*Journal of Thermal Analysis and Calorimetry, Vol. 59 (2000) 571–586*

*Dedicated to Prof. Edith A. Turi in recognition of her leadership in education*

# THE EFFECT OF HEAT AND MOISTURE TREATMENTS ON ENZYME DIGESTIBILITY OF *AeWx*, *Aewx* AND *aeWx* CORN STARCHES

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## Abstract

Thermal analysis was used to deduce the mechanism of resistance to enzymatic digestion by starches and to account for the extent of resistance at different enzymolysis reaction temperatures. Thermal analysis was also used to determine the most productive treatment temperature for exploration of the effects of 'heat-moisture treatment' of starches on their subsequent chemical and physical behavior, including enzyme digestibility. The starches were selected according to an experimental design based on a nontraditional description of genetically varied corn starches. As a result, each functional response to heat moisture treatments of the starches adjusted to different moisture contents could be assigned to the relevant causative structural factor in the experimental design.

**Keywords:** enzyme digestibility, genetically varied corn starches, heat moisture treatment, starch functional response, starch structure

## Introduction

Beyond genetic and agronomic variations in starches, the physicochemical properties of starches can also be changed by physical and/or chemical modification. The method of physical modification of starches by treatment at different temperatures and moisture contents is well-known [1–9]. When the treatment moisture content is sufficiently below 30%, and second stage swelling does not occur, the effects on the structure and function of starches result from an annealing process. Although annealing is a universally relevant mechanical relaxation process in this context, the term 'annealing' has been used in an unconventional way in the starch literature to refer to hydrothermal treatments in 'excess' water, and the special term 'heat-moisture treatment' (HMT) has been used to refer to treatment conditions at <30% moisture content for a given time at temperatures from 90 to 130°C [9]. Numerous studies [1–9] have demonstrated the effects of such treatments on the functional properties of starches, including significant changes in water uptake and starch granule crystallinity observed at room temperature, and swelling volume, viscosity, gelatinization properties, and enzyme digestibility observed at elevated temperatures. Starches from different botanical sources show different responses to these treatment conditions. Some of the studies cited above reported that the effect of HMT with regard to

*1418–2874/2000/ $ 5.00*

*Akadémiai Kiadó, Budapest*
*Kluwer Academic Publishers, Dordrecht*

crystallinity was greater for B-type starches, such as tuber starches, than for A-type starches, such as cereal starches. In fact, B-type native granular starches were converted to A-type HMT starches, which are characterized by crystalline regions having lower moisture contents than those of B-type starches [1, 6, 10, 11]. With regard to the amorphous fraction of cereal starches, some researchers have inferred complex formation between amylose and endogenous lipids from an observed decrease in apparent amylose content [6]. Other researchers have attributed the enhanced effects of HMT to decrease solubility and swelling power of potato starch, which is essentially free of endogenous lipids, to transformation of amorphous amylose into a helical structure [2].

To date, more studies [1, 3–7, 10, 12] on HMT have been carried out with common and waxy starches, such as corn, wheat, potato, tapioca, waxy corn, cassava, and several kinds of legumes, than with amylomaize starches. Regarding the HMT of amylomaize starches, one patent, for the preparation of granular resistant starch, has been published [13], as well as the report of Hoover and Manual [8]. In the patented case, the HMT condition was broadly defined, i.e. 10–80% water content and 60–160°C treatment temperature. Amylomaize starches of the amylose-extender type have been classified as B-type rather than A-type starches, even though their crystallinity by X-ray diffraction is different from that of potato starch, which is known as a typical B-type starch [14].

The selection of starches used to study the effect of heat and moisture treatments may be based on their composition, extent of crystallinity, type of crystals, and presence or absence of endogenous lipids. With respect to composition, the traditional approach for the selection of starches results from the consideration that they only vary in the types of the polymers: the amount of predominantly linear 'amylose' and highly branched 'amylopectin' chains. As a result, parent starches are typically described as normal, waxy, and high amylose. However, an alternative approach may take into account that such starches vary both in composition and structure of the polymers, particularly with respect to the length of the branched chains of amylopectin. Accordingly, the parent starches would be described, both functionally and genetically, as *AeWx, Aewx* and *aeWx* starches. This alternative approach was used to construct an experimental design for the present study. *AeWx, Aewx* and two *aeWx* corn starches were adjusted to different moisture levels (15–27%) and heated at 110°C for 16 h, in order to investigate the effects of heat and moisture treatments on the changes in their chemical and physical properties, including enzyme digestibility.

