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Crosslinked and stabilized in-kernel heat-moisture-treated and temperature-cycled normal maize starch and effects of reaction conditions on starch properties

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ABSTRACT

Kernels of normal maize were subjected to a heat-moisture treatment (HMT) followed by a temperature cycling (TC) regime that was previously shown to produce slowly digesting starch (SDS) and resistant starch (RS) (Wongsagonsup, Varavinit, & BeMiller, 2008). This starch (in-kernel HMT-TC normal maize starch [NMS]) was then subjected to a low level of crosslinking with phosphoryl chloride, to hydroxypropylation, and to crosslinking followed by hydroxypropylation to determine if it could be converted into a slowly digesting modified food starch. Five controls were used. Digestibility after cooking, RVA parameters, and DSC parameters of gelatinization and retrogradation were determined. Crosslinked in-kernel HMT-TC NMS had the greatest amount of SDS, but only 3.1% (compared to 1.3% in the native starch). Hydroxypropylated and crosslinked and hydroxypropylated in-kernel HMT-TC NMS had the greatest amount of RS, but only 35% and 33%, respectively (compared to 16% in the native starch). Derivatization changed the physical properties of in-kernel HMT-TC NMS. It was also found that subjecting the starches used in this study to the time, temperature, pH, and salt concentration conditions used for derivatization made significant changes to the physical properties of the starch. In fact, subjecting in-kernel HMT-TC NMS and HMT-TC laboratory-isolated NMS to the conditions used for derivatization but without any added reagent made greater changes in the peak viscosity than did derivatization. The same was not true, however, for laboratory-isolated NMS. And for the most part, the starches subjected to the conditions of derivatization alone also produced different final viscosity values, indicating that whatever changes in granule structure and behavior occurred as a result of treatment with reaction conditions were carried through the RVA cooling cycle.

1. Introduction

Increasing the content of slowly digesting starch (SDS) (Englyst & Hudson, 1996; Englyst, Kingman, & Cummings, 1992) in the human diet helps to maintain good health in both diabetic and non-diabetic persons (Lehmann & Robin, 2007; Zhang & Hamaker, 2009). Wongsagonsup et al. (2008) examined various temperature cycling and isothermal holding regimes of heat-moisture treated (HMT) (80 °C, 2 h) normal maize kernels and determined that holding the heated moist kernels at −24 °C for 1 h followed by holding at 30 °C for 2 h for 2 cycles (temperature cycling, TC) gave the greatest content of slowly digesting starch (SDS) (24%) and starch yield. They also concluded that such thermal treatment of maize kernels prior to isolating the starch is more effective in producing SDS than is the same treatment of isolated starch.

Resistant starch (RS), of which there are 4 types (Englyst et al., 1992), also exhibits desirable physiological effects (Bird, Lopez-Rubio, Shrestha, & Gidley, 2009; Birkett & Brown, 2007, 2008; Higgins, 2004; Sajilata, Singhal, & Kilkarni, 2006; Sharma & Yadav, 2008; Thompson, 2007). In-kernel HMT followed by TC also increased the content of RS3 resistant starch (which is retrograded, crystalline starch) over that of normal maize starch (Wongsagonsup et al., 2008). The HMT-TC conditions chosen for this work were those that had given the greatest percentage of SDS in the previous work.

Most of the starch used as an ingredient in processed foods is modified starch. Two common modifications are crosslinking with phosphoryl chloride and stabilization by reaction with propylene oxide. The objective of this research was to determine any effects of cooking and of these two modifications (which might produce RS4, which is starch that has been made resistant by derivatization), on the pasting, paste properties, and paste digestibilities of normal maize starch prepared using the conditions determined by Wongsagonsup et al. (2008) that were determined to be optimal

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for increasing the content of SDS to determine if a product with both an increased content of SDS and desirable physical properties could be made.

2. Materials and methods

2.1. Materials

Kernels of normal maize were obtained from the Purdue University Agronomy Center for Research and Education (West Lafayette, IN, USA). Commercial normal maize starch (NMS) was obtained from Tate & Lyle North America (Decatur, IL, USA). Sodium sulfate (Na_2SO_4) and sodium bisulfite were purchased from Mallinckrodt (Phillipsburg, NJ, USA). Propylene oxide (PO), phosphoryl chloride ($POCl_3$), and lactic acid were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Pancreatin (P-7545) and amyloglucosidase (A-7095) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA).

2.2. Thermal treatments of normal maize kernels (Wongsagonsup et al., 2008)

Normal maize kernels were soaked in deionized and distilled water at room temperature for 24 h. After 24 h, the moist kernels were placed into screw-capped glass bottles. The bottles were heated in a hot-air oven at 80 °C for 2 h. The heat-moisture treated (HMT) kernels were then distributed on an aluminum tray. The HMT kernels were treated by temperature cycling: first by holding at −24 °C for 1 h, then at 30 °C for 2 h for 2 cycles. The thermally treated kernels are referred to as heat-moisture treated, temperature-cycled (HMT-TC) kernels.

