The impact of single and dual hydrothermal modifications on the molecular structure and physicochemical properties of normal corn starch

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Abstract

Effect of single and dual hydrothermal modifications with annealing (ANN) and heat-moisture treatment (HMT) on molecular structure and physicochemical properties of corn starch was investigated. Normal corn starch was modified by ANN at 70% moisture at 50 °C for 24 h and HMT at 30% moisture at 120 °C for 24 h as well as by the combination of ANN and HMT. The apparent amylose content and swelling factor (SF) decreased on ANN and HMT, but amylose leaching (AML) increased. These changes were more pronounced on dual modification. The crystallinity (determined by X-ray diffraction), the gelatinization enthalpy (determined by differential scanning calorimetry) and ratio of 1047 cm⁻¹/1022 cm⁻¹ (determined by Fourier transform infrared spectroscopy) slightly increased on ANN and decreased on HMT. The ANN and subsequent HMT (ANN-HMT) resulted in the lowest crystallinity, gelatinization enthalpy and ratio of 1047 cm⁻¹/1022 cm⁻¹. The gelatinization temperature range decreased on HMT but increased on ANN. However, the gelatinization range of dually modified starches (ANN-HMT and HMT-ANN) was between ANN starch and HMT starch. Birefringence remained unchanged on ANN but decreased on HMT and dual modifications (ANN-HMT and HMT-ANN). HMT and dual modifications resulted in highly reduced pasting viscosity. ANN and HMT as well as dual modifications increased RDS content and decreased SDS and RS content.

Keywords:
- Corn starch
- Annealing
- Heat-moisture treatment
- Dual modification
- Molecular structure
- Functional properties

1. Introduction

Starch has been used in a wide variety of products, either as a food ingredient or as an industrial material. Total annual world production of starch is approximately 60 million MT [1]. Corn supplies over 80% of the world starch market [2], followed by potato, cassava and wheat. Starch digestibility has been known to vary among different starchy foods, which have been ascribed to various factors, including botanical source [3], food processing [4], particle size [5], amylose/amylopectin ratio [6], type of crystalline polymorphic (A, B or C) form [7], and presence of amylose–lipid complexes [8,9]. For nutritional purposes, starches are classified on the basis of their rate of enzymatic digestion into three categories: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) [10]. RDS is the starch fraction that causes a sudden increase in blood glucose level after ingestion, and SDS is a starch fraction that is digested completely in the small intestine at a lower rate as compared to RDS. RS is the starch portion that cannot be digested in the small intestine, but is fermented in the large intestine.

Native starches usually do not meet industrial needs in which starch should be able to withstand high shear rates and shear forces during processing, low acidity and high and low temperatures. Consequently, starches have been modified physically (heat-moisture treatment, annealing, pre-gelatinization, high pressure treatment) and chemically (cross-linking, substitution, acid hydrolysis, oxidation) in order to extend the range of starch applications in food, textiles, paper and pharmaceuticals [11]. At the present time, there is great interest in the use of hydrothermal treatment such as heat-moisture treatment (HMT) and annealing (ANN) which modify starch structure and properties without destroying its granular structure. Both HMT and ANN involve incubation of starch at a specific temperature and at a certain moisture level during a certain time period. The term HMT is used when low moisture levels (<35%, w/w) are applied, whereas, ANN refers to treatment of starch in excess water (<65%, w/w) or at intermediate water (40–55%, w/w). Both HMT and ANN occur below the onset temperature of gelatinization and have been shown to modify the structural arrangement of starch chains within the amorphous and crystalline domains to different extents [12,13]. ANN has been shown to increase granular stability, crystalline perfection and gelatinization
transition temperatures, and to decrease granular swelling, amylose leaching and the gelatinization temperature range. However, depending on the starch source and ANN conditions, crystallinity, amylose–lipid interactions, and susceptibility towards acid and enzyme hydrolysis have been shown to increase, decrease or remain unchanged on ANN [12–16]. Regardless of starch origin, increase in gelatinization transition temperatures, widening of the gelatinization temperature range, decrease in granular swelling and amylose leaching and an increase in thermal stability have been shown to occur on HMT. However, depending on botanical origin and treatment conditions, changes to the X-ray pattern (B to A + B), formation of amylose–lipid complexes, disruption of crystallinity, and increase or decrease in enzyme susceptibility have been shown to occur on HMT [12,17–20]. Although many studies have investigated the effect of ANN and HMT on starches individually, there is a dearth of information on the impact of dual hydrothermal treatment (ANN followed by HMT [ANN-HMT] and HMT followed by ANN [HMT-ANN]) on starch structure and properties. Stute [21] studied the effect of dual modification (ANN-HMT and HMT-ANN) on the gelatinization transition temperatures, X-ray pattern, pasting properties and sorption isotherm of potato starch (B-type unit cell). The above study showed that structural alterations on ANN are reversible when ANN potato starch is subjected to HMT and also that HMT potato starch can be annealed, since in addition to the structural changes in the elementary cell the interaction between the crystallites and the amorphous parts can still be modified under ANN conditions. However, it is difficult to interpret the data on the above study, due to lack of information on changes to amylopectin chain length distribution, crystallinity, amylose leaching and gelatinization enthalpy. Thus, the objective of this study was to determine to what extent the molecular structure and physico-chemical properties of normal corn starch (A-type unit cell) can be modified when subjected to single (ANN and HMT) and dual (ANN-HMT and HMT-ANN) modification. The results of this study may provide an alternative route for improving the thermal stability, shear stability, freeze–thaw stability and digestibility of corn starch. Research geared to hydrothermal treatment is important, may provide an alternative route for improving the thermal stability, shear stability, freeze–thaw stability and digestibility of corn starch. However, depending on the starch source and ANN conditions, crystallinity, transition temperatures, and to decrease granular swelling, amylose leaching and the gelatinization temperature range. Thus, the objective of this study was to determine to what extent the molecular structure and physico-chemical properties of normal corn starch (A-type unit cell) can be modified when subjected to single (ANN and HMT) and dual (ANN-HMT and HMT-ANN) modification. The results of this study may provide an alternative route for improving the thermal stability, shear stability, freeze–thaw stability and digestibility of corn starch.

