



Effects of annealing on gelatinization and microstructures of corn starches with different amylose/amylopectin ratios

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ABSTRACT

Corn starches with different amylose/amylopectin ratios (waxy 0/100, normal corn 23/77, Gelose 50 50/50, Gelose 80 80/20) were annealed at below their gelatinization temperatures in excess water. The effects of annealing on the gelatinization and microstructures of the starches were studied using DSC, XRD and a microscope equipped with both normal and polarized light. In addition, a high-pressure DSC pan was used to study the effects of high-temperature annealing on the multiphase transitions of starches with different water contents. The granular size of the starches increased after the annealing process, but the size variation rates were different, with higher amylopectin contents resulting in a higher diameter growth rates and final accretion ratios. DSC results showed that annealing increased the gelatinization enthalpy of the amylose-rich starches. The increased enthalpy was mainly attributed to endotherm G – there were no significant changes to endotherms M1, M2 or Z – indicating that annealing mainly affected the helical length of shorter or sub-optimal amylopectins, in particular the amylopectin in amylose-rich starches. The XRD traces of all starches after annealing remained unchanged.

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1. Introduction

In the study of conventional polymers, the term “annealing” is used to describe the process of heating a polymer to temperatures below its melting point, to induce a larger crystal area, the perfection of crystals, or a change to a more stable crystal structure (Wunderlich, 1976). In the study of starch, however, annealing is defined as the physical reorganization of starch granules in water at temperatures above the glass transition but below the gelatinization temperatures (Jacobs, Eerlingen, Rouseu, Colonna, & Delcour, 1998a; Knutson, 1990; Krueger, Knutson, Inglett, & Walker, 1987a; Larsson & Eliasson, 1991; Tester, Debon, & Karkalas, 1998). This definition implies that annealing only modifies the physicochemical properties of a starch without destroying its granular structure (Jacobs, Eerlingen, & Delcour, 1996; Jacobs et al., 1998b), and therefore no changes are observed in XRD traces of annealed starches (Gough & Pybus, 1971; Stute, 1992). However, it should be noted that annealing differs from heat/moisture treatments. In heat/moisture treatments, molecular mobility at high temperatures is controlled by limiting the amount of water (<35% w/w) and hence gelatinization (Jacobs et al., 1998a; Tester & Debon, 2000). The low water levels lead to high temperatures to induce physical reorganization within

granules (Franco, Ciacco, & Tavares, 1995; Stute, 1992; Tester, Debon, & Somerville, 2000). The effects of annealing and heat/moisture treatments can be distinguished by wide angle X-ray scattering (WAXS) and differential scanning calorimeter (DSC) results, as shown by Jacobs and Delcour (1998).

Gough and Pybus (1971) were probably the first to study the effects of annealing on the starch gelatinization. Using a Kofler hot-stage microscope, they studied wheat starch kept in water at 50 °C for three days, and observed higher gelatinization temperatures with much narrower ranges, compared to those of native (i.e. unannealed) starches. These results have been more recently supported by experimental DSC studies (Jacobs et al., 1998b; Tester et al., 1998, 2000; Yost & Hoseney, 1986).

Many studies have involved starches from various botanical sources, such as maize (Knutson, 1990; Krueger, Walker, Knutson, & Inglett, 1987b; Krueger et al., 1987a; Larsson & Eliasson, 1991; Tester et al., 2000), potato (Jacobs et al., 1998b; Stute, 1992), wheat (Kohyama & Sasaki, 2006; Tester et al., 1998; Yost & Hoseney, 1986), rice (Lin, Wang, & Chang, 2008; Seow & Teo, 1993), sago (Wang, Powell, & Oates, 1997) and barley (Andreev, Kalistratova, Wasserman, & Yuryev, 1999; Kiseleva et al., 2004). In general, results have shown that annealing raises the initial temperature of gelatinization and narrows the gelatinization temperature range. Such changes are typical in all starches irrespective of their molecular structure or amylose content. However, results centered on gelatinization enthalpies post-annealing – whether constant or in-

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creased – are sometimes contradictory. For example, Krueger et al. (1987) and some others (Kiseleva et al., 2004; Knutson, 1990) observed enhanced enthalpies for corn starches with normal and mutant genotypes, while Larsson and Eliasson (1991) and Tester and Debon (2000) reported that the enthalpy of normal maize starch was the same before and after annealing.

On the other hand, multiphase transitions in corn starch have been detected by DSC (Liu, Yu, Chen, & Li, 2007; Liu, Yu, Xie, & Chen, 2006; Russell, 1987a; Shogren, 1992; Yu & Christie, 2001). These transitions (e.g. endotherms G, M1, M2 and Z) represent the order–disorder in various microstructures of starch granules during gelatinization.

To the best of our knowledge, there is no other systematic study reported the annealing effect on the multiphase transitions of corn starches with amylose contents ranging from 0% to 80% (w/w). The aim of the current work is to carry out a comparative study on the properties of corn starches containing different ratios of amylose to amylopectin in the native and annealed states using DSC, XRD and microscope observations. The results will be used to study the mechanism of starch gelatinization and phase transitions.