## Materials and methods

Normal (*AeWx*) and waxy (*Aewx*) corn starches were obtained from American Maize Products Co., and two amylose extender corn starches, Hylon V (*ae5Wx*) and Hylon VII (*ae7Wx*) were obtained from National Starch and Chemical Co. Porcine pancreatic α-amylase, in the form of a crystallized suspension in 2.9 M NaCl solution containing 3 mM $CaCl_2$, was purchased from Sigma Chemical Co. (A-6255), and a

heat-stable α-amylase from Bacillus licheniformis was obtained from Novo Nordisk (Termamyl 120L).

*Heat-moisture treatment (HMT) of starch*

Water was added to 10 g of starch in a beaker to adjust its moisture content to 15, 18, 21, 24, or 27%, and the mixture was well-stirred. This mixture was transferred into a 100 mL pressure tube, sealed tightly with a cap, and held overnight at room temperature. The tubes were placed in an air oven at 110°C for 16 h and cooled to room temperature. The contents were removed from the tube and dried in air to about 10% moisture content. Based on the treatment moisture content, the resulting HMT starches will be referred to as HMT-15, HMT-18, HMT-21, HMT-24, and HMT-27. Thermal analysis (data not shown) confirmed that the 110°C treatment temperature was below the onsets of the gelatinization transition region for all of the starches at the lower treatment moisture contents, but above the onsets at the highest treatment moisture content.

*Apparent amylose content*

The apparent amylose content, which is the sum of the actual amylose content and the contribution from the long linear branches of the anomalous amylopectin [15], was measured by a blue value method modified from those of Chrastil [16] and Williams *et al*. [17].

*Water-retention capacity (WRC)*

The procedure for WRC was performed using the method of Medcalf and Gilles [18]. Starch (1 g) was weighed into a tared 15 mL centrifuge tube, 5 mL of distilled water was added, and the tube was stoppered. The tube was shaken vigorously, and the starch was permitted to hydrate for 20 min, with shaking at 5, 10, 15 and 20 min. Then the tube was centrifuged at 1000 g for 15 min, and the supernatant was decanted. The tube was drained for 5 min at a 45° angle, then drained for 5 min at a 90° angle (in inverted tube). The tube was then weighed, and WRC was calculated from the gain in mass.

*Modulated differential scanning calorimetry (MDSC)*

Thermal characteristics were measured using a modulated differential scanning calorimeter (DSC 2920, TA Instruments, USA). A sample of starch (about 5 mg) was placed in a stainless-steel, high-pressure pan (Perkin Elmer), and about 15 mg of water was added to the starch. The pan was then sealed and held at room temperature for about 1 h before heating at 5°C $min^{-1}$ from 30 to 150°C, with modulation of ±1.0°C every 60 s. An empty pan was used as a reference. Indium was used for temperature and DSC cell constant calibrations, and tin was also used for temperature calibration. High density polyethylene (average Mw, ca 125000, Aldrich Co.) was used for heat capacity calibration in the modulated mode.

*Enzyme digestibility*

Enzyme digestibility studies were carried out under different conditions, using different α-amylases. Porcine pancreatic α-amylase and Termamyl 120 L were used as a modification of the procedure of Knutson *et al*. [19]. For use of the former enzyme, a starch sample (10 mg) was suspended in 5 mL of water plus 5 mL of 0.02 M phosphate buffer (pH 6.9). Two μL of porcine pancreatic α-amylase was added, and the mixture was incubated at 37°C for 4 h with constant shaking. After the enzyme reaction, 30 mL of 60% ethanol was added, and the sample was centrifuged at 1000 g for 20 min. The supernatant was analyzed for hydrolyzed carbohydrate as maltose, using the phenol-sulfuric acid method [20].

For study of the hydrolysis by heat-stable α-amylase, a starch sample (10 mg) was suspended in 10 mL of 0.05 M phosphate buffer (pH 6.0), and 4 μL of Termamyl 120 L was added and mixed well. The mixture was incubated at 100°C for 30 min, cooled quickly with tap water, then 30 mL of 60% ethanol was added, and the procedure described above was followed.

*Polarization light microscopy*

The birefringence of starch granules was observed under polarized light at 400× magnification with a microscope (Carl Zeiss, Germany). The appearance of native and HMT *AeWx* starches was compared either in oil or upon subsequent addition of excess water, in order to explore how the ability to swell in water depended on the physical state of the starch. The appearance of native and HMT *AeWx* starch in water was also compared after enzyme hydrolysis by porcine pancreatic α-amylase. The appearance of native and HMT *ae7Wx* starch in water was compared before and after enzyme hydrolysis by Termamyl.