2. Materials and methods

2.1. Materials

Normal corn starch (cat. no. S-4126), pancreatin from porcine pancreas (cat. no. P-7545, activity 8×2.1), formation of amylose–lipid complexes, disruption of crystallinity, and increase or decrease in enzyme susceptibility have been shown to occur on HMT. However, depending on botanical origin and treatment conditions, changes to the X-ray pattern (B to A + B), formation of amylose–lipid complexes, disruption of crystallinity, and increase or decrease in enzyme susceptibility have been shown to occur on HMT [12–16]. Although many studies have investigated the effect of ANN and HMT on starches individually, there is a dearth of information on the impact of dual hydrothermal treatment (ANN followed by HMT [ANN-HMT] and HMT followed by ANN [HMT-ANN]) on starch structure and properties. Stute [21] studied the effect of dual modification (ANN-HMT and HMT-ANN) on the gelatinization transition temperatures, X-ray pattern, pasting properties and sorption isotherm of potato starch (B-type unit cell). The above study showed that structural alterations on ANN are reversible when ANN potato starch is subjected to HMT and also that HMT potato starch can be annealed, since in addition to the structural changes in the elementary cell the interaction between the crystallites and the amorphous parts can still be modified under ANN conditions. However, it is difficult to interpret the data on the above study, due to lack of information on changes to amylopectin chain length distribution, crystallinity, amylose leaching and gelatinization enthalpy. Thus, the objective of this study was to determine to what extent the molecular structure and physico-chemical properties of normal corn starch (A-type unit cell) can be modified when subjected to single (ANN and HMT) and dual (ANN-HMT and HMT-ANN) modification. The results of this study may provide an alternative route for improving the thermal stability, shear stability, freeze–thaw stability and digestibility of corn starch. Research geared to hydrothermal treatment is important, may provide an alternative route for improving the thermal stability, shear stability, freeze–thaw stability and digestibility of corn starch. However, depending on the starch source and ANN conditions, crystallinity, transition temperatures, and to decrease granular swelling, amylose leaching and the gelatinization temperature range. Thus, the objective of this study was to determine to what extent the molecular structure and physico-chemical properties of normal corn starch (A-type unit cell) can be modified when subjected to single (ANN and HMT) and dual (ANN-HMT and HMT-ANN) modification. The results of this study may provide an alternative route for improving the thermal stability, shear stability, freeze–thaw stability and digestibility of corn starch. Research geared to hydrothermal treatment is important, may provide an alternative route for improving the thermal stability, shear stability, freeze–thaw stability and digestibility of corn starch. However, depending on the starch source and ANN conditions, crystallinity, transition temperatures, and to decrease granular swelling, amylose leaching and the gelatinization temperature range.
sulfate (DTGS) detector using an attenuated total reflectance (ATR) accessory at a resolution of 4 cm\(^{-1}\) by 128 scans. Spectra were baseline-corrected, and then deconvoluted by drawing a straight line between 1200 and 800 cm\(^{-1}\) (using Win-IR Pro software). A half-band width of 15 cm\(^{-1}\) and a resolution enhancement factor of 1.5 with Bessel apodization were employed. Intensity measurements were performed on the deconvoluted spectra by recording the peak height of the absorbance bands from the baseline.