2.3. Starch isolation (Wongsagonsup et al., 2008)

HMT-TC kernels were steeped in a container with 0.5% sodium bisulfite solution adjusted to pH 4.0 with lactic acid. The containers containing the HMT-TC kernels and sodium bisulfite/sulfurous acid solution were incubated at 50 °C in a water bath for 24 h, after which the kernels were removed from the steepwater, put in a Waring blender cup with 0.05 M NaCl solution, and ground at low speed for approx. 20 s. The slurry was poured into a 325-mesh sieve over a beaker and worked through it with a soft brush. This process was repeated until the filtrate was no longer cloudy. The filtrate was then centrifuged at ∼700 × *g* for ∼10 min. The supernatant was discarded, and the yellowish or grayish layer was carefully scraped away using a spatula. Ethanol (70%) was poured into the tube, which was then vortexed for thorough mixing. The mixture was allowed to stand with occasional shaking for ∼1 h. The process of centrifuging, removing the undesirable layer, adding 70% ethanol, vortexing, and standing was continued until the supernatant was colorless. After the final 70% ethanol wash, the starch was slurried in 100% ethanol, collected using a Büchner funnel, allowed to air dry, and lightly ground in a mortar and pestle to powder it. The isolated starch is referred to as in-kernel HMT-TC starch.

Starch was also isolated from normal maize kernels that had not been subjected to the HMT-TC process using the same procedure. It is referred to as laboratory-isolated normal maize starch (LI-NMS).

2.4. Thermal treatment of laboratory-isolated normal maize starch

A weighed sample of LI-NMS was placed in a glass jar with a tight fitting screw cap. With frequent stirring, small amounts of deionized and distilled water were added until a total amount of water required to make the water equal to 30% of the total mixture was obtained (25.7 g of water added to 100 g of starch of 12% moisture). After thorough mixing, the jars were sealed and held in a refrigerator (4 °C) for 3 days. The equilibrated starch of 30% moisture was then subjected to the same heating and temperature-cycling regimes used to treat the normal maize kernels that had been soaked in water.

2.5. Hydroxypropylation of HMT-TC starch (Gray & BeMiller, 2005)

HMT-TC starch isolated from normal maize kernels (33 g, db) was mixed with 100 mL of 0.527 molal Na_2SO_4 solution and stirred for 5 min. With continued stirring, the pH was adjusted to pH 11.2 using 1 M NaOH. The reaction vessel was sealed tightly with a septum, and propylene oxide (2.6 mL) was added via a syringe. The flask was placed in a water bath at 49 °C, and the sample was stirred on a submergible magnetic stir plate for 24 h. After that, the starch slurry was stirred magnetically for 2 h at room temperature. The starch was recovered in a Büchner funnel, rinsed with 50% acetone (∼50 mL), and allowed to dry on the filter disk. Five controls were used, viz, LI-NMS derivatized (i.e., hydroxypropylated) in the same way, in-kernel HMT-TC NMS treated under the conditions used for derivatization (i.e., hydroxypropylation) without addition of the reagent, HMT-TC LI-NMS derivatized in the same way, HMT-TC LI-NMS treated under the conditions used for derivatization without addition of reagent, and LI-NMS treated under the conditions used for derivatization without added reagent.

2.6. Crosslinking of starch with phosphoryl chloride (Gray & BeMiller, 2004)

HMT-TC starch isolated from normal maize kernels (20 g, db) was reacted with 0.01% (v/w) $POCl_3$ (added slowly as a 3% solution in dioxane) at pH 11.3 for 2 h at 25 °C in 40 mL of deionized and distilled water. The reaction pH was maintained by addition of 1 M NaOH using a pH-stat autotitrator (Radiometer Titralab, TIM 854, Radiometer Analytical, Villeurbanne, France). After reaction, the starch slurry was neutralized to pH 6.5, and the starch was recovered by vacuum filtration, washed multiple times with water, washed with absolute ethanol, and air-dried. The same five controls as were used for hydroxypropylation were used.

2.7. Hydroxypropylation of crosslinked HMT-TC starch

Crosslinked starch (described above) was hydroxypropylated as described above. The same five controls were used.

2.8. Determination of pasting and paste properties

The pasting and paste properties of the starch samples were determined using a Rapid Visco Analyzer (RVA) (Series 4V, Newport Scientific Pty. Ltd., Warriewood, Australia) using standard profile 1. The samples were mixed with distilled water in the canister at a concentration of 7.0% (1.96 g, db in 28 g total) for at least 30 s. After placing the canister in the RVA, the sample was stirred 60 s at 50 °C, heated to 95 °C at rate of 13 °C/min (228 s), and finally held at 50 °C for 120 s, all at a rotation speed of 160 rpm. Total running time = 13 min. Triplicate analyses were performed on each sample, and the results were averaged.