## Results and discussion

*Apparent amylose content*

The apparent amylose contents measured for the starches are shown in Table 1. These values agreed well with the manufacturers' values of 28% for *AeWx*, 0% for *Aewx*, 55% for *ae5Wx*, and 70% for *ae7Wx* corn starches. Using literature values for the normal amylose contents of the *aeWx* and *aewx* corn starches [15], the contributions from the long linear branches of the anomalous amylopectin were calculated and shown in Table 1. The values in Table 1 are arranged so that the traditional approach, using an experimental design with apparent amylose as a single compositional factor, can be compared to the nontraditional approach, using an experimental design with two factors based on the composition and structure of the starch polymers. Although the *aewx* double mutant, which would have made it possible to complete the nontraditional factorial design, was not available for the present study, it is included in Table 1.

*WRC*

For the native starches, the WRC values at 23°C (Fig. 1) for the two *Ae* starches were lower (≤105%) than those for the two *ae* starches (120%). Although differences in the internal organization of the granules, such as the relative amounts of crystalline and amorphous components, or minor impurities such as lipids and protein, could contribute to WRC values, Table 1 shows a direct relationship between the pattern of WRC values for the native starches and contributions from the long linear branches of the anomalous amylopectin, but no relationship to the apparent amylose content or the normal amylose content of the starches.

**Table 1** Apparent amylose content, normal amylose content, and anomalous amylopectin content of corn starches

| | Traditional approach | Nontraditional approach | |
|---|---|---|---|
| Starch | Measured apparent amylose | Normal amylose | Calculated anomalous amylopectin |
| *AeWx* | 28 | 28 | 0 |
| *Aewx* | 0 | 0 | 0 |
| *ae5Wx*[a] | 57 | 33[b] | 24 |
| *ae7Wx*[a] | 71 | 33[b] | 38 |
| *aewx*[c] | 26[b] | 0[b] | 26 |

[a] Irregular granules were observed in the native starch sample, indicative that the mutant was derived from a dent corn background [15]
[b] Values from [15]
[c] The double mutant, which would have made it possible to complete a factorial design, was not available for the present study [15]

For heat treatment at moisture contents up to 18%, the WRC values for *AeWx* corn starches were not much affected, but the WRC increased remarkably from 82% for HMT-18 to 144% for HMT-27. In contrast, the WRC for *Aewx* corn starch was not affected by HMT, regardless of the moisture content of the starch. These results agreed with those of Franco *et al*. [7] for normal and waxy corn starches. The behavior of the two *aeWx* starches in response to HMT was similar to that of *AeWx*, but there was a less dramatic increase in WRC from 110% for HMT-18 to 140% for HMT-27. The WRC for *ae7Wx* corn starch was slightly higher than that for *ae5Wx* corn starch over the whole range of adjusted moisture levels. The literature reports that different starch sources with their characteristic compositions show different responses, in terms of WRC, to heat and moisture treatments, but the magnitude of the response is not related to amylose content. For example, Kulp and Lorenz [2] reported that potato and wheat starches, treated at 100°C for 16 h at different moisture levels from 18 to 27%, showed increased WRC over the entire range of treatment moisture levels, and that wheat starch showed a more pronounced increase than did

potato starch, even though their amylose contents were not significantly different. However, the present study suggests that the presence of amylose, rather than the amount of amylose in a particular starch, may determine the change in WRC resulting from HMT, since the WRC of the *Aewx* corn starch (no amylose) showed little response to HMT (Fig. 1), in contrast to the large responses of the other three starches, which did contain amylose (Table 1).

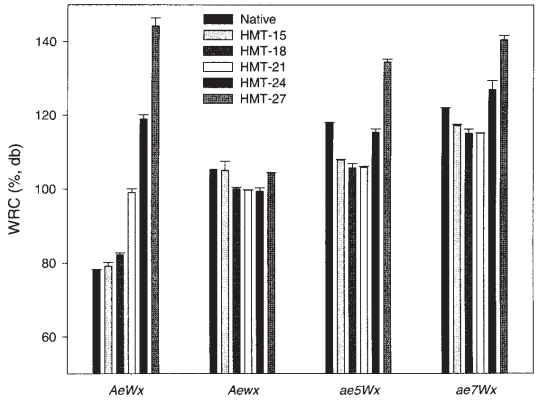


**Fig. 1** Water retention capacity *vs*. moisture content for native and HMT *AeWx*, *Aewx* and *aeWx* corn starches

A decrease in WRC for HMT starches could indicate that the crystalline portions become more perfect, by a relaxation process called annealing [21]. In the amorphous fraction, an analogous relaxation could result in a local intragranular densification, compared to the untreated native starch, when the HMT is conducted at low moisture content, such that subsequent rates of diffusion-limited processes would be decreased, even when excess water has been added. On the other hand, an increase in WRC could indicate that a local intragranular volume expansion can occur when the HMT is conducted at sufficiently high moisture content, and therefore such treated starches would exhibit greater rates of local diffusion than the untreated native starch, upon subsequent addition of excess water. Water uptake in amorphous regions allows polymer chains to become mobile via plasticization [21]. For a heat treatment at a given temperature, the greater the treatment moisture content, the greater the plasticization, and the greater the probability that local intragranular relaxations can occur. The local expansion volume also increases with increasing treatment moisture content, such that the relaxation may result in a local densification at lower treatment moisture contents, but a local expansion at sufficiently higher treatment moistures. Polarization light microscopy to compare the appearance of native and HMT *AeWx* starches at 23°C, either in oil or upon subsequent addition of excess water, supported these suggestions about the effects of HMT on the physical state of the starches (data not shown). No difference in swelling in the two media was observed for the native and HMT starches resulting from the lower treatment moisture contents, but greater swelling in water with loss of birefringence was observed after HMT at the highest