2. Experimental work

2.1. Materials and annealing treatment

Corn starches with different amylose/amylopectin ratios (waxy 0/100, corn 23/77, G50 50/50, G80 80/20) were used in the experimental work. The corn starch family provided both A-type (waxy and normal corn) and B-type (G50 and G80) crystal structures. All starches are commercially available and were kindly supplied by Penford (Australia). An infrared heating balance (Model DHS-20) was used to measure the moisture contents of samples during heating to 110 °C for 20 min.

Starch suspensions (1:10 w/w) were heated in a sealed container in a water bath kept at a constant temperature of either 30 or 50 °C. The temperature of 30 °C was chosen as it is close to that of the experimental environmental; 50 °C was chosen as it is just below the gelatinization temperatures of the starches used, and it has been widely used in previous studies of the effects of annealing (Karlsson & Eliasson, 2003; Knutson, 1990; Kohyama & Sasaki, 2006; Larsson & Eliasson, 1991; Waduge, Hoover, Vasanathan, Gao, & Li, 2006). After an incubation period of 72 h, the suspensions were centrifuged and the precipitates were dried over diphosphorus pentoxide. Annealed starches were stored for 10 days in a desiccator containing saturated magnesium nitrate, as described by Kohyama and Sasaki (2006).

2.2. Differential scanning calorimeter (DSC)

A Perkin-Elmer Diamond-I DSC with an internal coolant (Inter-cooler 1P) and nitrogen purge gas was used in the experimental work. The melting point and enthalpies of indium were used for temperature and heat capacity calibration. The transition temperatures (i.e. onset T_o , peak T_p and conclusion T_c) and enthalpic change (ΔH) were measured during heating. The gelatinization enthalpies were calculated individually and through summarization of all the gelatinization endotherms based on dry starch. A high-pressure DSC pan was used to study the effects of high-temperature annealing on the multiphase transitions in starches with intermediate water content. Since some gelatinization endotherms overlapped under measurement conditions, a mathematical curve-fitting technique was used to separate the peaks. The conventional mathematical deconvolution (Origin 7.0 software) was performed to fit peaks (Liu et al., 2006). The slow heating

rate of 5 °C/min was used to minimize any temperature lag due to the large mass of the steel pan. DSC measurements were performed in triplicate, and results were presented as the mean. A *t*-test was used to assess whether the data of two groups (native and annealed samples) were statistically different from each other.

Each sample was prepared by premixing a (native or annealed) starch with additional water in a glass vial that had been pre-weighed with its cover. Starch was placed into the vial, which was then weighed again to calculate the mass of starch. The desired volume of distilled water was added using a syringe and was mixed well with the starch using a small spatula. The vial was then sealed and weighed again to calculate the water content. To identify homogeneous samples, the mixed materials were equilibrated in the vial for 24 h and viable samples were then transferred into the high-pressure stainless steel pan (PE No. B0182901).

2.3. Light microscopy with hot stage

A polarization microscope (Axioskop 40 Pol, ZEISS) equipped with a 35 mm SLA camera and a hot-stage (CI94, Linkam Scientific Instruments Ltd.) thermosystem was used to characterize the starches with respect to the appearance, shape and size of granules. The magnification used throughout the work was 500× (50 × 10).

Starch granular was prepared by dispersing ~10 mg starch in 1 mL of distilled water in a glass vial. A drop of starch suspension was transferred onto a slide, covered with a slipcover, and then sealed with silicon glue to prevent water loss during heating. Each specimen was mounted on a hot stage and subsequently heated from 30 to 100 °C at a constant rate of 2 °C/min. Both normal and polarized light were used to investigate the phase transition of the starches. The camera interval timer was set as 30 s so that an image was captured at each 1 °C temperature increase.

The Gun Image Manipulation Program was used to measure the diameter variations of starch granules during heating, or isotherms at a certain temperature. The diameter of a granule was calculated using a sphere equal in area to the granule. The accretion ratio (*AR*) of starch swelling was calculated by $AR = (D_t - D_0)/D_0$, where D_0 and D_t represented the initial diameter of a starch granule and its diameter at a specific time, respectively.

2.4. X-ray diffractometry (XRD)

XRD traces of native and annealed starch granules were detected using a Bruker D8 Diffractometer operating at 40 kV, 40 mA, Cu K α radiation monochromatized with a graphite sample monochromator. The crystallinity of the starch granules was estimated from the summarized areas of crystalline peaks.

3. Results and discussion

Figs. 1 and 2 show the gelatinization endotherms of the various native and annealed corn starches in excess water (about 75%). It can be seen that all samples annealed at 30 °C showed a similar thermal behavior as their corresponding native samples, indicating that this temperature was not high enough to affect the microstructures and gelatinization behavior of the samples. Table 1 presents detail of the temperatures and enthalpies measured during the experimental work, there were no discernible difference in the samples after annealing at 30 °C. However, endotherm G for all samples shifted to higher temperature and became narrower after annealing at 50 °C.