2.9. Determination of thermal properties

The thermal properties of the starch samples were investigated using a differential scanning calorimeter (DSC TA 2920, TA Instruments, Wilmington, DE, USA). Starch (5 mg, db) was weighed into a pan. Distilled water was then added to 20 mg total weight (w/w starch to water ratio = 1:3). The pan was sealed and equilibrated at

Table 1
Digestibilities of gelatinized modified in-kernel HMT-TC NMS.[a]

Starch	SD_{20} (%)	SD_{120} (%)	RDS (%)	SDS (%)	RS (%)
Derivatized in-kernel HMT-TC NMS					
HP[b]	64.1 ± 1.5	64.8 ± 0.3	64.1	0.6	35.2
XL[c]	78.6 ± 1.3	81.6 ± 1.9	78.6	3.1	18.4
XL-HP[d]	66.4 ± 0.3	66.7 ± 0.8	66.4	0.3	33.3
Controls					
Derivatized HMT-TC LI-NMS					
HP[b]	67.8 ± 0.7	67.9 ± 1.1	67.8	0.2	32.1
XL[c]	80.9 ± 0.7	81.1 ± 0.6	80.9	0.2	18.9
XL-HP[d]	69.8 ± 0.5	72.1 ± 1.4	69.8	2.3	27.9
Derivatized LI-NMS[e]					
HP[b]	68.6 ± 1.3	71.1 ± 2.0	68.6	2.5	28.9
XL[c]	82.3 ± 1.4	83.2 ± 1.8	82.3	0.9	16.8
XL-HP[d]	64.1 ± 0.6	65.2 ± 0.8	64.1	1.1	34.8
In-kernel HMT-TC NMS (without derivatization)					
HMT-TC[f]	80.4 ± 1.0	82.4 ± 1.5	80.4	2.0	17.6
HP[g]	80.4 ± 1.2	82.6 ± 1.4	80.4	2.2	17.4
XL[g]	79.7 ± 0.9	80.9 ± 1.8	79.7	1.1	19.2
XL-HP[g]	80.9 ± 2.1	83.4 ± 0.6	80.9	2.5	16.6
HMT-TC LI-NMS (without derivatization)					
HMT-TC[f]	79.8 ± 1.0	81.8 ± 0.7	79.8	2.0	18.2
HP[g]	81.0 ± 0.6	82.0 ± 0.6	81.0	1.0	18.0
HL[g]	80.6 ± 0.5	81.3 ± 0.5	80.6	0.7	18.7
XL-HP[g]	81.6 ± 0.5	83.1 ± 1.2	81.6	1.4	17.0
LI-NMS (without derivatization)					
LI-NMS[f]	85.4 ± 0.5	88.2 ± 0.6	85.4	2.8	11.8
HP[g]	81.8 ± 1.2	81.8 ± 1.4	81.8	0.0	18.2
XL[g]	80.5 ± 0.9	81.0 ± 1.4	80.5	0.5	19.0
XL-HP[g]	80.6 ± 0.9	81.3 ± 0.9	80.6	0.7	18.7
Commercial NMS	82.7 ± 0.6	84.1 ± 0.2	82.7	1.3	15.9

[a] Values reported to 3 significant figures.
[b] Hydroxypropylated.
[c] Crosslinked with $POCl_3$.
[d] Crosslinked and hydroxypropylated.
[e] Laboratory-isolated normal maize starch.
[f] No additional treatment.
[g] Subjected to the same reaction conditions used for derivatization but without added reagent.

room temperature for 2 h. Samples were heated from 20 °C to 120 °C at a rate of 10 °C/min. A sealed empty pan was used as a reference, and indium was used as a calibration standard. All gelatinized samples were stored at 4 °C for 7 days. The samples were rescanned from 20 °C to 140 °C with a heating rate of 10 °C/min. The onset (T_o), peak (T_p) and conclusion (T_c) temperatures and gelatinization and retrogradation enthalpies (ΔH) were recorded for both initial and rescanned samples. Triplicate analyses were performed on each sample and the results were averaged.

2.10. Determination of digestibility

Rapidly digesting starch (RDS), slowly digesting starch (SDS), and resistant starch (RS) were determined using the method of Englyst et al. (1992). Starch slurries (550 mg dry basis in 10 mL of dd water) in 50-mL screw-capped centrifuge tubes were mixed by vortexing, then heated in a boiling water bath for 20 min with continuous stirring to simulate cooking of foods. After heating, 15 glass beads (0.5 mm dia.) were added for mechanical disruption of the sample. Also added was 50 mg of guar gum to keep the starch in suspension and to standardize the viscosity of the incubation mixture, 10 mL of 0.25 M sodium acetate buffer (pH 5.2). After equilibrating 25 min at rt, 50 units of pancreatic α-amylase (as pancreatin) and 35 units of glucoamylase (amyloglucosidase), each in 5.0 mL of the pH 5.2 buffer were added. The tubes were then incubated at 37 °C with shaking (150 rpm). After 20 and 120 min periods of incubation, 500-μL aliquots of hydrolyzate were removed and added to 80% ethanol (20 mL) in 50-mL, screw-capped, centrifuge tubes to stop the reaction. After vortexing, the tubes were centrifuged 15 min at 2300 × *g*. Aliquots of 100 μL from each of these tubes were placed in disposable glass tubes (12 mm × 75 mm), and 3 mL of GOPOD reagent (Megazyme, Wicklow, Ireland) were added to determine the concentration of glucose, following the directions supplied by Megazyme. The percentages of glucose after 20 min (G_{20}) and 120 min (G_{120}) incubation were calculated using a glucose standard curve to determine the percent starch digested after 20 min (SD_{20} (%)) and 120 min (SD_{120} (%)) of incubation. Each sample was analyzed in triplicate, and the results were averaged. Digestibility fractions were calculated as follows: RDS (%) = G_{20} (%), SDS (%) = G_{120} (%) − G_{20} (%), and RS (%) = 100% − (RDS (%) + SDS (%)).

