Amylose Content

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ABSTRACT: To determine the rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) contents in a starch sample, the addition of amyloglucosidase is often used to convert hydrolyzates from α-amylase digestion to glucose. The objectives of this study were to investigate the exact role of amyloglucosidase in determining the digestibility of starch and to understand the mechanism of enzymatic actions on starch granules. Four maize starches differing in amylose content were examined: waxy maize (0.5% amylose), normal maize (≈27% amylose), and two high-amylose starches (≈57 and ≈71% amylose). Notably, without amyloglucosidase addition, the RS content increased from 4.3 to 74.3% for waxy maize starch, 29.7 to 76.5% for normal maize starch, 65.8 to 88.0% for starch with 57% amylose, and 68.2 to 90.4% for the starch with 71% amylose. In the method without α-amylase addition, less RS was produced than without added amyloglucosidase, except in maize at 71% amylose content. Scanning electron microscopy (SEM) revealed the digestive patterns of pinholes with α-amylase and burrowing with amyloglucosidase as well as the degree of digestion between samples. To understand the roles of amyloglucosidase and α-amylase in the in vitro test, multiple analytical techniques including gel permeation chromatography, SEM, synchrotron wide-angle X-ray diffraction, and small-angle X-ray scattering were used to determine the molecular and crystalline structure before and after digestion. Amyloglucosidase has a significant impact on the SDS and RS contents of granular maize starches.

KEYWORDS: *resistant starch, alpha-amylase, amyloglucosidase, starch digestion, maize starch*

INTRODUCTION

Starch is a major component in cereal grain foods and the most important source of food energy. Understanding starch digestibility is of great interest to the food industry and of importance for diet-related disorders such as obesity, diabetes, and cardiovascular disease. Not all starch can be digested in the small intestine, where the portion of starch that is not digested is termed resistant starch (RS).[1]

Granular starch is considered a form of type 2 RS.[2−7] The mechanism of resistance to enzymatic digestion of starch granules is complex, and factors such as dense packing and restricted mobility of starch molecules, long amylopectin branches, helix form, crystallinity, lamellar organization, and structural features of granules are considered.[8] Physiological benefits have been correlated to RS consumption,[2,6] which notably alters fecal bulk and short-chain fatty acid metabolism, thus promoting colonic health.[6] In vitro measurement of rapidly digestible starch (RDS), slowly digestible starch (SDS), and RS in granular starches often employs both α-amylase (pancreatin) and amyloglucosidase;[9−18] however, the roles of each enzyme in the in vitro tests are not well documented. Amyloglucosidase converts oligomers produced from α-amylase digestion to glucose and is not believed to affect the SDS or RS content in normal maize starch.[2,4] In many cases, the level of glucose produced in the digest is measured and used to calculate the RDS, SDS, and RS contents;[4,11,14,15,19] whether the addition of amyloglucosidase affects the SDS and RS contents is not clear. Moreover, improved in vitro methods are needed to truly reflect starch digestibility in vivo systems.[20]

In this study, we selected four native maize starches ranging from 0 to about 70% amylose content and examined the roles of α-amylase and amyloglucosidase on digestibility of the starches. RS and SDS results have been published for normal maize and waxy maize starches[2,4,11,14,19,21] and the two high-amylose starches.[13,14,19,22] The high-amylose starches are generally more resistant to enzyme digestion. The maize starches with different amylose contents are known to have different morphology,[23−25] molecular structure,[4,13,26,27] molecular order and crystallinity,[12,28] and gelatinization properties.[27,29−33] The goals of this study were to determine if the addition of amyloglucosidase affects the RDS, SDS, and RS contents and if amyloglucosidase affects the digestibility of starches with different amylose contents. Multiple analytical techniques including gel permeation chromatography (GPC), scanning electron microscopy (SEM), synchrotron wide-angle X-ray diffraction (WAXD), and small-angle X-ray scattering (SAXS) were used to probe the short- and long-range orders of the structural changes of the starches after enzyme digestion and understand the mechanism of enzymatic actions on starch granules.

MATERIALS AND METHODS

Waxy, normal, and two high-amylose (HYLON V and HYLON VII) maize starches were obtained from National Starch LLC (Bridgewater, NJ), and their amylose contents were 0.5, 27.0, 56.8, and 71.0%, respectively, as determined by the potentiometric iodine method.[27] The moisture content for all samples was determined by AACC standard method 44-16.01.[34] Porcine pancreatin (catalogue no.