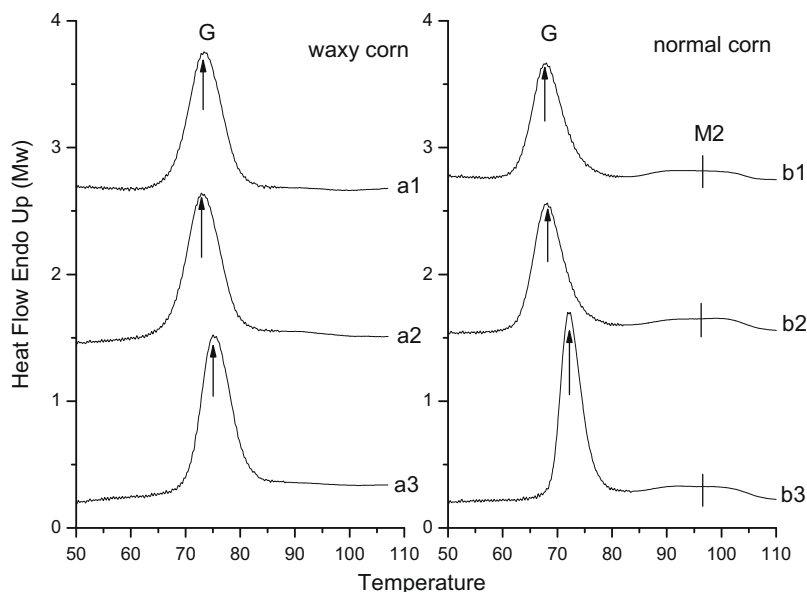


Fig. 1. Gelatinization endotherms of native and annealed waxy (left) and normal corn (right) starches in excess water: (a1) native waxy; (a2) waxy annealed at 30 °C; (a3) waxy annealed at 50 °C; (b1) native normal corn; (b2) normal corn annealed at 30 °C; and (b3) normal corn annealed at 50 °C.

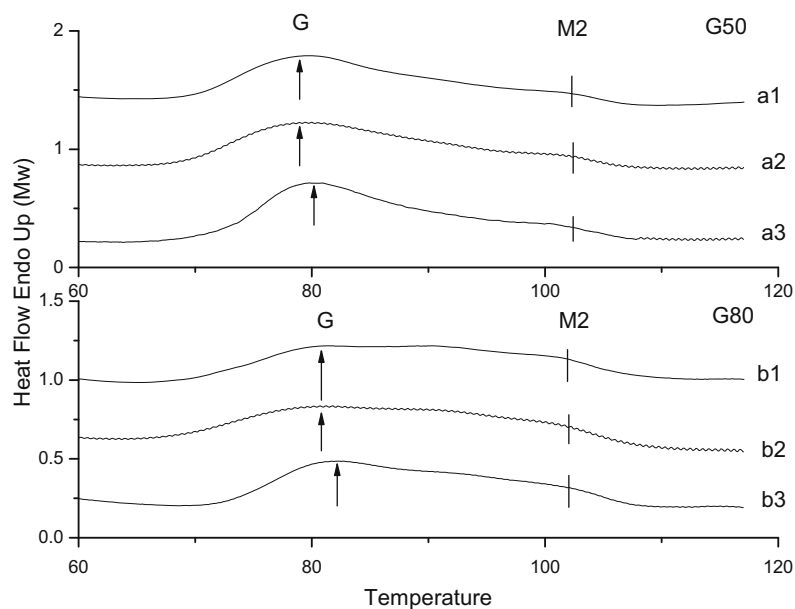


Fig. 2. Gelatinization endotherms of native and annealed high-amylose G50 (top) and G80 (bottom) corn starches in excess water: (a1) native G50; (a2) G50 annealed at 30 °C; (a3) G50 annealed at 50 °C; (b1) native G80; (b2) G80 annealed at 30 °C; and (b3) G80 annealed at 50 °C.

Table 1

Endothermic temperatures and enthalpies of various native and annealed corn starches with excess water.

Sample ^a	Endotherm G					Endotherm M2					Total ΔH (J/g)
	T_o (°C)	T_p (°C)	T_c (°C)	ΔT ($T_c - T_o$)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔT ($T_c - T_o$)	ΔH (J/g)	
Waxy (N)	67.0 ± 0.2	73.2 ± 0.3	79.7 ± 0.3	12.7 ± 0.2	15.18 ± 0.32						15.18 ± 0.32
Waxy (30)	66.7 ± 0.3	72.9 ± 0.3	79.5 ± 0.2	12.8 ± 0.2	15.36 ± 0.41						15.36 ± 0.41
Waxy (50)	69.6 ± 0.4	75.1 ± 0.2	79.9 ± 0.6	10.3 ± 0.1	15.51 ± 0.33						15.51 ± 0.33
Corn (N)	62.6 ± 0.4	67.7 ± 0.2	74.2 ± 0.2	11.6 ± 0.3	13.01 ± 0.35	84.9 ± 0.5	99.1 ± 0.4	105.9 ± 0.2	20.9 ± 0.3	2.01 ± 0.06	15.01 ± 0.27
Corn (30)	62.5 ± 0.2	67.9 ± 0.2	74.4 ± 0.2	11.9 ± 0.2	13.06 ± 0.06	85.5 ± 0.2	99.3 ± 0.3	106.1 ± 0.4	20.6 ± 0.3	2.00 ± 0.09	15.18 ± 0.31
Corn (50)	68.1 ± 0.4	71.9 ± 0.3	74.6 ± 0.4	6.5 ± 0.5	13.14 ± 0.27	85.2 ± 0.4	99.1 ± 0.4	106.5 ± 0.5	21.0 ± 0.3	2.05 ± 0.11	15.29 ± 0.43
G50 (N)	70.0 ± 0.3	79.6 ± 0.7	90.7 ± 0.5	20.7 ± 0.6	11.89 ± 0.52	88.2 ± 0.8	98.6 ± 0.6	109.1 ± 0.4	20.9 ± 1.1	3.09 ± 0.37	14.98 ± 0.65
G50 (30)	69.7 ± 0.6	79.4 ± 0.9	91.2 ± 0.8	20.5 ± 0.9	11.78 ± 0.36	87.6 ± 0.7	97.9 ± 1.0	108.9 ± 0.9	21.3 ± 0.5	3.29 ± 0.24	15.07 ± 0.74
G50 (50)	72.6 ± 0.5	81.2 ± 0.6	90.7 ± 0.7	18.1 ± 0.8	14.82 ± 0.57	88.9 ± 0.8	99.1 ± 0.9	110.3 ± 1.2	21.4 ± 0.7	3.21 ± 0.42	18.03 ± 0.78
G80 (N)	69.4 ± 0.5	82.4 ± 0.4	94.1 ± 0.8	24.7 ± 1.1	7.61 ± 0.51	91.6 ± 0.6	101.9 ± 0.6	110.2 ± 0.8	18.6 ± 0.8	4.47 ± 0.61	12.08 ± 0.95
G80 (30)	69.1 ± 0.7	82.1 ± 0.7	94.5 ± 0.6	25.4 ± 1.2	7.49 ± 0.64	91.0 ± 1.4	102.7 ± 0.5	109.7 ± 0.7	18.7 ± 0.6	4.34 ± 0.47	11.83 ± 0.66
G80 (50)	71.7 ± 0.4	83.4 ± 0.3	94.0 ± 0.9	22.3 ± 1.1	9.87 ± 0.47	91.2 ± 0.8	102.4 ± 1.5	109.1 ± 0.9	17.9 ± 1.2	4.52 ± 0.59	14.39 ± 0.71