2.9. Amylopectin chain length distribution

Isoamylase debranching of whole starch accompanied by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was used to determine the branch chain length distribution of the native and modified corn starches [25].

2.10. Differential scanning calorimetry (DSC)

The gelatinization transition temperatures and enthalpy of native and modified starches were determined using a differential scanning calorimeter (2920 Modulated DSC, TA Instruments, New Castle, DE, USA) equipped with a refrigerated cooling system. The starch (12 mg) and distilled water (28 ml) were added to a high-volume pan and immediately sealed. The sealed pans were allowed to stand overnight at room temperature before DSC experiment. The sample pans were heated from 5 to 180 °C at a heating rate of 10 °C/min. An empty pan was used as a reference. The transition temperatures reported are the onset (\(T_o\)), peak (\(T_p\)) and conclusion (\(T_c\)). The enthalpy of gelatinization (\(\Delta H\)) was estimated by integrating the area between the thermogram and a base line under the peak and was expressed in terms of Joules per gram (J/g) of dry starch.

2.11. Pasting properties

Pasting properties of native and modified corn starches (7.0% db, 30 g total weight) were determined using a Rapid Visco-Analyser (RVA-4, Newport Scientific, Warriewood, Australia). The starch slurries were equilibrated at 50 °C for 1 min, heated at 6 °C/min to 95 °C, held at 95 °C for 5 min, cooled at 6 °C/min to 50 °C, and held at 50 °C for 2 min.

2.12. Enzyme hydrolysis

Enzyme hydrolysis of native and modified granular starches was determined following the method described by Englyst et al. [10] with modifications [26]. Starch classifications based on the rate

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Fig. 1. Photomicrographs of native and modified corn starches. (A) Native corn starch, (B) annealed corn starch, (C) heat-moisture treated corn starch, (D) annealed corn starch subjected to heat-moisture treatment, and (E) heat-moisture treated corn starch subjected to annealing.
of hydrolysis were: rapidly digestible starch (RDS, digested within 20 min), slowly digestible starch (SDS, digested between 20 min and 120 min) and resistant starch (RS, undigested after 120 min).

2.13. Statistical analysis

The data reported are the means of duplicate measurements. Statistical analyses were carried out with Duncan’s multiple test \( (P<0.05) \) using SPSS V. 8.2 software (SPSS Institute Inc., Cary, NC).

3. Results and discussion

3.1. Granule morphology

Polarized light micrographs of native, annealed (ANN), heat-moisture treated (HMT), ANN-HMT and HMT-ANN corn starches are presented in Fig. 1. Native corn starch displayed a strong birefringence pattern (Fig. 1A). This is indicative of a great degree of order in the molecular orientation, a characteristic that is independent of crystallinity [27]. Birefringence also reflects the average radial orientation of helical structures [17]. The birefringence at the periphery and at the granule center remained unchanged on ANN (Fig. 1B). However, HMT resulted in the disappearance of birefringence at the periphery and at the granule center in some granules, whereas in others birefringence was retained at the periphery, but not at the granule center (Fig. 1C). Similar observations have been made on HMT (120–130 °C) of potato and corn starches [17,28]. Vermeylen et al. [17] have shown by studies on HMT of potato starch, that the formation of voids at the granule center becomes larger with increase in the temperature of HMT. This suggests that the thermal energy imparted to the starch chains at the granule center increases their mobility resulting in a loss in radial orientation. The retention of birefringence at the granule periphery, suggests that starch chains are probably more compactly packed at the periphery, and may require a higher input of thermal energy for chain mobility. ANN-HMT also resulted in retention of birefringence at the granule periphery and loss of birefringence at the granule center (Fig. 1D). However, loss of birefringence at the granule center was less evident in HMT-ANN corn starch (Fig. 1E). This suggests that increase in molecular order (Fig. 2) that occurs as a result of crystalline perfection on ANN may have compensated for the loss of molecular order that occurs on HMT.