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Table 1. RDS, SDS, and RS in Vitro Results As Determined by A-Amylase and Amyloglucosidase Digestion and Modified Digestive Methods[a]

maize starch	α-amylase and amyloglucosidase digestion (control method)			digestion with α-amylase			digestion with amyloglucosidase		
	RDS %	SDS %	RS %	RDS %	SDS %	RS %	RDS %	SDS %	RS %
waxy	29.0 ± 0.4 aA	67.2 ± 0.4	4.3 ± 0.8	10.2 ± 1.8	15.5 ± 2.2	74.3 ± 1.0 F	31.8 ± 2.2 aA	26.6 ± 4.1	41.5 ± 3.8
normal	15.1 ± 1.3 B	50.2 ± 2.8	29.7 ± 2.0 c	5.4 ± 0.7 bC	18.1 ± 2.8 D	76.5 ± 0.4 F	5.2 ± 0.7 bC	28.8 ± 1.5 c	66.0 ± 2.4
HYLON V	5.9 ± 0.3 C	28.3 ± 0.8	65.8 ± 0.7	2.2 ± 0.2 d	9.8 ± 0.8 DE	88.0 ± 0.7 F	1.6 ± 0.7 d	19.3 ± 0.8 D	79.1 ± 0.3 F
HYLON VII	7.7 ± 0.1 C	24.0 ± 1.8	68.2 ± 0.7	4.3 ± 1.0 eC	5.3 ± 1.5 eE	90.4 ± 2.1 G	0.8 ± 0.5	4.7 ± 1.2 eE	94.4 ± 1.8 G

[a]Data with lowercase letters indicate no significant difference at $P > 0.05$ for the sample. Capital letters denote no significant difference among category groups ($P > 0.05$). *Values shown are means ± standard deviations. **Starch portions are reported in percentage of sample.

Table 2. RS as Determined by the α-Amylase and Amyloglucosidase Digestion Method and Modified Methods in Vitro Results Analyzed via HPAEC[a]

maize starch	enzymes used in starch digestion	RS %
waxy	amyloglucosidase and α-amylase	2.7 ± 1.1
	α-amylase	71.2 ± 4.8 ab
	amyloglucosidase	56.7 ± 4.3 a
normal	amyloglucosidase and α-amylase	32.1 ± 4.7
	α-amylase	78.4 ± 0.5 b
	amyloglucosidase	69.5 ± 2.5 b

[a]Data with like letters are not significantly different ($p > 0.05$). *Values shown are means ± standard deviations. **Starch portions are reported in percentage of sample.

P7545) and amyloglucosidase (catalogue no. A7255) were obtained from Sigma-Aldrich, Inc. (St. Louis, MO). The enzyme activity was calculated spectrometrically by measuring substrate decrease over time at 520 nm, using a procedure described previously.[35,36] α-Aamylase (EC 3.2.1.1; 1,4-α-D-glucan glucanohydrolase) had an activity of 300 U, with 0.9 mg of glucose released from soluble starch in 3 min at 37 °C and pH 5.8. One U is defined as the amount of the enzyme that hydrolyzed the conversion of one micromole of starch per minute to glucose. Amyloglucosidase (EC 3.2.1.3; 1,4- α-D-glucosidase) had an activity of 234 U, with 0.7 mg of glucose released from soluble starch in 3 min at 37 °C and pH 5.8. Amyloglucosidase (catalogue no. A7255) has been recently discontinued from Sigma-Aldrich, Inc. The replacement is amyloglucosidase catalogue no. A9228, which is reported as 40000 units/g solid where 1 mg of glucose is released from soluble starch in 3 min at pH 4.5 at 55 °C.[36] Other chemicals were reagent grade.

Methods. *Digestion Method and Modifications.* The control samples were analyzed via the Englyst method.[10] Modified digestion methods were as follows: digestion samples were prepared with only one enzyme, referred to throughout as "digestion with α-amylase", and "digestion with amyloglucosidase". Vials designated as digestion with amyloglucosidase were prepared at the same volume as the control and were run according to the Englyst method of RS determination, only without α-amylase added. At 20 and 120 min intervals, 250 μL of supernatant was added to 10.0 mL of 66.6% ethanol solution. All 10.0 mL 66.6% ethanol vials were centrifuged at 1580*g* for 5 min and analyzed by the same colorimetric method as the control digestion method samples. For the digestion with α-amylase samples, the vials were also prepared at the same volume and run under the same method as the control,[10] only without amyloglucosidase initially added. At 20 and 120 min, 18 mg of amyloglucosidase was added to all 10.0 mL 66.6% ethanol vials containing a 250 μL sample from the digestion with α-amylase experiment and held at 37 °C for 30 min. We found that the added amyloglucosidase was able to completely convert oligomers to glucose under the test conditions. All colorimetric determinations of glucose were performed by AACC Method 76-13.01,[34] using a glucose assay kit (Megazyme, International Ireland Ltd. Wicklow, Ireland).

RDS and RS were collected and calculated at 20 and 120 min, respectively, for all samples. The values for SDS and RS were produced from equations. Equations for the two calculated values are SDS = digestible starch (DS) − RDS and RS = 100 − DS, which are explained in detail in the original publication.[10]

High-Performance Anion-Exchange Chromatography (HPAEC). The results of the in vitro digestion methods were verified by HPAEC with pulsed amperometric detection (Dionex ICS-3000, Dionex Corp., Sunnyvate, CA). To ensure all digested material was converted to glucose, at 20 and 120 min, 0.5 mL of supernatant from all digestion experiments was introduced to 100 μL of amyloglucosidase (aqueous; 10 μg/10 μL H_2O), and the vials were held for 30 min at 70 °C. Amyloglucosidase is active on carbohydrates at 70 °C.[37] The digest