2.11. Statistical and data analysis

Means and standard deviations were obtained using Microsoft Excel software. The data in Tables 2–4 were analyzed in 4 ways: (1) as is, (2) by comparing the values for in-kernel HMT-TC NMS (see below for abbreviations) alone with those for products from HMT-TC NMS following derivatization and by doing the same for LI-NMS and HMT-TC LI-NMS reacted in the same way, (3) by comparing the values for in-kernel HMT-TC NMS alone with those for products from in-kernel HMT-TC NMS subjected to the conditions of derivatization but without added reagent and doing the same for the LI-NMS and HMT-TC LI-NMS reacted or treated in the same way, and (4) by comparing the values for the products of derivatized in-kernel HMT-TC NMS to those for in-kernel HMT-TC NMS subjected to the conditions of derivatization but without added reagent and doing the same for the LI-NMS and HMT-TC LI-NMS reacted or treated in the same way.

Table 2
RVA analysis.

Sample	Peak η (mPa s)[a]	Breakdown (loss of peak η, %)	Setback (gain over trough η, %)	Final η (mPa s)[a]	Pasting temp. (°C)[b]
Derivatized in-kernel HMT-TC NMS					
HP[c]	1059 ± 7	−21	+82	1524 ± 17	68.3 ± 0.4
XL[d]	901 ± 8	−2.3	+27	1116 ± 20	79.9 ± 2.9
XL-HP[e]	1074 ± 3	−19	+97	1724 ± 21	70.0 ± 0.4
Controls					
Derivatized HMT-TC LI-NMS[f]					
HP[c]	923 ± 5	−12	+27	1843 ± 39	73.2 ± 0.5
XL[d]	924 ± 4	−2.3	+23	1115 ± 14	78.6 ± 0.5
XL-HP[e]	967 ± 2	−13	+22	1872 ± 27	72.2 ± 0.5
Derivatized LI-NMS[f]					
HP[c]	1511 ± 3	−50	+129	1741 ± 18	66.8 ± 0.4
XL[d]	1175 ± 9	−19	+50	1419 ± 44	74.3 ± 0.0
XL-HP[e]	1345 ± 14	−40	+130	1846 ± 13	67.8 ± 0.8
In-kernel HMT-TC NMS (without derivatization)					
HMT-TC[g]	892 ± 10	−2.9	+19	1034 ± 25	86.6 ± 1.3
HP[h]	728 ± 51	−2.7	+39	984 ± 45	77.5 ± 1.4
XL[h]	848 ± 10	−3.8	+29	1053 ± 9	78.8 ± 1.7
XL-HP[h]	683 ± 9	−3.7	+41	930 ± 16	78.3 ± 0.0
HMT-TC LI-NMS (without derivatization)					
HMT-TC[g]	943 ± 10	−3.0	+16	1064 ± 28	84.4 ± 4.6
HP[h]	792 ± 5	−2.4	+38	1068 ± 14	78.0 ± 0.5
XL[h]	905 ± 11	−2.1	+24	1095 ± 23	78.4 ± 0.8
HP-XL[h]	800 ± 4	−3.5	+36	1052 ± 3	78.3 ± 0.1
LI-NMS[f] *(without derivatization)*					
NT[i]	1050 ± 13	−9.4	+26	1203 ± 33	82.6 ± 1.3
HP[h]	1025 ± 8	−13	+51	1352 ± 21	74.4 ± 0.0
XL[h]	1118 ± 23	−19	+45	1317 ± 37	75.2 ± 0.8
XL-HP[h]	957 ± 7	−9.4	+59	1378 ± 39	74.9 ± 1.0

[a] Values reported to 4 significant figures.
[b] Values reported to 3 significant figures.
[c] Hydroxypropylated.
[d] Crosslinked with $POCl_3$.
[e] Crosslinked and hydroxypropylated.
[f] Laboratory-isolated NMS.
[g] HMT-TC only.
[h] Subjected to the same reaction conditions used for derivatization but without added reagent.
[i] No treatment.

3. Results and discussion

To present the results and to discuss them, the following abbreviations are used. NMS refers to commercial normal maize starch. In-kernel HMT-TC NMS refers to starch isolated from in-kernel, heat-moisture-treated, and temperature-cycled NMS. HP refers to hydroxypropylated starch. XL refers to phosphoryl chloride-crosslinked starch. HP refers to starch that was hydroxypropylated. (Previously, it was determined that NMS hydroxypropylated under the conditions used here had a molar substitution (MS) value of 0.10 [Gray & BeMiller, 2005].) XL-HP refers to starch that was first cross-linked, then hydroxypropylated. Because the in-kernel HMT-TC NMS was not isolated by a commercial process, starch was isolated from kernels of normal maize by the same laboratory procedure used to isolate the in-kernel HMT-TC starch (LI-NMS) and used as a control. Also used as a control (to determine if in-kernel HMT-TC makes a difference) was LI-NMS subjected to the same HMT-TC procedure (HMT-TC LI-NMS).