treatment moisture content. The microscopy also revealed the basis for the WRC values at 23°C shown in Fig. 1. WRC values for the native and HMT starches resulting from the lower treatment moisture contents do not reflect granule swelling, whereas granule swelling was the direct cause of the high WRC values after HMT at the highest moisture content.

*MDSC*

The gelatinization transition comprises the glass transition and the subsequent non-equilibrium melting of amylopectin, and the temperature location of the glass transition is determined by: the moisture content and its distribution in the starch, and the extent of crystallinity and branch chain length and distribution in the amylopectin [21]. For this study, MDSC analysis was conducted with a great excess of water (75%, w/w), more than sufficient for pasting by second stage swelling of the starches below 135°C, so that the thermal behavior (Figs 2 and 3) could be related to the susceptibility of the starches to enzyme digestion in excess water. For the native starches, the temperature regions of the gelatinization transition could be classified into two types of behavior, which were not related to apparent amylose content nor normal amylose. Rather, these MDSC behavior types depended on the anomalous amylopectin contribution. The *AeWx* and *Aewx* corn starches (Fig. 2) exhibited a narrow gelatinization temperature region from about 57.5 to 84°C, whereas the *ae5Wx* and *ae7Wx* corn starches (Fig. 3) exhibited a broad temperature region from 59 to 110°C (Table 2). For native *AeWx*, the analytical MDSC condition of great excess water (about 75%) allowed the gelatinization events to occur in a narrow region of temperature and time after the onset of the transition, resulting in the complete

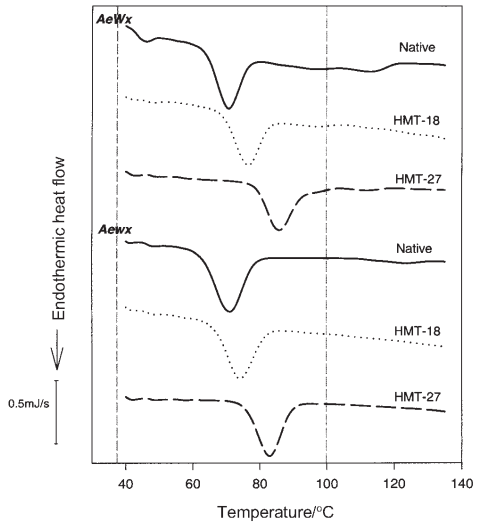


**Fig. 2** MDSC curves for native and HMT *AeWx* and *Aewx* corn starches at 25% solids

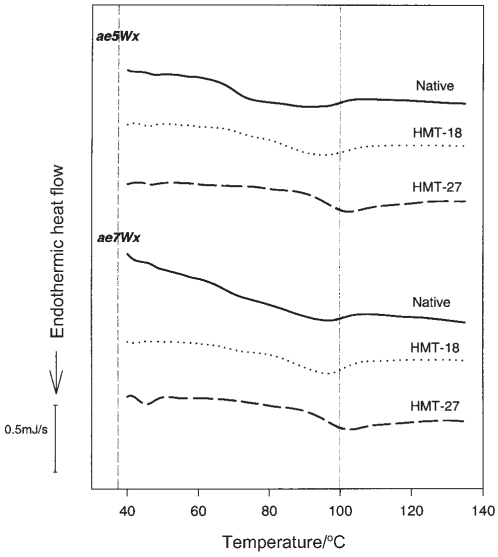


**Fig. 3** MDSC curves for native and HMT *aeWx* corn starches at 25% solids

deconvolution of the gelatinization temperature region from the lipid-amylose melting temperature region. The effect of the excess water on the gelatinization events was similar for native *Aewx*, but the lipid-amylose transition was missing, due to the absence of amylose. In contrast, in spite of the great excess water, the deconvolution of the two transition regions was not observed for the native *aeWx* starches, perhaps because the gelatinization transition region was so broad that it overlapped with the lipid-amylose transition region. This suggestion is supported by the fact that the transition region for these *aeWx* starches extended to above 105°C, whereas Yuan *et al*. [22] reported for the double mutant *aewx*, which does not form normal lipid-amylose complexes due to the absence of amylose, that the transition region ended below 100°C. Although the *aewx* double mutant was not available for the present study, the DSC results of Yuan *et al*. [22] show that it exhibited the same MDSC behavior type as the *aeWx* starches in our study, due to the contributions of the anomalous amylopectin.