3.2. Apparent amylose content

The apparent amylose content of native and modified starches is shown in Table 1. The amylose content marginally decreased on ANN and HMT. The extent of the decrease in apparent amylose content was 0.2% for annealed (C-ANN) or heat-moisture treated starch (C-HMT). The dually modified starch (ANN-HMT and HMT-ANN) exhibited a much greater decrease in apparent amylose content (0.9% for ANN–HMT and 0.6% for HMT–ANN). Lan et al. [15] and Nakazawa and Wang [29] also observed a decrease in apparent amylose content determined colorimetrically using iodine on ANN. However, the amylose content determined by high performance size exclusion chromatography [15] or complex formation between concanavalin A (Con A) [30] and amylopectin showed no decrease on ANN. Lan et al. [15] claimed that decreased apparent amylose content on ANN could be associated with a decrease in the number of helical turns as a result of a change in amylose conformation and restriction in the ability of amylose to form longer or more ordered helical segments by amylose–amylose (AM–AM) and/or amylose–amylopectin (AM–AMP) interactions formed on ANN, thereby decreasing the color intensity of the amylose–iodide complex. Consequently, the larger decrease in apparent amylose content of dually modified starch when compared to individually treated starch by ANN and HMT could be explained by a combination effect between ANN and HMT in decreasing the ability of amylose to form a complex with iodine.

3.3. Branch chain length distribution of amylopectin

The branch chain length distribution of native, ANN, HMT, ANN-HMT and HMT-ANN corn starches is presented in Table 1. According to the classification proposed by Hanashiro et al. [31], the A, B1, B2 and B3 amylopectin branch chains correspond to chains with a degree of polymerization (DP) of 6–12, 13–24, 25–36 and ≥37, respectively. The chain length distribution remained unchanged on ANN (Table 2). This suggests that the crystalline structure of amylpectin was not disrupted by the thermal energy or by double helical realignment during ANN. A similar finding was reported by Kohyama and Sasaki [30] and by Lan et al. [15] for potato, corn, and wheat starches on ANN. However, HMT, ANN-HMT and

![Image](350x632 to 589x784)

**Fig. 2.** X-ray diffraction patterns and relative crystallinity (in parenthesis) of native and modified corn starches. C, native corn starch; C-ANN, annealed corn starch; C-HMT, heat-moisture treated corn starch; C-ANN-HMT, annealed corn starch subjected to heat-moisture treatment; C-HMT-ANN, heat-moisture treated corn starch subjected to annealing.

**Table 1**

<table>
<thead>
<tr>
<th>Starch</th>
<th>Apparent amylose content (%)</th>
<th>Average chain length</th>
<th>Distribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>DP6–12</td>
</tr>
<tr>
<td>C</td>
<td>29.6 ± 0.2a</td>
<td>19.8 ± 0.1a</td>
<td>24.2 ± 0.5b</td>
</tr>
<tr>
<td>C-ANN</td>
<td>29.4 ± 0.4ab</td>
<td>19.7 ± 0.1a</td>
<td>24.3 ± 0.1b</td>
</tr>
<tr>
<td>C-HMT</td>
<td>29.4 ± 0.1ab</td>
<td>19.5 ± 0.1b</td>
<td>24.9 ± 0.3a</td>
</tr>
<tr>
<td>C-ANN-HMT</td>
<td>28.7 ± 0.5c</td>
<td>19.5 ± 0.1b</td>
<td>24.9 ± 0.2a</td>
</tr>
<tr>
<td>C-HMT-ANN</td>
<td>29.0 ± 0.3bc</td>
<td>19.5 ± 0.1b</td>
<td></td>
</tr>
</tbody>
</table>

C, native corn starch; C-ANN, annealed corn starch; C-HMT, heat-moisture treated corn starch; C-ANN-HMT, annealed corn starch subjected to heat-moisture treatment; C-HMT-ANN, heat-moisture treated corn starch subjected to annealing.

* Values within each column with different letters (a–c) are significantly different \( (P<0.05) \).

b Degree of polymerization.
The X-ray diffraction patterns and RC of native, ANN, HMT, ANN-HMT and HMT-ANN corn starches are presented in Fig. 2. All starches displayed the characteristic ‘A’-type crystalline pattern. RC remained unchanged in ANN starch, whereas it decreased in the other starches (HMT~ANN-HMT > HMT-ANN). Starch crystallinity has been shown to be influenced by: (1) amylopectin content, (2) average amylopectin chain length, (3) orientation of the double helices (within the crystallites) to the X-ray beam, and (4) crystallite size [15,23]. Decrease in RC on HMT could be attributed to disruption of amylopectin crystallites, which was evidenced by a decrease in gelatinization enthalpy (Table 3) since gelatinization enthalpy reflects the overall crystallinity (quality and amount of starch crystallites) of amylopectin [23], shorter average amyllopectin chain length (Table 2), and lower proportion of longer chains (DP ≥37, Table 2). The reduction in RC was lower in HMT-ANN than in ANN-HMT corn starch, which was in agreement with trend of gelatinization enthalpy (Table 3), since double helices disrupted on HMT may have become better aligned and/or may have formed larger crystals on ANN.