^a (N) = native (unannealed) samples; (30) = samples annealed at 30 °C; (50) = samples annealed at 50 °C.

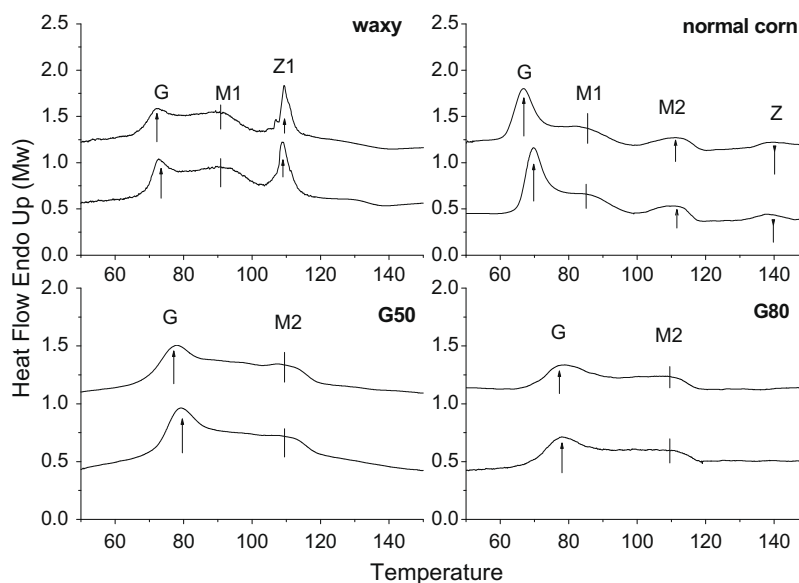


Fig. 3. Gelatinization endotherms of all native (top line) and annealed corn starches (bottom line) at 50 °C with intermediate water content.

As shown by the results of endotherm G in Table 1, the amylopectin-rich (waxy and normal corn) starches displayed increasing T_o and T_p (examined by t -test; $p < 0.001$), constant T_c ($p > 0.05$) and decreasing ΔT after being annealed at 50 °C. For the waxy and normal corn starches, respectively, T_o was increased by about 2.6 and 5.5 °C, while ΔT was decreased by about 2.4 and 5.1 °C. As observed in previous studies (Jacobs et al., 1998b; Knutson, 1990; Kohyama & Sasaki, 2006; Larsson & Eliasson, 1991; Yost & Hoseney, 1986), there were no observable changes in the enthalpies of either starch after annealing. No discernible temperature shifting and stable enthalpy were observed for endotherm M2 for normal corn starch (see Table 1).

For the amylose-rich starch (G50 and G80), broad dual endotherms composed of endotherms G and M2 were observed (Liu & Shi, 2006; Russell, 1987b) (see Fig. 2). While increasing T_o and endotherm narrowing were observed, stable T_p and T_c were detected (see Table 1). A stable T_c indicated that near-perfect double helices in the granules were not affected by the annealing process. An important difference in the amylose-rich starches was that ΔH increased after annealing at 50 °C by about 3.3 and 2.4 J/g for G50 and G80, respectively. The increased enthalpy was mainly attributed to endotherm G, since only a slight change in endotherm M2 ($p > 0.05$) was detected.