Two treatment conditions were selected to explore the effect of HMT on the MDSC behavior, because they resulted in the least (for HMT-18) and the greatest (for HMT-27) accessibility of the HMT starches to α-amylase digestion at 37°C (Fig. 4). For all four starches at both treatment conditions, the effect of HMT was to delay the gelatinization transition region to higher temperatures, but the magnitude of the delay was greater at the higher treatment moisture content. The delay of transition temperature regions of HMT starches has been explained as an annealing process in the amorphous and crystalline regions of amylopectin [23]. In the present study, there was no recognizable trend in the effect of HMT on the heats of transition (Table 2). As observed for the native starches, the HMT starches could be classified into two types of MDSC behavior which depended on

the anomalous amylopectin contribution, but not on the apparent or normal amylose contents. At the higher moisture treatment condition, the magnitude of the delay of the gelatinization transition region was greater for the two *ae* starches than for the two *Ae* starches, because the effect on the onset temperature was greater for the two *ae* starches, resulting in a pronounced decrease in the width of the transition region. To date, many reported results for HMT of starches from corn, potato, wheat, barley, arrowroot, cassava, etc. have shown a similar trend in changes in the temperatures of the gelatinization transition region, and a similar absence of a trend in the effects on the heats of transition, depending on the starch source [1, 2, 10, 11]. A decrease in the heat of gelatinization was observed after HMT of potato starch [6, 9, 10, 11]. However, cereal starches showed a slight [10] or no decrease [6, 11] in the heat of gelatinization after HMT.

**Table 2** MDSC total heat flow characteristics of corn starches at 25% solids before and after HMT

| Starch | Gelatinization transition region | | | |
|---|---|---|---|---|
| | $T_{onset}$/°C | $T_{peak}$/°C | $T_{completion}$/°C | Heat of transition $\Delta Q$/J g$^{-1}$ |
| Native | | | | |
| *AeWx* | 59.2 | 70.6 | 81.0 | 10.6 |
| *Aewx* | 57.5 | 71.0 | 84.0 | 12.8 |
| *ae5Wx* | 59.0 | 87.4 | 107.0 | 12.0 |
| *ae7Wx* | 61.0 | 93.3 | 110.0 | 10.3 |
| Heat-moisture treated at 18% MC | | | | |
| *AeWx* | 64.0 | 76.4 | 87.8 | 10.6 |
| *Aewx* | 60.5 | 74.1 | 87.0 | 13.0 |
| *ae5Wx* | 65.5 | 95.1 | 116.0 | 11.2 |
| *ae7Wx* | 64.0 | 96.9 | 116.0 | 10.2 |
| Heat-moisture treated at 27% MC | | | | |
| *AeWx* | 75.0 | 85.9 | 102.8 | 10.0 |
| *Aewx* | 70.2 | 82.8 | 94.0 | 10.7 |
| *ae5Wx* | 85.0 | 101.5 | 126.0 | 8.2 |
| *ae7Wx* | 86.0 | 101.8 | 128.0 | 8.8 |

### *Enzyme digestibility*

The effect of HMT of starch on enzyme digestibility by porcine pancreatic α-amylase is shown in Fig. 4. The enzyme reaction incubation temperature was 37°C, well below the onset of the gelatinization transition region of the native starches and the analogous transition region of the HMT starches (Figs 2 and 3). For the native starches, the two *Ae* starches were very susceptible to the enzyme reaction, while the two *ae* starches were very resistant, which demonstrates the role of the anomalous amylopectin to decrease susceptibility to enzyme digestion at 37°C. The greatest digestibility of the native

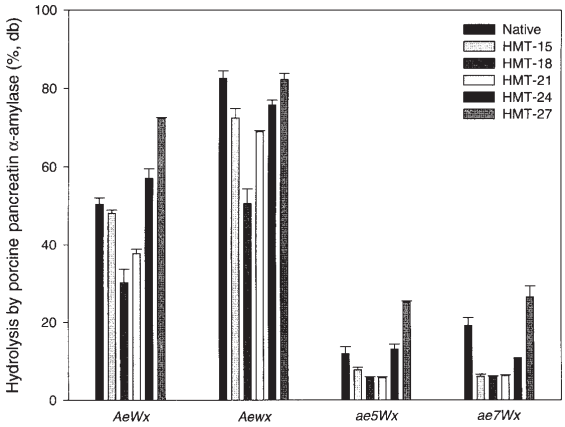


**Fig. 4** Enzyme digestibility by porcine pancreatic α-amylase for native and HMT *AeWx*, *Aewx* and *aeWx* corn starches

starches was observed for *Aewx* starch, which suggests the absence or presence of amylose also influences enzyme susceptibility. Similarly for the HMT starches, at each treatment moisture level, the two *Ae* starches were more susceptible to enzyme digestion than the two *ae* starches. The two *Ae* starches showed a distinct minimum in enzyme susceptibility after treatment at 18% moisture content, but the two *ae* starches showed no significant differences after treatment at 15–21% moisture contents. At each treatment moisture level, the greatest digestibility of HMT starches was observed for *Aewx* starch, consistent with the behavior of the native starches.