3.5. ATR-FI-IR spectroscopy

The ATR-FI-IR spectral data on native and modified starches are presented in Fig. 3. This technique has been suggested to be sensitive to changes in structure on a molecular level (short-range order), such as starch chain conformation, helicity, crystallinity as well as water content [33], as opposed to long-range order (detected by X-ray diffraction) which is related to the packing of double helices into ordered crystalline arrays. Furthermore, the ATR-FI-IR technique yields information on the structural organization of starch chains near the granule surface, since the IR beam penetrates only into a depth of 2 μm in the granule [34]. The IR bands at 1047 and 1022 cm⁻¹ have been shown to be associated with ordered and amorphous structures of starch, respectively [33]. Studies on potato starch have shown that the band at 1022 cm⁻¹ increases with decreasing crystallinity, whereas the band at 1047 cm⁻¹ increases with increase in crystallinity [33]. Thus, the ratio of the heights of

![Fig. 3. Ratio between 1047 and 1022 cm⁻¹ of FT-IR spectra of native and modified corn starches. Values on the bars with different letters are significantly different (P<0.05). C, native corn starch; C-ANN, annealed corn starch; C-HMT, heat-moisture treated corn starch; C-ANN-HMT, annealed corn starch subjected to heat-moisture treatment; C-HMT-ANN, heat-moisture treated corn starch subjected to annealing.](image)
the bands at 1047 and 1022 cm\(^{-1}\) expresses the amount of ordered starch to amorphous starch\[33–35\]. The results (Fig. 3) showed that the 1047 cm\(^{-1}\)/1022 cm\(^{-1}\) ratio increased on ANN, but decreased on HMT. The increased ratio suggests that the moderate thermal energy and the high moisture content prevailing during ANN cause more efficient packing of double helices within the crystalline lamella. The decrease in the ratio on HMT could be attributed to dissociation and unraveling of double helices forming the crystalline array. The decrease in the ratio is more pronounced in ANN-HMT than in HMT starch, since in the former, the decreased ratio also reflects disruption of those crystallites that were perfected on ANN. The increase in the ratio, when ANN follows HMT (HMT-ANN) reflects perfection of crystallites that were disrupted on HMT.

3.6. Swelling factor (SF) and amylease leaching (AML)

SF and AML in the temperature range 60–90 °C are presented in Table 2. ANN and HMT decreased SF, whereas AML increased on ANN and HMT. The extents of the above changes were more pronounced on HMT. For instance at 90 °C, SF decreased by 15.9% and 30.3%, and AML increased by 0.3% and 3.9% on ANN and HMT, respectively. Many researchers have shown a reduction in granular swelling\[16,20,36–39\] and amylease leaching\[16,20,36,40\] on ANN and HMT. However, an increase in AML has been reported on ANN in wheat\[39\] and in certain cultivars of barley starches\[36\]. Crystalline perfection, interaction involving amyleose chains and V-amylose lipid complex formation have been shown to be factors influencing the reduction in SF on ANN\[16,36,41,42\]. However, in this study, increased AML (Table 2) and the unchanged X-ray pattern (Fig. 2) on ANN suggest that the main causative factor influencing the decreased SF on ANN is crystalline perfection (Fig. 3 and Table 3). Decreased SF on HMT has been attributed to the interplay of: (1) crystallite disruption, (2) increased crystallinity, (3) amyleose–lipid interactions and (4) interactions involving amyleose–amylose and/or amyleose–amylopectin chains\[20,43\]. In this study, the increase in proportion of short A chains and the reduction in the proportion of long B3 chains (DP ≥ 37) of amylopectin suggest that the main causative factor influencing decreased SF on HMT is crystallite disruption. This seems plausible, since X-ray pattern showed no evidence of amyleose–lipid complex formation (Fig. 2), and AML data (Table 2) provided no evidence of interactions involving amyleose chains on HMT. The increase in AML on ANN suggests that the thermal energy imparted to loosely packed amyleose chains may have increased their mobility, thereby facilitating their diffusion out of the granule. However, in HMT starch, increased AML reflects leaching of both loosely packed amyleose chains and short chains (capable of forming complexes with iodine) resulting from the disrupted crystallites. The decrease in SF and increase in AML were further enhanced (Table 2) when HMT followed ANN (ANN-HMT). For instance at 90 °C, SF decreased from 21.1 to 15.6 and AML increased from 17.8% to 22.4% (Table 2). However, when HMT followed ANN (HMT-ANN), the decrease in SF and increase in AML were only marginal (Table 2). The results suggest that when ANN starch is subjected to HMT (ANN-HMT) both perfected crystallites as well as the imperfect crystallites would disrupt resulting in further decreases in SF and increased AML. The marginal changes in SF and AML, when HMT follows ANN (ANN-HMT), suggest that the extent of crystallite perfection on ANN may not have been large enough to make any significant impact on SF or AML.