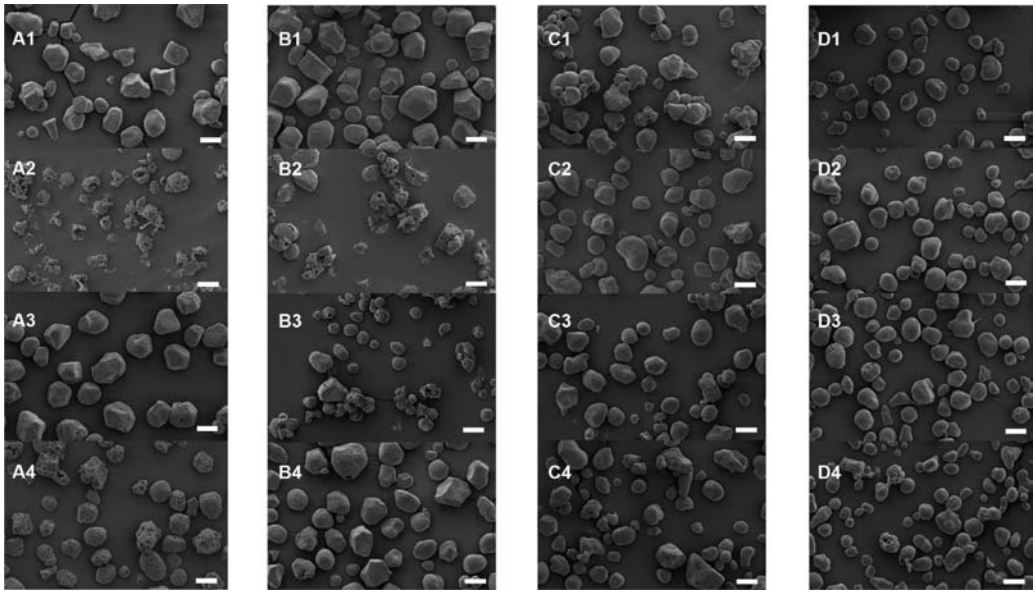

Figure 1. Scanning electron micrographs of maize starch at 1000× magnification with 10 μm scale bars: (A) waxy, (B) normal, (C) HYLON V, and (D) HYLON VII maize starch are all represented with (1) native maize starch, (2) maize starch after digestion with α-amylase and amyloglucosidase (control method), (3) maize starch digestion with α-amylase only, and (4) maize starch after digestion using only amyloglucosidase in the method.

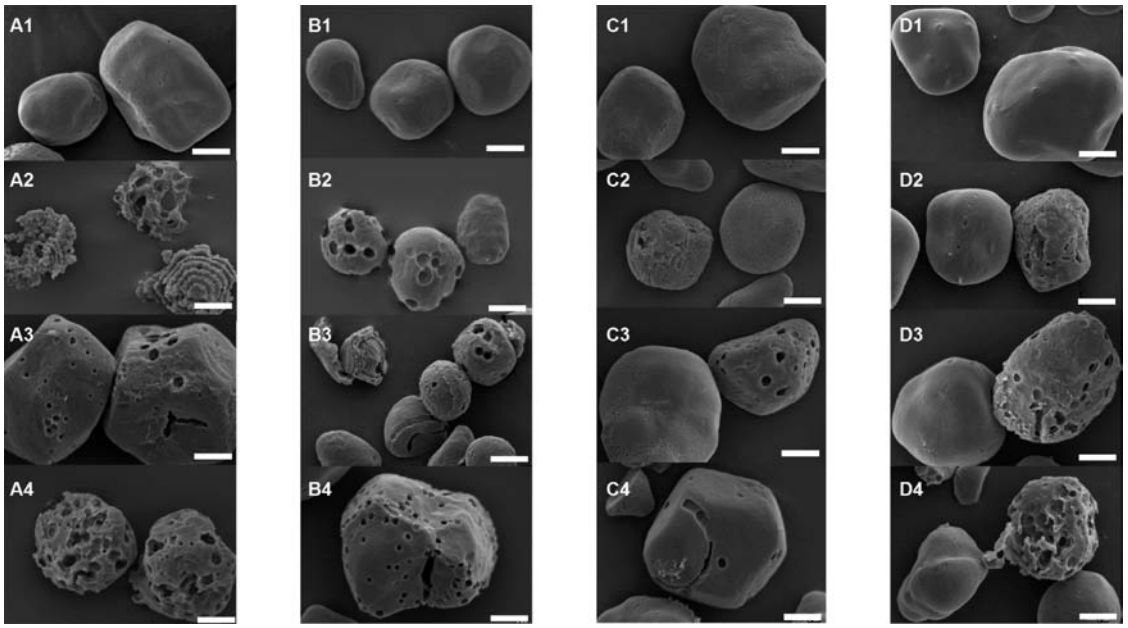

Figure 2. Scanning electron micrographs of maize starch at 5000× magnification with 3 μm scale bars: (A) waxy, (B) normal, (C) HYLON V, and (D) HYLON VII maize starch are all represented with (1) native maize starch, (2) maize starch after digestion with α-amylase and amyloglucosidase (control method), (3) maize starch digestion with α-amylase only, and (4) maize starch after digestion using only amyloglucosidase in the method.

was diluted to 1:10000 with deionized water and analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The eluent was prepared as previously reported.[7] The eluent was 150 mM NaOH, and the separations were carried out at 25 °C with a flow rate of 1 mL/min. Glucose (catalogue no. P3761, Sigma-Aldrich, Inc., St. Louis, MO) was used as a standard.