In contrast, the incubation temperature for the reaction with Termamyl heat-stable α-amylase was 100°C, well above the gelatinization transition region for the two *Ae* starches (Fig. 2), resulting in complete gelatinization and hydrolysis by the enzyme. Therefore, only the two *ae* starches were used to investigate the hydrolysis by heat-stable α-amylase, and the results in Fig. 5 show that the extent of hydrolysis of each starch at 100°C by Termamyl progressively decreased with increasing moisture level for the

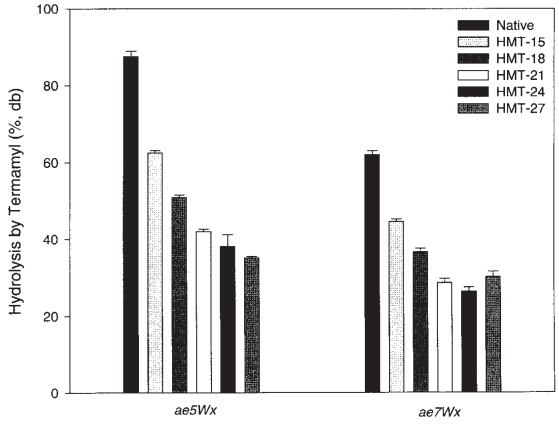


**Fig. 5** Enzyme digestibility by Termamyl, heat-stable α-amylase, for native and HMT *aeWx* corn starches

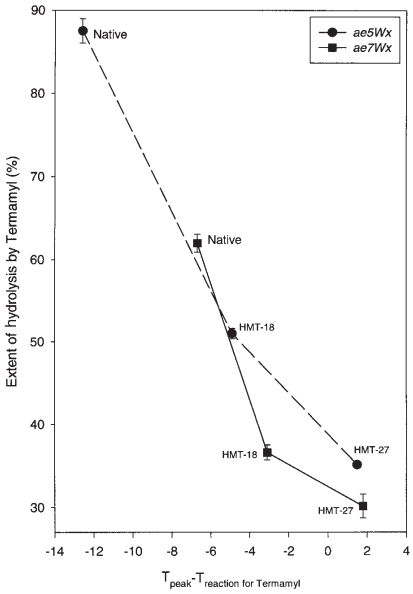


**Fig. 6** Relationship of the extent of hydrolysis by Termamyl to the temperature increment between the transition peak temperature and the 100°C Termamyl reaction temperature ($T_{peak}$–$T_{reaction}$), for native and HMT *aeWx* corn starches

HMT. As described above for the HMT starches, the effect of annealing to delay the onset of the gelatinization transition was so much greater for the two *ae* starches than for the two *Ae* starches, that the peak transition temperatures after HMT at the highest treatment moisture level occurred above the reaction temperature for digestion by Termamyl (Fig. 3 and Table 2). Figure 6 shows that with increasing treatment moisture level, the progressive increase in annealing accounted for the progressive decrease in enzyme susceptibility at 100°C for both *ae* starches. The enhanced effect of the increased level of anomalous amylopectin (Table 1) can be seen by pairwise comparison of the native starches and the HMT starches treated at each moisture level (Fig. 6). The combination of a lower level of anomalous amylopectin with a greater extent of HMT can be used to achieve the same extent of resistance to enzyme digestion as a higher level of anomalous amylopectin with a lower extent of HMT. The temperature increment between the transition peak temperature and the 100°C Termamyl reaction temperature ranged from –12.6 to 1.8°C for the series of starch samples. In contrast, the temperature increment ($T_{peak}$–$T_{reaction}$) between the transition peak temperature and the 37°C pancreatic amylase reaction temperature was greater than 50.4–64.8°C for the two *ae* starch samples, which accounts for the fact that the extent of digestion of each *ae* sample was less by the pancreatic amylase than by Termamyl.