The effects of annealing on multiphase transitions were studied using starches with intermediate water content. Previous studies (Liu et al., 2006, 2007) have shown that up to four endotherms can be detected by DSC for native corn starches with about 50% water level. As shown in Fig. 3, under intermediate water condition, the gelatinization endotherms detected in the present study depended on the amylose/amylopectin ratios of the various samples. For the waxy and normal corn starches with excess water (Fig. 1), endotherm G covered endotherm M1, therefore only one apparent endotherm could be detected. As reducing water content, endotherm M1 became apparent and shifted to a higher temperature (Liu & Shi, 2006; Russell, 1987b; Seow & Teo, 1993), and further decreased the water content to intermediate level (about 50%) dual peaks were clearly visible. However, the amylose-rich starches showed a single endotherm (G) with intermediate water content, and dual endotherms (G and M2) in excess water.

Three endotherms (G, M1 and Z1) were observed for the waxy starch with 50% water content – G and M1 are associated with amylopectin disruption, and Z1 is attributed to annealing of the amylopectin crystallites during heating (Liu et al., 2006; Maurice, Slade, Sirett, & Page, 1985; Russell, 1987b; Shogren, 1992). For the normal corn starch with 50% water content, endotherms G, M1, M2 and Z were clearly detectable, and M2 and Z are attributed to the melting of amylose–lipid complexes and non-complex amylose crystalline (Biladeris, Page, Slade, & Sirett, 1985; Jovanovich & Anon, 1999; Liu et al., 2006; Raphaelides & Karkalas, 1988).

Data on the endothermic temperatures and thermal transition enthalpies of the amylopectin-rich corn starches in intermediate water condition are presented in Table 2. It can be seen that post-annealing, the temperatures of endotherms G and M1 for the waxy starch were increased by about 3 and 2.9 °C, respectively, while those for the normal corn starch were increased by about 6.6 and 5.2 °C, respectively. According to the results, it seems that the annealing temperature of 50 °C was not high enough to affect the two additional endotherms (M2 and Z) those are detected for the normal corn starch. Similar to the results of the samples with excess water, total enthalpies of both the waxy and normal corn starches remained constant, indicating that these residual structures are essentially independent of previous heat treatment.

Table 3 lists the thermal characteristics of the amylose-rich (G50 and G80) corn starches with intermediate water content. An increased T_o and narrowed ΔT was found for endotherm G after annealing at 50 °C, however no changes were observed in endotherm M2. Total enthalpy was increased after annealing, which was mainly attributed to endotherm G.

From above results, it can be seen that, regardless of water content, annealing increased and narrowed the temperature range of endotherms G and M1 (which are related to amylopectin). The narrowed peaks were an indication of better homogeneity in the annealed samples. However, the T_c of amylopectin and amylose melting remained unchanged, meaning that near-perfect internal structures were not altered by annealing. An important phenomenon is that annealing only increased the enthalpy of amylopectin in the amylose-rich samples rather than in the amylopectin-rich sam-

Table 2
Endothermic data for native and annealed waxy and normal corn starches with intermediate water content.

Sample ^a	Endotherm G				Endotherm M1				Endotherm M2				Endotherm Z				Total ΔH (J/g)	
	T_0 (°C)	T_p (°C)	T_c (°C)	ΔT (°C)	ΔH (J/g)	T_0 (°C)	T_p (°C)	T_c (°C)	ΔT (°C)	ΔH (J/g)	T_0 (°C)	T_p (°C)	T_c (°C)	ΔT (°C)	ΔH (J/g)	T_0 (°C)	T_p (°C)	ΔH (J/g)
Waxy (N)	66.9 ± 0.3	73.5 ± 0.3	78.9 ± 0.3	12.0 ± 0.5	6.14 ± 0.43	80.5 ± 0.6	90.5 ± 0.8	99.1 ± 0.9	18.6 ± 1.1	5.59 ± 0.46	100.3 ± 0.9	112.5 ± 0.6	118.5 ± 0.5	18.2 ± 0.8	1.90 ± 0.09	132.8 ± 0.8	138.5 ± 0.6	145.2 ± 0.6
Waxy (50)	69.9 ± 0.3	75.1 ± 0.5	80.6 ± 0.6	10.7 ± 0.4	6.22 ± 0.21	83.4 ± 0.8	91.7 ± 0.4	98.6 ± 0.7	15.2 ± 0.5	5.63 ± 0.35	101.9 ± 0.4	112.8 ± 0.4	119.4 ± 0.5	17.7 ± 0.8	1.94 ± 0.10	133.2 ± 0.6	138.2 ± 0.3	145.2 ± 0.4
Corn (N)	61.5 ± 0.4	67.1 ± 0.3	72.4 ± 0.6	10.9 ± 0.5	10.15 ± 0.56	77.4 ± 0.6	85.2 ± 0.5	93.4 ± 0.9	16.0 ± 0.7	2.38 ± 0.21	101.9 ± 0.4	112.8 ± 0.4	119.4 ± 0.5	17.7 ± 0.8	1.94 ± 0.10	133.2 ± 0.6	138.2 ± 0.3	145.2 ± 0.4
Corn (50)	68.1 ± 0.5	72.3 ± 0.5	77.7 ± 0.2	9.6 ± 0.6	10.91 ± 0.41	82.6 ± 0.7	89.1 ± 0.5	94.6 ± 0.4	12.0 ± 0.3	2.03 ± 0.11	101.9 ± 0.4	112.8 ± 0.4	119.4 ± 0.5	17.7 ± 0.8	1.94 ± 0.10	133.2 ± 0.6	138.2 ± 0.3	145.2 ± 0.4

^a (N) = native (unannealed) samples; (50) = samples annealed at 50 °C.

ples. Endotherms M2 and Z, which are related to amylose, were little affected.