SEM. At the conclusion of the in vitro digestions, the 120 min vials were collected from the water bath and centrifuged at 1480*g* for 10 min. The supernatant was discarded, and the remaining starch in the vials was washed three times with deionized water and centrifuged at 1480*g* for 10 min. After the starch was cleaned and the supernatant was removed, vials were stored at 4 °C for 24 h and freeze-dried for 16 h. The freeze-dried samples were mounted on carbon paper and gold−palladium sputter-coated with a Desk II Sputter/Etch Unit (Denton Vacuum LCC, Moorestown, NJ). Images were collected at 1000× and 5000× resolution by an S-3500N SEM with an absorbed electron detector (S-6542) (Hitachi Science Systems, Chiyoda, Tokyo, Japan) operating at an accelerating voltage of 20 kV.

Molecular Size Distribution by GPC. All native and freeze-dried digested samples from SEM preparation were used for GPC as previously described.[38]

WAXD and SAXS. WAXD and SAXS experiments were carried out at the Advanced Polymers Beamline (X27C) in the National Synchrotron Light Source, Brookhaven National Laboratory, in Upton, NY. Details of the experimental setup of the X27C beamline were previously reported.[39−42] The wavelength used was 0.1371 nm, and the sample-to-detector distances were 129.37 and 2392.70 mm for

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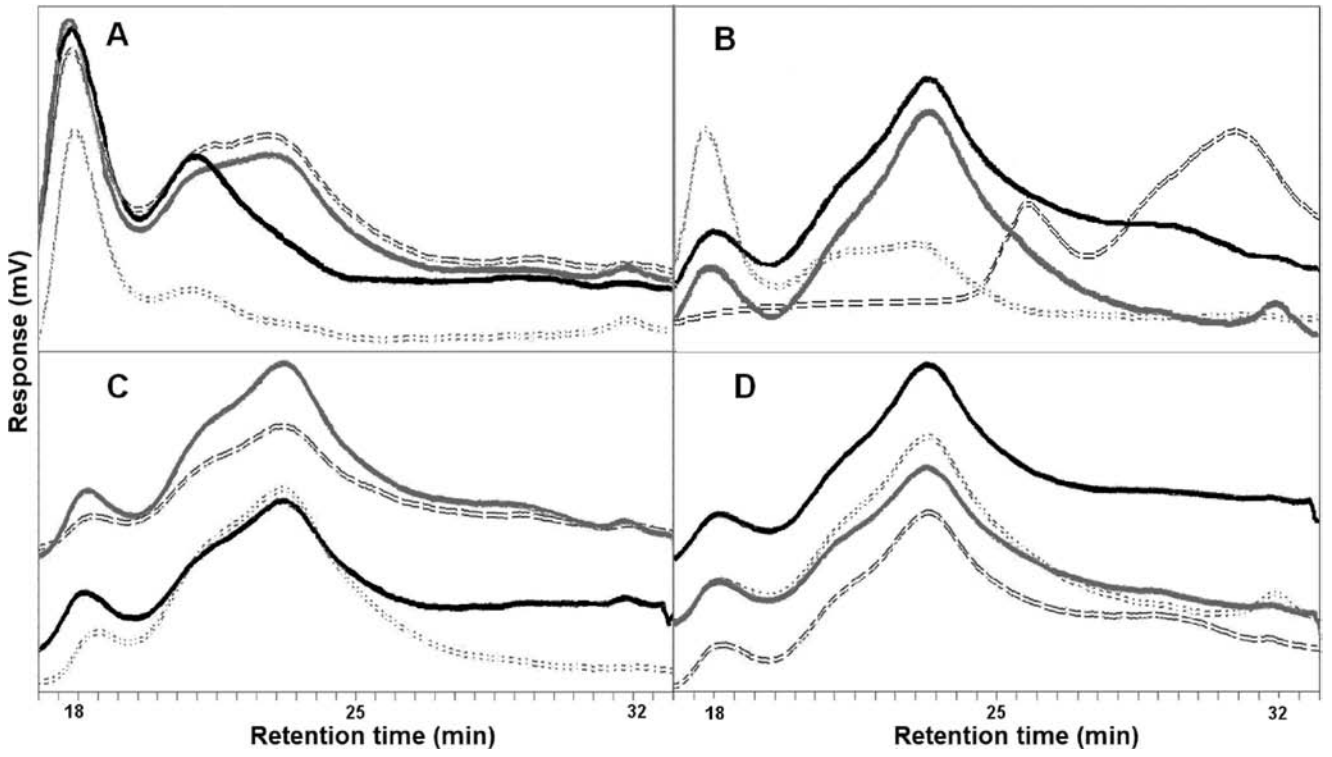

Figure 3. GPC plots of retention time vs response: (A) waxy, (B) normal, (C) HYLON V, and (D) HYLON VII maize starch. For all chromatographs, the patterns are as follows: native (dot), maize starch after digestion with α-amylase and amyloglucosidase (dashed), maize starch digestion with only α-amylase (gray), and maize starch after digestion using only amyloglucosidase (black).