3.1. Digestibility of the modified starch products

The data in Table 1 show that the digestibility parameters reported by Wongsagonsup et al. (2008) for starch isolated from in-kernel HMT-TC normal maize kernels were not maintained when the starch was either cooked (during the assay) or converted into modified food starch, then cooked. None of the products had a significant amount of SDS. HP and XL-HP in-kernel HMT-TC NMS had 35% and 33%, respectively, RS. RS of cooked, in-kernel HMT-TC NMS was 18% and that of cooked commercial NMS 16%. However, more was learned from this research than the fact that the SDS nature of the starch was not maintained when the in-kernel HMT-TC NMS was cooked or derivatized and cooked.

3.2. RVA analysis

In Table 2, the values for breakdown are given as percent loss of the peak viscosity and the values for setback are given as percent increase over the trough viscosity in order to better compare the effects because of relatively large differences in the values for the various parameters. Several aspects of the RVA data stand out in Table 2. (1) When in-kernel HMT-TC NMS was hydroxypropylated, lightly crosslinked, and lightly crosslinked and hydroxypropylated, the relative pasting and paste properties of the products are as would be expected from these modifications. (2) The three derivatizations increased the values for peak viscosity, final viscosity, percent breakdown (except for XL), and percent setback and reduced pasting temperature (except for XL) for the in-kernel HMT-TC NMS products (derivatized in-kernel HMT-TC NMS as compared to unmodified in-kernel HMT-TC NMS). The same changes were found for derivatized HMT-TC LI-NMS as compared to HMT-TC LI-NMS except that peak viscosities decreased. (3) In comparing the products of derivatization for HMT-TC LI-NMS with those of derivatized LI-NMS, changes in the final viscosity values were mixed. Otherwise, the peak viscosity, breakdown, and setback values decreased. (4) In comparing the products of derivatization of in-kernel HMT-TC LI-NMS with those of HMT-TC LI-NMS, it was found that the RVA values for XL were quite similar. For HP and HP-XL, peak viscosity, breakdown, and setback values

Table 3
Thermal properties of gelatinization.[a]

Sample	Onset temp. (°C)	Peak temp. (°C)	Conclusion temp. (°C)	Temp. range (°C)	Enthalpy (J/g)
Derivatized in-kernel HMT-TC NMS					
HP[b]	59.0 ± 0.1	65.3 ± 0.0	72.4 ± 0.2	13.4 ± 0.2	8.5 ± 0.0
XL[c]	67.2 ± 0.1	71.4 ± 0.1	76.7 ± 0.0	9.5 ± 0.0	13.3 ± 0.1
XL-HP[d]	56.7 ± 0.5	65.3 ± 0.1	73.2 ± 0.1	16.5 ± 0.4	8.5 ± 0.2
Controls					
Derivatized HMT-TC LI-NMS[e]					
HP[b]	64.3 ± 0.0	69.1 ± 0.2	73.1 ± 0.2	8.8 ± 0.2	14.1 ± 0.9
XL[c]	68.0 ± 0.1	72.5 ± 0.0	76.6 ± 0.2	8.6 ± 0.2	15.5 ± 0.3
XL-HP[d]	62.5 ± 0.0	68.8 ± 0.2	73.1 ± 0.2	10.6 ± 0.2	13.7 ± 1.0
Derivatized LI-NMS[e]					
HP[b]	56.7 ± 0.8	61.8 ± 0.3	68.4 ± 0.3	11.8 ± 0.5	14.0 ± 0.7
XL[c]	65.8 ± 0.6	69.6 ± 0.2	75.1 ± 0.2	9.3 ± 0.5	15.3 ± 0.4
XL-HP[d]	57.9 ± 1.1	62.8 ± 0.1	69.1 ± 0.1	11.2 ± 1.2	13.3 ± 0.2
In-kernel HMT-TC NMS (without derivatization)					
HMT-TC[f]	67.6 ± 0.3	71.8 ± 0.2	78.3 ± 0.1	10.7 ± 0.4	14.9 ± 0.2
HP[g]	69.6 ± 0.3	74.4 ± 0.1	79.7 ± 0.3	10.1 ± 0.5	15.0 ± 0.2
XL[g]	67.4 ± 0.7	71.4 ± 0.1	76.7 ± 0.1	9.2 ± 0.8	12.5 ± 0.0
XL-HP[g]	68.8 ± 0.2	74.1 ± 0.1	80.0 ± 0.1	11.2 ± 0.1	15.8 ± 0.3
HMT-TC LI-NMS (without derivatization)					
HMT-TC[h]	67.6 ± 0.5	73.1 ± 0.0	77.3 ± 0.4	9.7 ± 0.8	15.9 ± 0.5
HP[g]	69.0 ± 0.0	74.7 ± 0.1	78.6 ± 0.3	9.6 ± 0.3	14.5 ± 0.5
XL[g]	68.0 ± 0.0	72.7 ± 0.1	76.8 ± 0.3	8.8 ± 0.3	15.4 ± 1.2
XL-HP[g]	66.9 ± 0.1	74.3 ± 0.2	78.1 ± 0.2	11.2 ± 0.3	15.2 ± 0.0
LI-NMS[d] *(without derivatization)*					
LI-NMS[h]	65.5 ± 0.1	69.7 ± 0.3	75.4 ± 0.4	10.0 ± 0.3	16.2 ± 1.2
HP[g]	66.2 ± 0.3	70.1 ± 0.2	75.0 ± 0.0	8.8 ± 0.3	14.8 ± 0.2
XL[g]	64.9 ± 0.1	69.1 ± 0.2	74.0 ± 0.4	9.1 ± 0.3	15.1 ± 0.5
XL-HP[g]	67.2 ± 0.7	71.4 ± 0.1	76.1 ± 0.1	8.9 ± 0.8	13.5 ± 0.3