3.7. Gelatinization

The gelatinization parameters (\(T_0, T_p, T_c\), and \(\Delta H\)) of native and modified corn starches are presented in Table 3. ANN increased \(T_0, T_p,\) and \(T_c\) and decreased \(T_c - T_p\) (gelatination temperature range). However, the enthalpy of gelatinization (\(\Delta H\)) remained unchanged on ANN. HMT increased \(T_0, T_p, T_c\) and \(T_c - T_p\) but decreased \(\Delta H\) (Table 3). Increased \(T_0, T_p,\) and \(T_c\) on ANN and HMT have been partly attributed to interaction between amyleose–amylose (AM–AM) and/or amyleose–amylopectin chains (AM–AMP), and to the formation of additional complexes between amyleose and starch lipids\[15,16,20,36\]. Evidence for interaction between AM–AM and AM–AMP chains have come from the observation that AML decreases during both ANN and HMT. However, in this study, AML increased during both ANN and HMT (Table 2). As discussed earlier, X-ray diffraction (Fig. 2) showed no evidence of amyleose–lipid interactions during ANN and HMT. Consequently, the increase in \(T_0, T_p,\) and \(T_c\) on ANN could be attributed solely to crystalline perfection\[12,15,16,36,41\], whereas the above increase on HMT suggests that crystallites disrupted on HMT may have aggregated to form larger crystallites. The decrease in relative crystallinity on HMT (Fig. 2) suggests that these large crystals are probably not arranged in a crystalline array. The decrease in \(T_c - T_p\) on ANN could be attributed to crystalline perfection, whereas the increase in \(T_c - T_p\) on HMT suggests melting of crystallites of different stability. The constancy of \(\Delta H\) pre and post ANN suggests that no new double helices were formed on ANN. The decrease in \(\Delta H\) on HMT (Table 3) suggests that the high temperature (120 °C) prevailing during HMT may have increased the mobility of double helices forming the crystalline structure, resulting in disruption of hydrogen bonds among double helices and between adjacent double helices. This seems plausible, since the crystallinity was slightly decreased (Fig. 2), the ratio of 1047 cm\(^{-1}\)/1022 cm\(^{-1}\) was decreased (Fig. 3), and the intensity of birefringence in some starch granules was decreased (Fig. 1) after HMT of corn starch. Consequently, the number of double helices that unravel and melt during gelatinization of HMT starch would be lower than its native counterpart.

Increase in \(T_0, T_p,\) and \(T_c\) was observed in ANN-HMT starch (Table 3). However, the extent of this increase was lower than in the ANN starch for \(T_0\) and \(T_p,\) but higher for \(T_c.\) Furthermore, \(T_c - T_p\) and \(\Delta H\) of ANN-HMT starch were wider and lower, respectively, than in the ANN starch. In addition, among the native and modified starches, ANN-HMT starch showed the lowest gelatinization enthalpy (Table 3), crystallinity (Fig. 2), and ratio of 1047 and 1022 cm\(^{-1}\) (Fig. 3). This suggests that HMT of ANN starch may promote disruption of some of the crystallites that were perfected on ANN when compared to HMT of native starch. In HMT-ANN starch, the increase in \(T_0, T_p, T_c\) was higher than in HMT starch, whereas \(T_c - T_p\) was lower and \(\Delta H\) higher (Table 3). This indicates that crystallites disrupted on HMT may have become better aligned on ANN. The dually modified starches (ANN-HMT and HMT-ANN) displayed different gelatinization parameters (Table 3). HMT-ANN had higher gelatinization temperatures and enthalpy than ANN-HMT. This suggests that the crystalline structure of dually modified starch could be influenced by the treatment sequence.