*Changes in starch birefringence caused by enzyme treatment*

*AeWx* corn starch was selected as an example of the less enzyme accessible *Ae* starch (Fig. 4) to observe the birefringence of starch granules hydrolyzed by porcine pancreatic α-amylase before and after HMT (Fig. 7). As shown in Fig. 7A, the native starch before enzyme treatment clearly exhibited the characteristic Maltese cross, but the birefringence decreased after enzyme treatment at 37°C (Fig. 7B). Some granules were severely affected, and the enzyme attacked the apparent center of these granules more readily than it did the peripheral portions of the granules; so that the center of the granules appeared as a black hole. Upon plasticization of the amorphous regions by temperature and moisture uptake [21], the contiguous crystalline regions become free to melt and disorganize under the influence of further heating and moisture uptake, resulting in the development of a central cavity [24]. Before enzyme treatment, the appearance of HMT starches (Figs 7C, 7E) was indistinguishable from the native starch (Fig. 7A), but thermal analysis (Fig. 2) revealed the differences in the physical states of the same three samples. After enzyme treatment, the birefringence of the

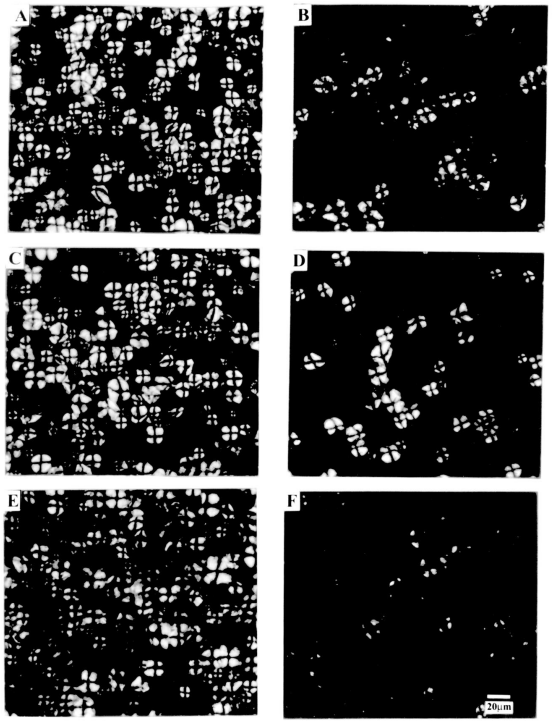


**Fig. 7** Photomicrographs of native and HMT *AeWx* corn starch in water under polarized light, before and after pancreatic α-amylase treatment. A) native starch before enzyme treatment; B) native starch after enzyme treatment; C) HMT-18 before enzyme treatment; D) HMT-18 after enzyme treatment; E) HMT-27 before enzyme treatment; F) HMT-27 after enzyme treatment

HMT-18 starch showed that only a few granules were hydrolyzed by the enzyme, so that HMT-18 starch after enzyme treatment (Fig. 7D) looked similar to the native starch before enzyme treatment (Fig. 7A). In contrast, most of the HMT-27 granules were severely affected by enzymatic hydrolysis, and only a few granules remained slightly birefringent in the peripheral regions (Fig. 7F). These birefringence patterns revealed the changes in the structure of the starch granules resulting from the dependence of the extent of enzyme digestion at 37°C (Fig. 4) on the physical states of the native and HMT starches (Fig. 2).

The patterns of the extents of digestion of native and HMT *ae* corn starches treated with Termamyl were similar (Fig. 5), and *ae7Wx* corn starch was selected as an example of the less enzyme accessible *ae* starch to illustrate the changes in birefringence caused by the enzyme treatment at 100°C (Fig. 8). Before enzyme treatment, the birefringence of the native and HMT starches (Figs 8A, 8C, 8E) was similar, even though thermal analysis (Fig. 3) revealed differences in the physical states of the samples. Of the granules that retained a low level of residual birefringence, when the native starch was incubated at 100°C in the absence of enzyme, most were similar in size to the native granules (data not

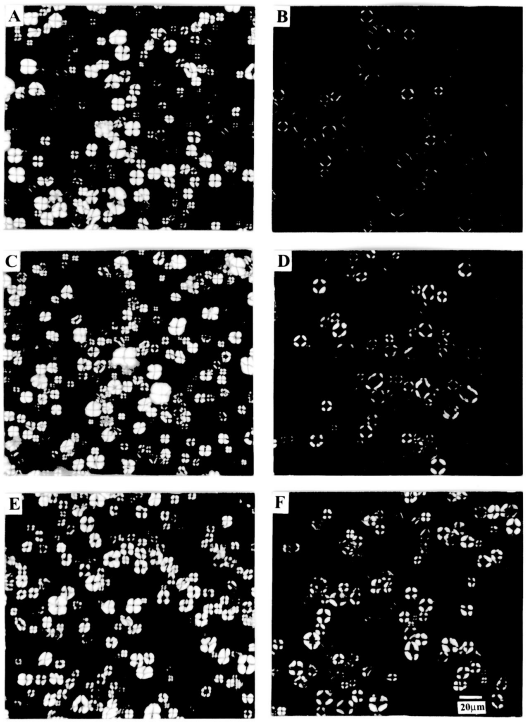


**Fig. 8** Photomicrographs of native and HMT *ae7Wx* corn starch in water under polarized light, before and after Termamyl treatment. A) native starch before enzyme treatment; B) native starch after enzyme treatment; C) HMT-18 before enzyme treatment; D) HMT-18 after enzyme treatment; E) HMT-27 before enzyme treatment; F) HMT-27 after enzyme treatment