WAXD and SAXS, respectively. A 2D MAR-X-ray detector CCD (Rayonix, LLC, formerly Mar USA, Inc., Evanston, IL) was used for data collection. To prepare the samples for WAXD and SAXS, the native maize starches (ca. 11% moisture) and the digested, freeze-dried maize starches (ca. 4% moisture) were mixed with water to form starch pastes (45% moisture) prior to X-ray detection. The relative crystallinity was calculated by the ratio of the peak areas to the total diffractogram area.[43]

Statistics. Means and standard deviations were calculated for all collected digestive data. All values are expressed as means ± the standard deviations. The significances of differences between groups were compared using two-sample *t* tests (Excel 2003). *P* values (two-tailed) of less than 0.05 were considered to be a sign of statistical significance.

■ RESULTS AND DISCUSSION

RDS, SDS, and RS Content of Maize Starches. Because the molecular structure of maize starch is determined by the amylose and amylopectin fine structure,[4] starches with a full range of amylose content percentages were studied. All digestions with α-amylase and amyloglucosidase (Table 1) were within the acceptable range of previously reported data.[2,4,10,11,19] Results from the modified digestion methods with amyloglucosidase only or with α-amylase only are also presented in Table 1. The RS content increased, whereas the SDS content decreased, with the increasing percentage of amylose present in the maize starch samples. The RS content of the waxy maize and normal maize starches increased from 4.3 to 74.3%, and 29.7 to 76.5%, respectively, when amyloglucosidase was not used. In contrast, when α-amylase was not used, the RS content of the waxy maize and normal starches was 41.5 and 66.0%, respectively, suggesting that amyloglucosidase plays a more important role in digestion of starches with low amylose content.

Amyloglucosidase was thought not to affect the digestion results. In the determination of RS content, α-amylase is believed to be the most important enzyme to measure digestion of the starch fractions, whereas amyloglucosidase is employed to combat any potentially inhibitory factors on α-amylase;[4,10] however, when amyloglucosidase or α-amylase was not used during the digestion, results were dramatically different (Table 1). When comparing digestion with α-amylase and amyloglucosidase, the digestion without amyloglucosidase yielded an RS content increase in all starches, but the increase in RS content was not equal among the starches: A greater increase in RS was observed in waxy maize and normal maize starches. Our results are in agreement with the work by Kimura and Robyt, who reported that waxy and normal maize starches were more susceptible to amyloglucosidase hydrolysis than high amylose maize starch.[44] Previously, in studying wheat starch, Shetty et al. showed that pure amyloglucosidase can attack native starch.[45]

Studying the effects of amyloglucosidase in conjunction with the effects of α-amylase was essential to determine the synergy of the enzymes as they work together. Amyloglucosidase and α-amylase worked together in digestion of native starch granules. The impact of amyloglucosidase was greater on waxy maize and normal maize starches as compared with high-amylose starches. No stepwise, linear correlation was seen in the rapidly digestible or slowly DS content in methods of digestion with α-amylase added.

HPAEC was used as a secondary method to confirm the results of the colorimetric in vitro test. Of the HPAEC RS determinations (Table 2), only normal and waxy maize are reported for comparison. HPAEC data were within range of both our results reported from the colorimetric determination and previously reported data.[4,19] To samples without amyloglucosidase initially added, amyloglucosidase was later