Previous studies (Hizukuri, 1985; Kohyama & Sasaki, 2006; Koizumi, Fukuda, & Hizukuri, 1991; Nagamine & Komae, 1996) have shown no observable differences in the amylose content and close chain length distribution in amylopectin after annealing, which indicates that annealing is purely a physical process. Cooke and Gidley (1992) compared the results from XRD, DSC and nuclear magnetic resonance (NMR) measurements, and concluded that DSC endothermic values primarily reflect a loss of double-helical order, rather than a loss of crystalline register. The different results obtained for annealed samples with various amylose/amylopectin ratios could be explained by their microstructures. Native G80 and G50 have loosely packed B-type formations, which contain 36 water molecules in each unit cell, compared to 8 water molecules per cell in A-type starches. Although annealing may not transform starches from B-type to A-type structures (Stute, 1992), more hydrogen bonds were formed, resulting in higher transition enthalpy. Since only annealed low-amylose starches maintained constant enthalpy, amylose content is a possible explanation, in which amylose molecules interact with other amylose or amylopectin to form new double helices. Increased double helical content of annealed high-amylose starch has been confirmed by ¹³C-CP/MAS-NMR observations (Shi, Capitani, Trzasko, & Jeffcoat, 1998; Tester & Debon, 2000; Tester et al., 2000). The contribution of both short-range ordering and partial incorporation of amylose into amylopectin crystallites could affect gelatinization behavior (Russell, 1987a, 1987b). The diverse composition of the starches resulted in the different degrees of change (Knutson, 1990; Krueger et al., 1987b; Waduge et al., 2006).

While little mixing of amylose and amylopectin occurs in typical A-type starches (Knutson, 1990; Zobel, 1988), flexibility and mobility within the internal structures of high-amylose starches means that, during annealing, amylose and amylopectin molecules more readily interact with other amylose or amylopectin, forming new double helices. However, Knutson (1990) suggested that the strongest interactions during annealing occur when the amylose and amylopectin concentrations are approximately equal, which would explain the higher increase rate of ΔH for G50 than for G80 recorded in the present study (see Table 3).

As noted, the endotherms related to amylose (M2 and Z) showed no clear changes after annealing in any of the corn starches studied (see Tables 3 and 4), indicating that neither amylose–lipid complexes nor amylose aggregations were influenced by the annealing process. The new double helices that formed during annealing were attributed to “free” amylose in the starches and, as expected, the volume of free amylose chains increased with increasing amylose content, which would further explain the increased enthalpy observed in the amylose-rich starches. Furthermore, previous studies have suggested that endotherms G and M1 are related to shorter and longer double helices of amylopectin, respectively (Liu & Thompson, 1998; Liu et al., 2007). This work has further proved that improvement in helical length only occurs between shorter or sub-optimal amylopectins.

Fig. 4 presents data on the average size and size distribution of the native and annealed starch granules used in this study. The size order (largest to smallest) of the native granules was waxy > normal corn > G50 > G80, which corresponded with amylopectin content. It can be seen that the annealing treatment increased the granule sizes in all samples, but at different rates. The results of laser diffraction particle size analysis also demonstrated similar trends in size change after annealing (see Table 4). The size threshold for all samples shifted to a higher position post-annealing, but the same size order was observed. It was found that the average rate of size change was increased with increasing amylopectin con-

Table 3

Endothermic data for native and annealed G50 and G80 corn starches with intermediate water content.

Sample ^a	Endotherm G					Endotherm M2					Total ΔH (J/g)
	T_o (°C)	T_p (°C)	T_c (°C)	ΔT ($T_c - T_o$)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔT ($T_c - T_o$)	ΔH (J/g)	
G50 (N)	70.0 ± 0.1	77.8 ± 0.4	85.9 ± 0.4	15.9 ± 0.4	11.09 ± 0.81	102.6 ± 0.5	113.0 ± 0.4	119.8 ± 0.5	17.2 ± 0.3	3.59 ± 0.13	14.68 ± 1.07
G50 (50)	72.8 ± 0.1	78.2 ± 0.2	86.0 ± 0.1	13.2 ± 0.2	13.04 ± 0.67	102.4 ± 0.4	112.5 ± 0.4	120.0 ± 0.5	17.7 ± 0.5	3.67 ± 0.24	16.71 ± 1.15
G80 (N)	70.3 ± 0.1	78.2 ± 0.2	89.2 ± 0.5	18.9 ± 0.4	8.73 ± 0.41	102.2 ± 0.4	110.7 ± 0.4	119.7 ± 0.4	18.9 ± 0.4	3.61 ± 0.39	12.34 ± 0.89
G80 (50)	72.9 ± 0.4	78.5 ± 0.7	89.8 ± 0.4	16.8 ± 0.4	10.31 ± 0.65	102.4 ± 0.3	110.9 ± 0.6	120.1 ± 0.4	17.7 ± 0.6	3.47 ± 0.55	13.78 ± 1.27

^a (N) = native (unannealed) samples; (50) = samples annealed at 50 °C.**Table 4**

Average particle size of native and annealed corn starches.