3.8. Pasting properties

The RVA pasting curves of native and modified corn starches are presented in Fig. 4. The RVA parameters (peak viscosity [PV], breakdown, setback, final viscosity [FV], pasting temperature [PT], and the time taken to reach peak viscosity [TPV]) are listed in Table 4. PV, breakdown, setback and FV decreased on ANN (Table 4). However, PT and TPV remained unchanged (Fig. 4 and Table 4). A similar finding has been reported for wheat\[15,40,46\]. The decrease in PV and the increase in thermal stability on ANN reflect the decrease in SF (Table 2). Theoretically, an increase in AML (Table 2) on ANN should have
increased PV. This result suggests that the decrease in SF negates the influence of increased AML on PV. The extent of setback has been shown to be influenced by the extent of AML, granule size, amylopectin chain length, and presence of unfragmented rigid swollen granules embedded in the leached amylose network [15,16,40].

As shown in Table 1, the amylopectin chain length distribution remained unchanged on ANN, and the increase in AML on ANN was only marginal (Table 2). Therefore, the decrease in setback mainly reflects the presence of some intact granules that may have survived fragmentation. This seems plausible since granule stability increases on ANN. Intact granules may have hindered rapid association between leached amylose chains. PV, breakdown, setback and FV also decreased on HMT (Fig. 4). The extent of these decreases was more pronounced than on ANN (Table 4). This could be attributed to a greater decrease in granular swelling and to a decrease in the proportions of long amylopectin chains (DP > 37) on HMT (Table 1). PT and TPV also remained unchanged on HMT (Table 4). Significant decrease in pasting viscosity has been reported for maize [19,47], rice [48], potato, oat, lentil and yam [20] starches. In ANN-HMT starch, the extent of decrease in PV and setback and the increase in thermal stability was higher than in either ANN or HMT starch (Table 4). This could be attributed to the greater reduction in granular swelling than the ANN and HMT starches (Table 2). There was no significant difference in PV and setback between ANN-HMT and HMT-ANN starches (Table 4). However, the thermal stability of HMT-ANN was slightly higher than that of ANN-HMT starch.

3.9. Enzyme hydrolysis

The amounts of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) in native and modified corn starches are presented in Table 5. ANN increased RDS levels by 3.5% and decreased SDS and RS levels by 2.1% and 1.4%, respectively. Whereas, HMT increased RDS levels by 10.8% and decreased SDS and RS levels by 8.0% and 2.8% respectively. HMT of the ANN starch (ANN-HMT) resulted in a further increase in RDS (6.2%), and decreases of 5.1% and 1.0% in SDS and RS levels, respectively. ANN of the HMT starch (HMT-ANN) decreased RDS by 5.1%, and increased SDS and RS levels by 2.3% and 2.8%, respectively. It is difficult to find a consensus on the impact of ANN and HMT from previous other studies on enzyme susceptibility due to differences in enzyme source and concentration, time of hydrolysis, number of annealing steps, time and temperature of HMT and starch source. ANN has been shown either to increase or decrease enzyme susceptibility. Decreased hydrolysis on ANN has been attributed to the interplay of the following factors: (1) crystalline perfection, (2) interaction between AM–AM and or AM/AMP chains and (3) amylose–lipid complex formation [16,42]. Whereas, an increase in hydrolysis of ANN has been attributed to an increase in granule porosity [49]. In this study, there was no evidence of AM–AM, AM–AMP (Table 2) and amylose–lipid interactions on ANN (Fig. 2). This suggests that the increase in RDS and the decreases in SDS and RS levels could only be attributed to an increase in granule porosity (facilitates the entry of the enzyme into the granule interior) which probably negates the impact of crystalline perfection on hydrolysis. The effect of HMT on enzyme hydrolysis has been shown to be influenced by: (1) starch source, (2) moisture content during HMT, (3) temperature and duration of HMT, (4) amylose–lipid interactions and (5) AM–AM and/or AM–AMP interaction [18,20,50]. The increase in RDS and decreases in SDS and RS levels on HMT suggest that crystallite disruption on HMT, which was evidenced by decreased crystallinity (Fig. 2) and gelatinization enthalpy (Table 3), may have exposed the (1–4) glycosidic that were previously buried within the starch crystallites, and hence not readily accessible to enzyme attack in native starch. As discussed earlier, the increase or decrease in RDS levels on ANN reflects the interplay between crystalline perfection (decreases hydrolysis) and increase in granule porosity (increases hydrolysis). Further increases in the RDS level and decreases in SDS and RS levels in ANN-HMT starch (Table 5) are indicative of disruption of those crystallites that were perfectly on ANN. Whereas the decrease in the RDS level and increases in SDS and RS levels in HMT-ANN starch (Table 5) reflect perfection of disrupted crystallites.