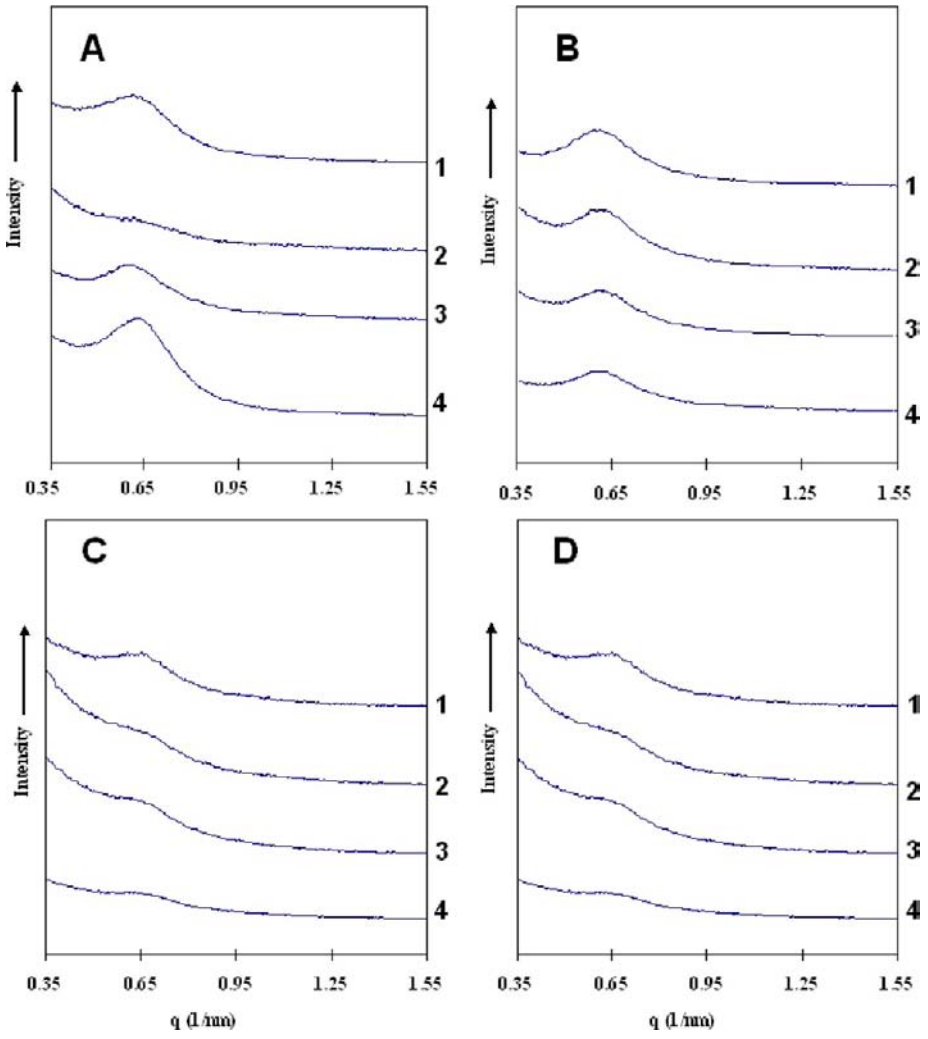

Figure 4. Synchrotron SAXS curves of native starch (1) and native starch digested by α-amylase and amyloglucosidase (2), by α-amylase only (3), and by amyloglucosidase only (4) in descending order for (A) waxy, (B) normal, (C) HYLON V, and (D) HYLON VII maize starch.

added, remote to any remaining starch granules, to convert the α-amylase digested material in solution to glucose for analysis via the colorimetric method and HPAEC. Without full conversion to glucose, digested material would be present but would remain unrecognized by the colorimetric or identification of glucose by HPAEC.

Significant differences were found between enzyme addition methods. Notably, amyloglucosidase could change the outcome 30−60% of the expected SDS fraction. No official AOAC method exists for SDS measurement. Of the available methods, the Englyst method of digestion[10] is often used to measure SDS because it was designed to calculate this nutritional concept. Only from in vivo studies has SDS been found to alter gastrointestinal response.[42]

Starch Granular Morphology before and after Enzyme Digestion. SEM photomicrographs provide an overall picture of digestion for multiple granules and are grouped together for comparison (Figures 1 and 2). Maize starches containing amylose are naturally irregularly shaped.[46] Classically, SEM starch digestion analysis focuses on comparing the uniformity of sample, pore, and digestive residue geometry.[24,42,46] The digestion of native starches by amylases seems to be inversely related to the amylose content.[15,46] For high-amylose starches, granules with pinholes were observed after 2 h of in vitro digestion, but the majority of digestive residues still resembled the native starch granules (Figures 1D and 2D), suggesting that digestion on high-amylose maize starches was heterogeneous. Using transmission electron microscopy (TEM), Evans and Thompson[47] reported that after digestion, most of the residual granules from high-amylose maize starches showed little evidence of digestion,[6,15] and partially digested granules had a radial digestion pattern[15,46,48] in the interior.

Figures 1 and 2, A1−D1, show the native starches and provide objective views of the basic morphology in undigested starches. In Figure 2, magnification is at 5000× to examine the details of digestion within the same sample and across the sample set. Degradation and digestive progression decreased as the amylose content increased. Consistent with the level of RS content (Table 1), waxy maize starch granules were extensively digested by α-amylase and amyloglucosidase (Figures 1A2 and 2A2). Amyloglucosidase alone generated more and large pinholes on waxy and normal maize starches (Figures 1A4 and 2A4) as compared with the granules digested by α-amylase only (Figures 1A3 and 2A3). It has been suggested that during digestion, enzymes migrate inside the granule.[4,46,49] The digestion of the starch material has been theorized to occur from the inside, and enzymes return to the surface after all