shown). In the presence of the enzyme, most of these granules were attacked, and the loss of birefringence was exaggerated in the central portion of the granules (Fig. 8B). However, many of the granules exhibited excessive swelling and loss of birefringence in the absence of enzyme, and these granules disappeared completely in the presence of enzyme (data not shown). The HMT-18 and HMT-27 *ae7Wx* corn starches (Figs 8D, 8F) exhibited lower susceptibility to Termamyl digestion and greater residual birefringence than the native starch (Fig. 8B). The difference in birefringence due to enzyme treatment was not significant for the two HMT starches. These birefringence patterns revealed the differences in the structures of the granules that were expected from the pattern of the extents of enzyme digestion at 100°C (Fig. 5).

## Conclusions

*AeWx*, *Aewx* and two *aeWx* corn starches adjusted to different moisture levels (15–27%) were heated at 110°C for 16 h, in order to investigate the effect of 'heat-moisture treatment' on the changes in physical properties and enzyme digestibility of starch granules. General observations can be summarized as follows. The effect of these heat-moisture treatments on WRC depended both on the type of starch and the moisture content used for the heat treatment. MDSC curves showed that all of the HMT starches gelatinized at higher temperatures than did the corresponding native starches, and the extent of the temperature increase depended on the moisture content used for the heat treatment. Similarly, the effect of these heat-moisture treatments on enzyme digestibility, assayed by incubation at 37°C with porcine pancreatic α-amylase, showed analogous trends for all the starches. The starches were least digestible after the heat-moisture treatment at 18% moisture content, but were most digestible after the heat-moisture treatment at 27% moisture content. On the other hand, the impact of these heat-moisture treatments on enzyme digestibility, assayed by incubation at 100°C with a heat-stable α-amylase from Bacillus licheniformis, depended both on the type of starch and the moisture content used for the heat treatment, as observed for the effect on water retention capacity. Additionally, observation of the birefringence patterns for *AeWx* starch samples, before and after incubation with porcine pancreatic α-amylase, and *aeWx* starch samples, before and after incubation with heat-stable bacterial α-amylase, revealed the morphological effect of the digestion of the granules in the region around the hilum.

The critical conclusions of the present study are summarized in Table 3, which demonstrates that each functional response of the native and HMT starches could be assigned to the relevant causative structural factor in the experimental design based on the nontraditional description of the genetically varied corn starches. None of the functional responses could have been accounted for by using the traditional approach based on the apparent amylose content. Only one of the functional responses required both nontraditional factors to account for the behavior of the native and HMT starches. Enzyme digestibility at 37°C, which was well below the gelatinization tran-

**Table 3** Functional responses of native and HMT starches assigned to apparent amylose content, normal amylose content, or anomalous amylopectin contribution

| Functional response | Starch | Traditional approach | Nontraditional approach | |
|---|---|---|---|---|
| | | Apparent amylose content | Presence or absence of normal amylose | Anomalous amylopectin contribution |
| WRC | | | | |
| | Native | No | No | Yes |
| | HMT | No | Yes | No |
| MDSC | | | | |
| | Native | No | No | Yes |
| | HMT | No | No | Yes |
| Enzyme digestibility | | | | |
| 37°C | Native | No | Yes | Yes |
| | HMT | No | Yes | Yes |
| 100°C | Native | No | No | Yes |
| | HMT | No | No | Yes |

sition region for all of the samples, depended on both the presence or absence of normal amylose and the anomalous amylopectin contribution. Only one of the functional responses, WRC, showed contrasting behavior for the native *vs*. HMT starches. WRC for the native starches depended only on the anomalous amylopectin contribution, but WRC for the HMT starches depended only on the presence or absence of normal amylose. Two functional responses, MDSC and enzyme digestibility at 100°C, depended only on the anomalous amylopectin for both native and HMT starches, which reveals the controlling effect of the elevated glass transition temperature of the anomalous amylopectin, due to the long linear branches.

The practical importance of the increased understanding provided by the present study relates to the growing interest in enzyme-resistant starches (RS) and their nutritional benefits [25–27]. The role of anomalous amylopectin contributions in the control of enzyme resistance explains the value of *ae* starches as raw materials used in HMT processes for the commercial production of RS.

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