Sample	Average particle diameter (μm)			
	Waxy	Corn	G50	G80
Unannealed	15.00	12.88	12.11	10.90
Annealed at 50 °C	17.31	14.11	12.39	11.11

tent. The increase in particle size can be explained by the ingress of moisture through the amorphous regions of the starches during annealing.

Figs. 5 and 6 show the size variation of low-amylose native and annealed starches with excess water, in which the initial and end temperatures of birefringence loss are indicated. There was little change in granule size at temperatures lower than 60 °C. However, as expected, the growth rate was increased sharply in the temperature range of birefringence loss, since crystalline order is considered as the main factor restricting granular swelling. It should be noted that the granular growth rates and final accretion ratios of the annealed samples were lower than those of the native samples, i.e. the final sizes of native granules were significantly larger than those of annealed granules. One explanation for this could be that the thermal treatment perfected the crystalline order and strengthened the granular structure, leading to decreased variability. However, this phenomenon was not observed in the high-amylose starches (see Fig. 7), possibly because these starches did not fully swell and retained their granular structure at higher temperatures.

The XRD results of the different native and annealed corn starches are shown in Fig. 8, where it can be seen that the native waxy and normal corn starches showed typical patterns of A-type starch, whereas the native G50 and G80 exhibited B-type patterns. The results indicate that the crystal types and structures of the corn starches investigated were unaffected by annealing, which is in agreement with previous studies (Jacobs et al., 1998a,

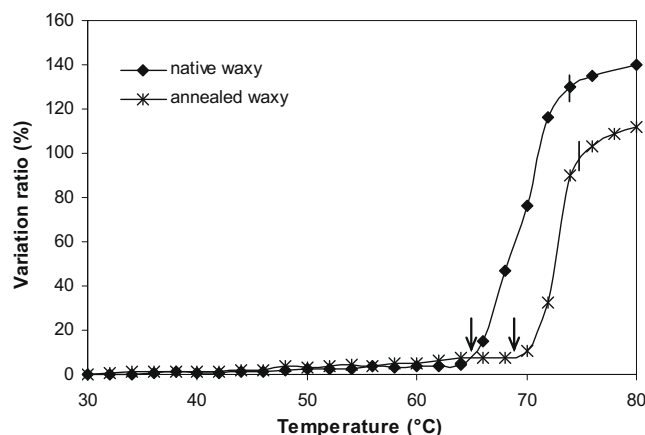


Fig. 5. Comparison of diameter variation of native and annealed waxy corn starches during heating, as observed by microscope. “↓” and “|” represent the initial and end temperatures of birefringence loss, respectively.

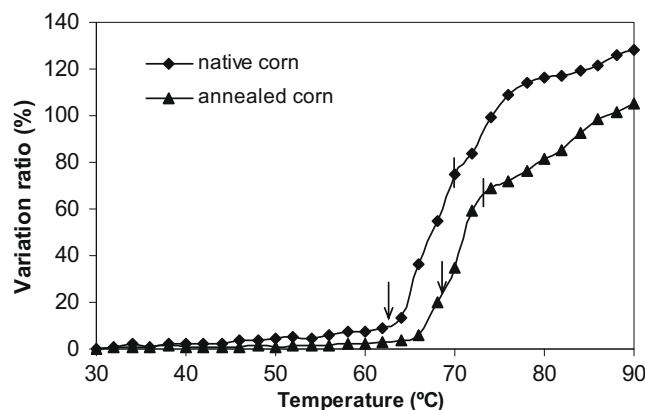


Fig. 6. Comparison of diameter variation of native and annealed normal corn starches during heating, as observed by microscope. “↓” and “|” represent the initial and end temperatures of birefringence loss, respectively.

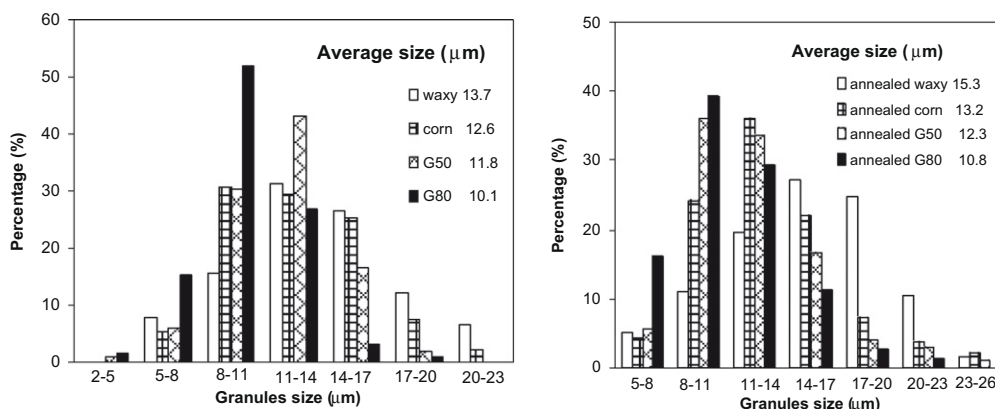


Fig. 4. Average granule sizes and size distributions of native (left) and annealed (right) corn starches.