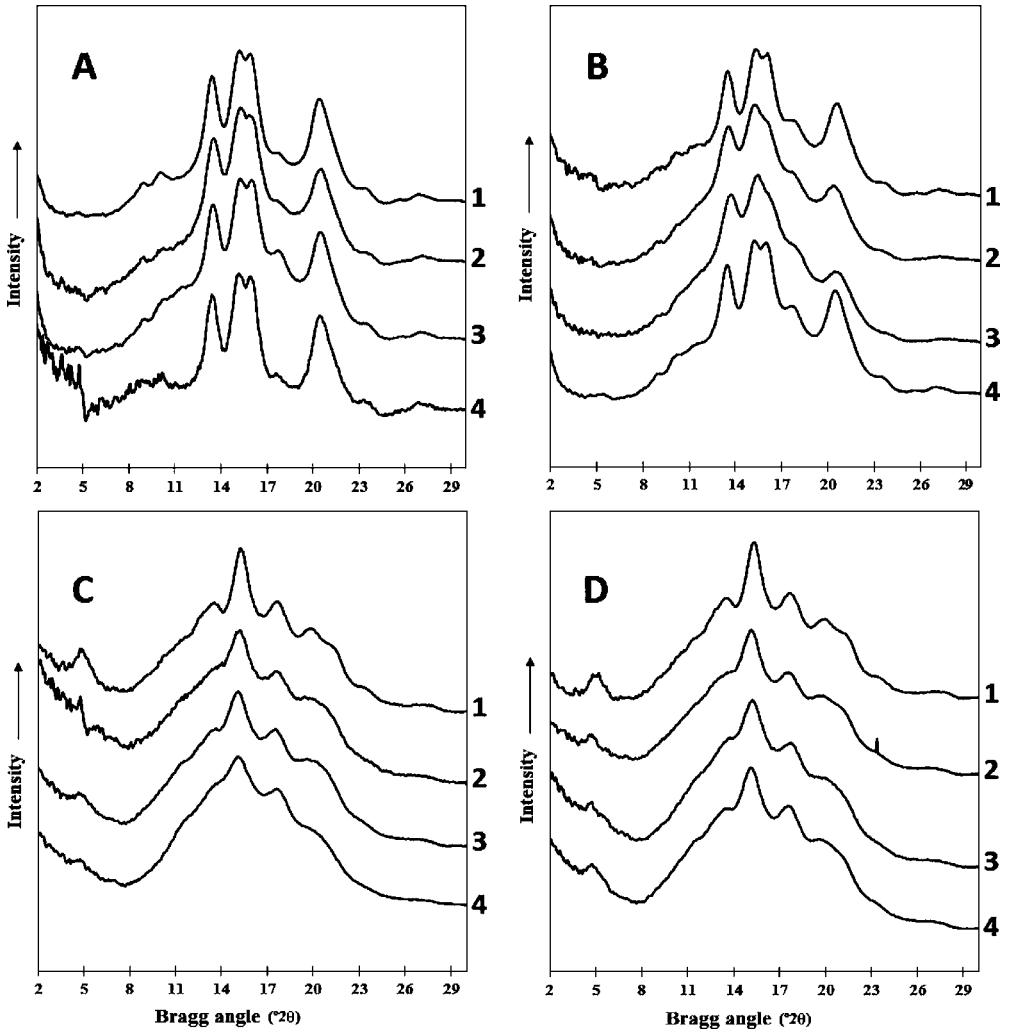

Figure 5. Synchrotron WAXD curves of native starch (1), native starch digested by α-amylase and amyloglucosidase (2), by α-amylase only (3), and by amyloglucosidase only (4) in descending order for (A) waxy, (B) normal, (C) HYLON V, and (D) HYLON VII maize starch.

Table 3. Crystallinity of Native Starch and Native Starch Digested by α-Amylase and Amyloglucosidase, by α-Amylase Only, And by Amyloglucosidase Only in Descending order for (A) Waxy, (B) Normal, (C) HYLON V, and (D) HYLON VII Maize Starch

	crystallinity (%)			
maize starch	undigested	α-amylase and amyloglucosidase digestion	digestion with α-amylase	digestion with amyloglucosidase
waxy	37.8 ± 0.3	29.2 ± 0.4	26.9 ± 0.1	56.0 ± 0.6
normal	34.7 ± 0.3	26.4 ± 0.1	23.5 ± 0.4	32.6 ± 0.1
HYLON V	28.3 ± 0.4	21.3 ± 0.3	17.7 ± 0.4	17.4 ± 0.1
HYLON VII	23.7 ± 0.3	17.5 ± 0.1	17.6 ± 0.3	17.2 ± 0.4

material is consumed.[50] A side-by-side mechanism has been proposed to explain the digestion of amylose and amylopectin as well as crystalline and amorphous regions in normal maize starch granules.[4] The digestion via pores route[4] is applicable only in A type starches.[46] Amyloglucosidase degrades maize starch surfaces in a surface pattern resembling the native starch pore distribution.[4,48,49]

Molecular Size Distribution. The GPC chromatograms visually portray the molecular size distribution for each starch and enzyme combination before and after digestion (Figure 3A–D). Dextrans with different molecular weights (MW) were used to calibrate the columns in this study. An equivalent molecular size to dextran standards was used. However, the absolute MW was not obtained.[51] For native starches, the peak around the retention time (RT) of 18 min represented amylopectin, whereas the peak around the RT of 23 min (about 1.6×10^5 g/mol) was amylose. Although SEM micrographs provide a visual of the degradation, GPC analysis displays the relative MW distribution of products digested by the enzymes.[4,14,38] The molecular size distribution of waxy maize with amyloglucosidase appeared to follow the same trend as the native starch, reflecting the localized attack of the granules by amyloglucosidase. The waxy maize digested by α-amylase had more low MW fraction and was detected around the same retention time (RT) as the samples digested with amyloglucosidase and α-amylase (Figure 3A). Chromatograms