[a] DSC values reported to 3 significant figures.
[b] Hydroxypropylated.
[c] Crosslinked with $POCl_3$.
[d] Crosslinked and hydroxypropylated.
[e] Laboratory-isolated NMS.
[f] HMT-TC only.
[g] Subjected to the same reaction conditions used for derivatization but without added reagent.
[h] No additional treatment.

increased and final viscosity values and pasting temperatures decreased upon derivatization. (5) When in-kernel HMT-TC NMS was subjected to the same reaction conditions used for derivatization but without added reagent, values for all pasting and paste parameters (except for pasting temperature) were reduced over those for LI-NMS that had not been subjected to the HMT-TC treatment. (6) Subjecting the isolated starches to the same conditions used for derivatization but without added reagent changed all the pasting and paste parameters. For the final three controls in Table 2, the top line gives the values for the starch as isolated; the bottom three lines report the values for the starch that had been subjected to the conditions of derivatization without added reagent. Major changes are evident. With two exceptions, the differences between the final viscosity values for the three types of derivatized starches (in-kernel HMT-TC NMS, LI-NMS, HMT-TC LI-NMS) as compared to the final viscosity values for the same starches subjected to the same reaction conditions but without added reagent were greater than the differences in peak viscosity values for the same three pairs of starches. (The two exceptions were XL HMT-TC LI-NMS, for which the differences were the same, and HP LI-NMS, for which the difference in peak viscosity values was greater than was the difference in final viscosity values.) This finding indicates that whatever changes in granule structure that presumably strengthened granules during treatment under reaction conditions alone were maintained, but not completely maintained, through the RVA cooking cycle. Maintenance of the increased viscosity was greatest for HP. (7) In comparing differences effected by derivatization vs. differences effected by the reaction conditions alone, crosslinking with $POCl_3$ at the level used had the least effect. Hydroxypropylation and crosslinking followed by hydroxypropylation had greater effects, with increases in peak and final viscosities being greater for the LI-NMS products as compared to the in-kernel HMT-TC NMS products.

3.3. DSC analysis of gelatinization (Table 3)

(1) Generally, in every comparison, the values for XL in-kernel HMT-TC NMS were either unchanged or changed only slightly and not significantly over the control (XL HMT-TC NMS without derivatization). (2) Values for onset temperature, peak temperature, conclusion temperature, and enthalpy for HP and XL-HP in-kernel HMT-TC NMS decreased when (a) derivatized in-kernel HMT-TC NMS was compared to in-kernel HMT-TC NMS subjected to the conditions of modification but without reagent, (c) derivatized LI-NMS was compared to LI-NMS alone, and (d) derivatized LI-NMS was compared to LI-NMS subjected to the conditions of modification but without added reagent. (3) The enthalpy values for derivatized in-kernel HMT-TC NMS were considerably lower than those for derivatized LI-NMS and derivatized HMT-TC LI-NMS.

3.4. DSC analysis of retrogradation (Table 4)

The differences observed in making the various comparisons were small and exhibited no particular trends except that the amounts of retrogradation (as determined by enthalpy values) were unchanged when LI-NMS was treated with the conditions of derivatization but without added reagent; HP and XL-HP in-kernel HMT-TC NMS showed greatly reduced amounts of retrogradation, while XL in-kernel HMT-TC NMS showed a slightly increased amount of retrogradation; and for the derivatized starches only, the order of enthalpy values paralleled the order of conclusion temper-

Table 4
Thermal properties of retrograded pastes.[a]