Table 4
Pasting characteristics of native and modified corn starches.

<table>
<thead>
<tr>
<th>Starch</th>
<th>PV (cp)</th>
<th>Breakdown (cp)</th>
<th>Setback (cp)</th>
<th>FV (cp)</th>
<th>PT (°C)</th>
<th>TPV (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1117 ± 30a</td>
<td>389 ± 7a</td>
<td>419 ± 24a</td>
<td>1147 ± 15a</td>
<td>84.7 ± 0.1a</td>
<td>8.9 ± 0.1a</td>
</tr>
<tr>
<td>C-ANN</td>
<td>994 ± 27b</td>
<td>304 ± 6b</td>
<td>315 ± 17b</td>
<td>1005 ± 38b</td>
<td>84.7 ± 0.1a</td>
<td>8.9 ± 0.0a</td>
</tr>
<tr>
<td>C-HMT</td>
<td>319 ± 13c</td>
<td>161 ± 0c</td>
<td>111 ± 3c</td>
<td>269 ± 26c</td>
<td>85.0 ± 0.2a</td>
<td>8.7 ± 0.1ab</td>
</tr>
<tr>
<td>C-ANN-HMT</td>
<td>241 ± 6d</td>
<td>144 ± 6d</td>
<td>70 ± 3d</td>
<td>169 ± 2d</td>
<td>84.7 ± 0.0a</td>
<td>8.6 ± 0.0b</td>
</tr>
<tr>
<td>C-HMT-ANN</td>
<td>224 ± 18d</td>
<td>112 ± 4e</td>
<td>78 ± 6d</td>
<td>190 ± 21d</td>
<td>84.8 ± 0.1a</td>
<td>8.8 ± 0.0ab</td>
</tr>
</tbody>
</table>

PV, peak viscosity; FV, final viscosity; PT, pasting temperature; TPV, time to reach peak viscosity; C, native corn starch; C-ANN, annealed corn starch; C-HMT, heat-moisture treated corn starch; C-ANN-HMT, annealed corn starch subjected to heat-moisture treatment; C-HMT-ANN, heat-moisture treated corn starch subjected to annealing.

* Values within each column with different letters (a–e) are significantly different (P < 0.05).

Table 5
Starch nutritional fractions of native and modified corn starches.

<table>
<thead>
<tr>
<th>Starch</th>
<th>RDS (%)</th>
<th>SDS (%)</th>
<th>RS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>23.4 ± 0.2d</td>
<td>56.9 ± 1.1a</td>
<td>19.7 ± 1.2a</td>
</tr>
<tr>
<td>C-ANN</td>
<td>26.9 ± 0.8c</td>
<td>54.3 ± 1.0b</td>
<td>18.3 ± 0.6b</td>
</tr>
<tr>
<td>C-HMT</td>
<td>34.2 ± 0.9a</td>
<td>48.9 ± 0.6d</td>
<td>16.9 ± 0.4c</td>
</tr>
<tr>
<td>C-ANN-HMT</td>
<td>33.1 ± 1.0a</td>
<td>49.7 ± 0.6d</td>
<td>17.3 ± 0.2bc</td>
</tr>
<tr>
<td>C-HMT-ANN</td>
<td>29.1 ± 0.9b</td>
<td>51.2 ± 0.5c</td>
<td>19.7 ± 1.0a</td>
</tr>
</tbody>
</table>

RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch; C, native corn starch; C-ANN, annealed corn starch; C-HMT, heat-moisture treated corn starch; C-ANN-HMT, annealed corn starch subjected to heat-moisture treatment; C-HMT-ANN, heat-moisture treated corn starch subjected to annealing.

* Values within each column with different letters (a–d) are significantly different (P < 0.05).
4. Conclusions

Molecular structure and physicochemical properties of corn starch could be modified to varying extents by hydrothermal treatment. The extent of this modification was most pronounced in the HMT and ANN-HMT starches. This suggests that crystallite disruption in HMT starch has a greater impact on starch properties than crystalline perfection in ANN starch. The results showed that HMT, ANN-HMT and HMT-ANN were more effective than ANN in increasing thermal stability and decreasing the extent of setback. None of the modification techniques were effective in increasing the resistant starch content. Research is underway to understand the impact of dual modification on starches of different crystal structures (A, B, C) and on starches of varying amylose contents.

Acknowledgements

The authors thank Mrs. Elizabeth Donner for her technical support. Financial support for this study through The Saskatchewan Pulse Crop Development Board and Agriculture and Agri-Food Canada MII program is gratefully acknowledged.

References