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for normal maize with amyloglucosidase and with α-amylase had a large peak at 23 min RT (about 1.6×10^5 g/mol), whereas the normal maize starch digested by both α-amylase and amyloglucosidase had more low MW molecules appearing at 31 min RT (about 1.5×10^3 g/mol) (Figure 3B), suggesting a synergistic attack by the two enzymes. For the moderately high-amylose (57%) maize starch, the area under the peak starting at 18 min RT for the combined digestion with amyloglucosidase and α-amylase was notably smaller than that of the native and two modified digestions (Figure 3C). For the starch with 71% amylose, a shoulder was noted at 29 min RT for the residues digested by α-amylase and amyloglucosidase, and a smaller shoulder was observed for the residues digested by α-amylase only (Figure 3D). The relative small changes in molecular size distribution between native high amylose starches and digestive residues suggest that digestion on high-amylose starches was heterogeneous and limited.

Starch Structure by SAXS and WAXD. Figure 4 shows the synchrotron SAXS curves of four native starches and their digestion residues. For native waxy maize and normal maize starches, the 9 nm lamellar peak was clearly observed at q 0.65 1/nm (Figure 4A,B). This peak was thought to be attributable to the alternative repeating stacks of amorphous and crystalline lamella in starch granules.[52−54] Because of the lower amylopectin content, fewer crystalline lamella were formed for high-amylose starch granules, resulting in a broader and less clear lamellar peak (Figure 4C,D).

For waxy maize starch (Figure 4A), the 9 nm lamellar peak remained intact when digested by amyloglucosidase only, decreased by α-amylase only, and disappeared with the combination of α-amylase and amyloglucosidase. These results indicated that α-amylase had more profound effects on the lamellae structure of waxy maize starch than amyloglucosidase. For normal maize starch (Figure 4B), the 9 nm lamellar peak remained the same as that of native starch when treated by amyloglucosidase only, α-amylase only, and a combination of α-amylase and amyloglucosidase, signifying that these two enzymes had similar digestion effects on normal maize starch. After digestion by amyloglucosidase only, α-amylase only, and combined α-amylase and amyloglucosidase, the 9 nm lamellar peak of the high-amylose maize starches was still evident but decreased (Figure 4C,D), suggesting that bulk amorphous starch within the amorphous growth rings was hydrolyzed.[55] However, this hydrolysis was relatively small because little change in molecular size was observed (Figure 3), and the degree of crystallinity of the starch was not increased (Figure 5D).

Synchrotron WAXD curves of four native starches and their digestion residues are shown in Figure 5. Native waxy maize starch and normal maize starch showed a typical A type X-ray diffraction pattern (Figure 5A,B), whereas two native high-amylose maize starches displayed a B type starch structure (Figure 5C,D). As compared with native starch, the relative crystallinity of waxy maize starch decreased from 37.8 to 29.2 and 26.9%, respectively, after digestion by both α-amylase and amyloglucosidase and α-amylase only but increased to 56% when digested by amyloglucosidase only (Table 3 and Figure 5A). The degree of crystallinity of normal maize starch undigested, digested by both α-amylase and amyloglucosidase, α-amylase only, and amyloglucosidase only, was 34.7, 26.4, 23.5, and 32.6%, respectively (Table 3 and Figure 5B). Similar trends were observed for high-amylose maize starches (Table 3 and Figure 5C,D). Our results suggested that α-amylase was able to hydrolyze both crystalline and amorphous regions for all maize starches and were consistent with the side-by-side mechanism.[4] Previous studies also show that α-amylase hydrolyzes both amorphous and crystalline domains of wheat starch[56] and maize mutant starches.[57] In addition, a new α-amylase from *Anoxybacillus flavothermus*[58] and a fungal α-amylase from *Rhizomucor* sp.[59] were reported to be very efficient in hydrolyze the crystalline fraction of maize starch.

Our experiments noted significant differences between enzyme addition methods. Amyloglucosidase had a significant impact on SDS and RS contents of granular maize starches. Amyloglucosidase digestion was greater for waxy maize and normal maize starches than for high-amylose maize starches. The extent of enzyme digestion is largely controlled by the granule architecture and diffusion of the enzymes within densely packed starch granules. Future project aspirations involve applying knowledge of enzyme digestion and synergy to starch morphological impacts on digestion to diagram the influence of RSs in the human digestive system as functional foods.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

DS, digestible starch; RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch; SAXS, synchrotron small-angle X-ray scattering; WAXD, wide-angle X-ray diffraction; HPAEC, high-performance anion-exchange chromatography; HPAEC-PAD, high-performance anion-exchange chromatography with pulsed amperometric detection; SEM, scanning electron microscopy; TEM, transmission electron microscopy; MW, molecular weight; GPC, gel permeation chromatography

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