Sample	Onset temp. (°C)	Peak temp. (°C)	Conclusion temp. (°C)	Temp. range (°C)	Enthalpy (J/g)
Derivatized in-kernel HMT-TC NMS					
HP[b]	39.2 ± 0.7	52.2 ± 0.3	59.5 ± 0.7	20.3 ± 0.0	3.2 ± 0.4
XL[c]	39.9 ± 0.4	50.6 ± 1.0	64.6 ± 0.2	24.7 ± 0.6	8.7 ± 0.5
XL-HP[d]	40.0 ± 0.1	51.9 ± 0.1	61.5 ± 0.5	21.5 ± 0.5	4.1 ± 0.2
Controls					
Derivatized HMT-TC LI-NMS[e]					
HP[b]	42.7 ± 0.2	54.0 ± 0.2	63.7 ± 0.8	21.1 ± 0.6	4.9 ± 0.0
XL[c]	40.4 ± 0.1	51.0 ± 0.5	66.1 ± 0.4	25.7 ± 0.3	8.9 ± 0.1
XL-HP[d]	43.6 ± 0.5	54.3 ± 0.2	63.9 ± 0.6	20.3 ± 1.1	4.8 ± 0.0
Derivatized LI-NMS[e]					
HP[b]	41.9 ± 0.1	52.7 ± 0.5	61.2 ± 1.3	19.3 ± 1.2	3.4 ± 0.2
XL[c]	43.0 ± 0.1	52.9 ± 0.7	62.9 ± 0.3	19.9 ± 0.4	9.1 ± 0.5
XL-HP[d]	40.6 ± 0.2	52.2 ± 0.0	60.9 ± 1.2	20.4 ± 0.9	3.3 ± 0.6
In-kernel HMT-TC NMS (without derivatization)					
HMT-TC[f]	39.8 ± 0.6	51.0 ± 0.2	65.0 ± 0.7	25.2 ± 1.2	8.3 ± 0.3
HP[g]	40.2 ± 0.9	51.4 ± 0.3	63.8 ± 0.8	23.5 ± 0.1	8.7 ± 0.2
XL[g]	39.4 ± 0.5	50.3 ± 0.6	63.4 ± 0.3	24.0 ± 0.2	7.9 ± 0.3
XL-HP[g]	38.8 ± 0.8	49.9 ± 0.7	62.9 ± 0.1	24.1 ± 0.7	8.9 ± 0.0
HMT-TC LI-NMS[e] *(without derivatization)*					
HMT-TC[h]	38.2 ± 1.9	49.7 ± 1.7	65.8 ± 0.0	27.7 ± 1.9	8.6 ± 0.1
HP[g]	42.5 ± 0.2	53.0 ± 0.3	65.9 ± 0.8	23.4 ± 0.6	9.7 ± 0.1
XL[g]	41.0 ± 0.5	52.2 ± 0.2	65.7 ± 0.9	24.7 ± 1.3	9.0 ± 0.5
XL-HP[g]	43.5 ± 0.1	53.6 ± 0.1	66.0 ± 0.3	22.5 ± 0.2	9.9 ± 0.2
LI-NMS[e] *(without derivatization)*					
LI-NMS[h]	43.4 ± 0.3	52.8 ± 0.4	62.4 ± 0.5	19.0 ± 0.6	8.4 ± 0.3
HP[g]	40.4 ± 0.8	50.9 ± 0.1	64.7 ± 0.5	24.2 ± 1.2	8.5 ± 1.0
XL[g]	40.6 ± 0.9	50.8 ± 0.1	65.5 ± 0.2	25.0 ± 0.7	8.5 ± 0.2
XL-HP[g]	39.8 ± 0.3	50.6 ± 0.8	62.4 ± 0.1	22.7 ± 0.3	8.4 ± 0.1

[a] DSC values reported to 3 significant figures.
[b] Hydroxypropylated.
[c] Crosslinked with $POCl_3$.
[d] Crosslinked and hydroxypropylated.
[e] Laboratory-isolated NMS.
[f] HMT-TC only.
[g] Subjected to the same reaction conditions used for derivatization but without added reagent.
[h] No additional treatment.

atures, indicating that the amount of retrograded starch paralleled the perfectness of crystals.

3.5. Discussion

Books, book chapters, and review articles (a recent one by Huber & BeMiller, 2010) have been devoted to chemically modified starches, their properties, and the modification reactions. A new aspect is presented here. The finding that the conditions used for modification changed starch pasting and hot paste properties to a greater extent than did the crosslinking or stabilization reactions (at the levels of added reagent/modification used in this project, e.g., the very low level of crosslinking reagent used [0.01% $POCl_3$ v/w of starch]) means that the properties of modified starches studied and produced for many years may have been due at least in part, perhaps a large part, to factors that have not been previously considered. This finding may have far-reaching consequences, since the products of such treatments would be classified as physically modified starches as opposed to chemically modified starches.

Under the conditions of modification without added reagent, the starch granules may have undergone a type of annealing (Jacobs & Delcour, 1998; Tester & Debon, 2000), i.e., annealing at 48 °C under conditions of high pH, which would cause the granules to swell, in the presence of sodium sulfate, which would restrict granule swelling. Chung, Liu, and Hoover (2009) reported that HMT of normal maize starch increased gelatinization temperatures and the phase transition temperature range of normal maize starch, a finding confirmed in this study using LI-NMS. They also reported that annealing increased gelatinization temperatures but decreased the phase transition temperature range. In this work, we found the same pattern (comparing peak temperatures) when conditions for hydroxypropylation were used, while conditions for crosslinking with $POCl_3$ decreased both values. Conditions for XL-HP also increased both values except for the gelatinization temperature range. These results seem to indicate that an annealing that produced more perfect crystal structures occurred only (or primarily) when the conditions for hydroxypropylation were used. Major differences between the conditions for hydroxypropylation vs. the conditions for crosslinking were a higher concentration of sodium sulfate and a longer reaction time in the former. Except for XL-HP of in-kernel HMT-TC NMS, the enthalpies of gelatinization (ΔH) were reduced by subjecting the starches to the conditions of derivatization, indicating a reduction in the amount of crystallinity. Chung et al. (2009) reported a decrease in gelatinization enthalpy upon HMT, which is what we found for LI-NMS. They also reported an increase in ΔH upon annealing, which is not what we generally found upon subjecting the starches to the conditions of derivatization without added reagent, so the question of what happened and how remains unanswered.