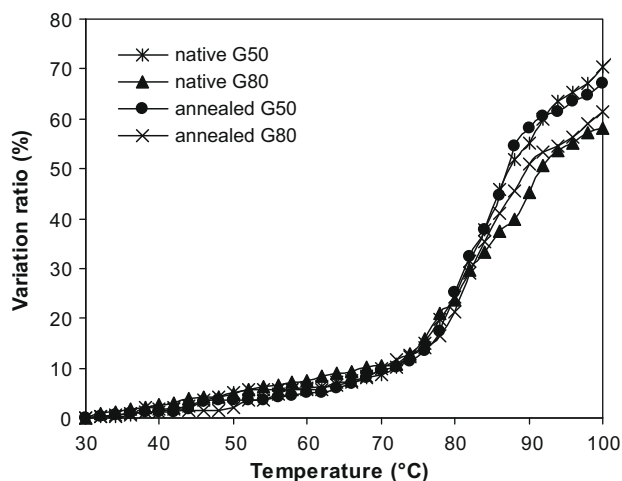


Fig. 7. Comparison of diameter variation of native and annealed high-amylose G80 and G50 corn starches during heating, as observed by microscope.

1998b; Stute, 1992). The XRD results also supported the conclusion that annealing does not significantly increase the crystalline material within starch granules (Tester, Ansell, Snape, & Yusuph, 2005; Tester et al., 2000).

4. Conclusions

- (1) For all annealed (50 °C) corn starches with excess water, endotherm G shifted to a higher temperature and became narrow, and enthalpy was increased in the amylose-rich starches (G50 and G80). There was no observable difference in endotherm M2.

- (2) For all annealed (50 °C) corn starches with intermediate water content, endotherm G shifted to a higher temperature and narrowed. The enthalpies of the amylopectin-rich starches remained constant, while those of the amylose-rich starches were increased. Endotherm M1 detected for waxy and normal corn starches also shifted to a higher temperature and narrowed, and enthalpy kept stable. No discernible differences were detected in endotherm M2 for the normal, G50 and G80 corn starches.
- (3) It is interesting to note that annealing only influenced the total enthalpy of the amylose-rich starches, but that the effect was mainly attributed to amylopectin endotherm G. The milder changes in endotherms M1, M2 and Z support the theory that annealing only improves the helical length of shorter or sub-optimal amylopectins. The endotherms related to amylose (M2 and Z) showed no discernible changes after annealing in either the amylose- or amylopectin-rich starches, indicating that neither amylose–lipid complexes nor amylose aggregations were affected by annealing. The new double helices formed during annealing are attributed to “free” amylose in starch.
- (4) The granular sizes of samples were increased after annealing, with the diameter growth rates and final accretion ratios being more prominent in amylopectin-rich starches. The XRD patterns remained unchanged.

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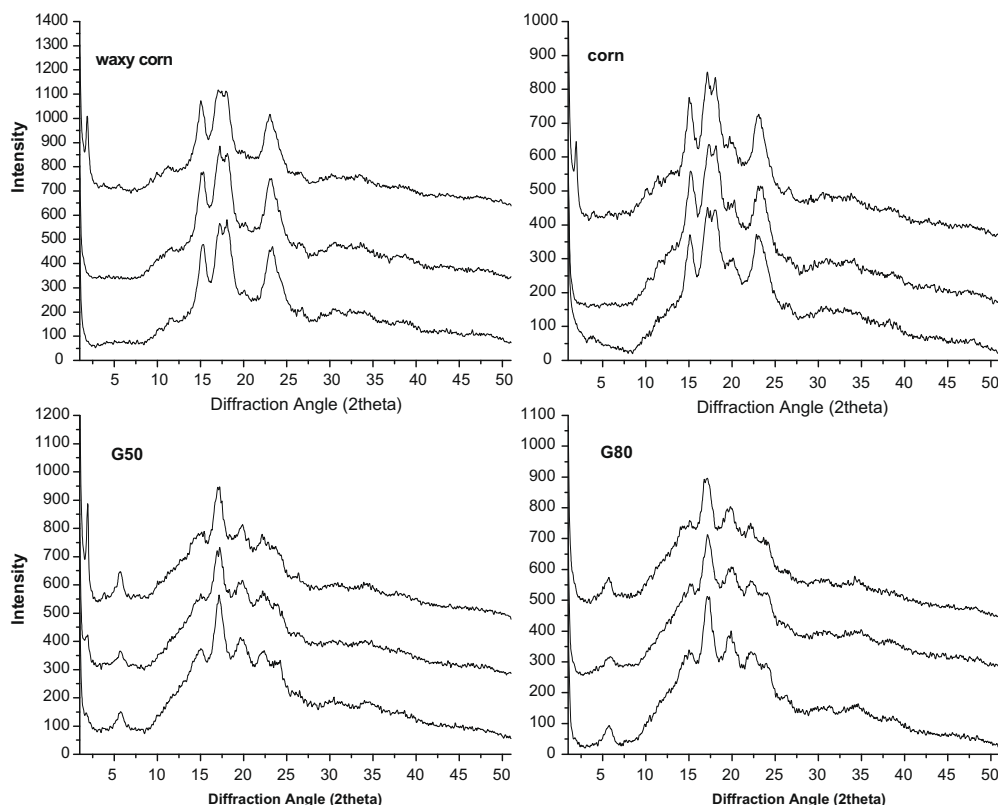


Fig. 8. XRD traces of native and annealed corn starches: (top traces) native starch; (middle traces) starch annealed at 30 °C; and (bottom traces) starch annealed at 50 °C.

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