4. Conclusions

After cooking, neither the native in-kernel HMT-TC NMS nor any of the derivatized in-kernel HMT-TC NMS products had significant amounts of SDS. Only two of the three products had even a modest amount of RS. Pastes of XL and XL-HP made from in-kernel HMT-TC NMS exhibited lower degrees of setback (a desirable characteristic in a food starch) as compared to the controls (LI-NMS) modified in the same ways, while their final paste viscosities were a little lower

than those of the controls. The surprising finding was that in-kernel HMT-TC NMS itself and in-kernel HMT-TC NMS, HMT-TC LI-NMS, and LI-NMS treated with the conditions of modification but without any reagent exhibited reductions in both percentage breakdown and setback as compared to the derivatized starches. In fact, treatment of in-kernel HMT-TC NMS with the conditions used for modification but without any reagent made greater changes (as compared to LI-NMS) in the peak viscosity than did HMT-TC alone or derivatization. The same was true of HMT-TC LI-NMS, but not of LI-NMS. In terms of final viscosity, which reflects the combined effects of paste viscosity, breakdown and setback, HP and XL-HP resulted in much higher final viscosities (XL-HP > HP), while XL resulted in a lower final viscosity; treatment of HMT-TC using the conditions used for modification but without reagent resulted in decreased viscosities for all three products (XL-HP < HP < XL); and all three treatments produced greater changes than did HMT-TC alone.

References

Bird, A. R., Lopez-Rubio, A., Shrestha, A. K., & Gidley, M. J. (2009). Resistant starch in vitro and in vivo: Factors determining yield, structure, and physiological relevance. In S. Kasapis, I. T. Norton, & J. B. Ubbink (Eds.), *Modern biopolymer science* (pp. 449–510). New York: Academic Press.

Birkett, A. M., & Brown, I. L. (2007). Resistant starch. In C. J. K. Henry (Ed.), *Novel food ingredients for weight control* (pp. 174–197). Cambridge: Woodhead Publishing.

Birkett, A. M., & Brown, I. L. (2008). Resistant starch and health. In B. R. Hamaker (Ed.), *Technology of functional cereal products* (pp. 63–85). West Palm Beach, FL: CRC Press.

Chung, H.-J., Liu, Q., & Hoover, R. (2009). Impact of annealing and heat-moisture treatment on rapidly digestible, slowly digestible and resistant starch levels in native and gelatinized corn, pea and lentil starches. *Carbohydrate Polymers*, *75*, 436–447.

Englyst, H. N., & Hudson, G. J. (1996). The classification and measurement of dietary carbohydrates. *Food Chemistry*, *57*, 15–21.

Englyst, H. N., Kingman, S. M., & Cummings, H. J. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, *46*, 533–550.

Gray, J. A., & BeMiller, J. N. (2005). Influence of reaction conditions on the location of reactions in waxy maize starch granules reacted with a propylene oxide analog at low substitution levels. *Carbohydrate Polymers*, *60*, 147–162.

Gray, J. A., & BeMiller, J. N. (2004). Development and utilization of reflectance confocal laser scanning microscopy to locate reaction sites in modified starch granules. *Cereal Chemistry*, *81*, 278–286.

Higgins, J. A. (2004). Resistant starch: Metabolic effects and potential health benefits. *Journal of AOAC International*, *87*, 761–768.

Huber, K. C., & BeMiller, J. N. (2010). Modified starch: Chemistry and properties. In A. C. Bertolini (Ed.), *Starches* (pp. 145–203). Boca Raton, FL: CRC Press.

Jacobs, H., & Delcour, J. A. (1998). Hydrothermal modifications of granular starch with retention of the granular structure: A review. *Journal of Agricultural and Food Chemistry*, *46*, 2895–2905.

Lehmann, U., & Robin, F. (2007). Slowly digestible starch – Its structure and health implications: A review. *Trends in Food Science & Technology*, *18*, 346–355.

Sajilata, M. G., Singhal, R. S., & Kilkarni, P. R. (2006). Resistant starch – A review. *Comprehensive Reviews in Food Science and Food Safety*, *5*, 1–17.

Sharma, A., & Yadav, B. S. (2008). Resistant starch: Physiological roles and food applications. *Food Reviews International*, *24*, 193–234.

Tester, R. F., & Debon, S. J. J. (2000). Annealing of starch: A review. *International Journal of Biological Macromolecules*, *27*, 1–12.

Thompson, D. B. (2007). Resistant starch. In C. G. Biliaderis, & M. S. Izydorczyk (Eds.), *Functional food carbohydrates* (pp. 73–95). Boca Raton: CRC Press.

Wongsagonsup, R., Varavinit, S., & BeMiller, J. N. (2008). Increasing slowly digestible starch content of normal and waxy maize starches and properties of starch products. *Cereal Chemistry*, *85*, 738–745.

Zhang, G., & Hamaker, B. R. (2009). Slowly digestible starch: Concept, mechanism, and proposed extended glycemic index. *Critical Review in Food Science and Nutrition*, *49*, 852–867.