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Impact on molecular organization of amylopectin in starch granules upon annealing

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ABSTRACT

This study investigated the influence of the internal structure of amylopectin on annealing (3 h, 24 h) of starches from four different types of amylopectin (Bertoft, Koch, & Aman, 2012; Bertoft, Piyachomkwan, Chatakanonda, & Sriroth, 2008). Regardless of the starch source and incubation time, annealing significantly increased the onset gelatinization temperature (To) and narrowed and deepened the amylopectin endotherm. However, the extent of the change in the melting temperature (Tm) and the enthalpy of gelatinization (ΔH) differed among the types. In terms of the To and Tm, starches from type 1 (oat, rye, barley, and waxy barley) showed the most significant response to annealing. The Tm of starches belonging to type 2 (waxy maize, rice, waxy rice, and sago) remained unchanged after 3 h of annealing. Type 1 and type 2 starches with the lowest gelatinization temperatures showed the greatest increase in melting temperature after annealing. However, type 3 (tapioca, mung bean, and arrowroot) and type 4 (potato, waxy potato, canna, and yam) starches were not in line with these observations. Instead, starches from type 3 and type 4 showed a pronounced increase in the ΔH. The inter-block chain length (IB-CL) (distance between tightly branched units within a cluster) correlated positively ($r = 0.93$, $p < 0.01$) with the change in enthalpy after 24 h of annealing. These data indicate that a short IB-CL affects the optimum registration of double helices within the crystalline lamellae. The relationship between the gelatinization parameters before and after annealing suggests that type 1 and 2 starches might possess a high number of unpacked double helices (type 1 > type 2) compared to other types. Longer IB-CLs, which facilitate the parallel packing of splayed double helices, and the lengthening of double helices likely increased the ΔH in type 3 and type 4 starches. It is concluded that annealing can be used as a probe for visualizing the organization of glucan chains (alignment of double helices/degree of perfection) within crystalline lamellae.

1. Introduction

Starch is a semicystalline composite of biopolymers of glucose composed of linear α-(1,4)-linked residues with α-(1,6)-linked branch chains. These glucan chains are categorized mainly as amylose, which is a relatively long, linear α-glucan with few branches [1% α-(1,6)], and amylopectin, which has a highly branched structure [5% α-(1,6)] (Pérez & Bertoft, 2010). Starch granules exhibit alternating zones of semi-crystalline and amorphous rings (French, 1984). Semi-crystalline rings are formed from alternating crystalline and amorphous lamellae with a repeat distance of 8.5–10 nm (Jenkins, Cameron, & Donald, 1993; Sanderson, Daniels, Donald, Blennow, & Engelsen, 2006). The crystalline lamella is 5–6 nm thick (Robin, Mercier, Charbonniere, & Guilbot, 1974) and is formed primarily of the double helices of external amylopectin chains (Pérez & Bertoft, 2010). The double helices consist of six residues per turn, with a pitch height of 2.1 nm (Gernat, Radosta, Anger, & Damaschun, 1993). The amorphous lamella is composed mainly of internal amylopectin chains (long chains) and branch points (Bertoft et al., 2008). The location of amylose in relation to amylopectin and the structure of the amorphous growth ring are still obscure (Pérez & Bertoft, 2010). The organization of glucan chains in the amylopectin molecule is characterized by the internal unit chain length profile. Four different types of amylopectin have been reported based on the internal unit chain profile obtained from φ,β-limit dextrins of amylopectin. These types differ principally with respect to the quantity and distribution of short and long chains, the size of the clusters of chains, the number of building blocks (tightly branched units) within the clusters, the distribution pattern of the branching points, and the degree of polymerization (DP) of the inter-block chain segments (Bertoft et al., 2008, 2012).

However, no general agreement about or clear understanding of the organization of the glucan chains and their impact on the

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granular architecture has been established, and the structural basis of the functional properties of starches from different botanical origins is also poorly understood. Since the first cluster model of amylopectin was proposed, several modified structure models have been developed over the years. One of the most widely accepted todate was proposed by Hizukuri (1986), who developed a model based on the cluster models originated by French (1972) and Nikuni (1978). In this traditional model, the clusters are interconnected through long amylopectin chains, which participate in the crystalline and amorphous lamellae. Bertoft (2004) proposed a two-directional backbone model. In this alternative model, long chains of amylopectin are not integrated as parts of the clusters, and the direction of the clustered chains is perpendicular to the direction of the amorphous backbone. Although no experimental evidence of the validity of either model has been obtained, a recent study (Chauhan & Seetharaman, 2013) of the iodine binding of lintners and limit dextrins demonstrated that the molecular mechanism behind iodine binding could be visualized by the backbone model of amylopectin.

The organization of glucan chains and the formation of crystalline lamellae are governed mainly by a combination of enzyme activity and environmental conditions during biosynthesis. The influence of environmental conditions on lamellar organization has been examined in numerous studies that focused primarily on gelatinization behavior (Alvani, Qi, & Tester, 2012; Kiseleva et al., 2003, 2004; Protserov et al., 2002; Tester, 1997). These studies revealed that hydrothermal treatment could be used as a tool for acquiring an understanding of the nature of granule organization.

Annealing (ANN) is a hydrothermal treatment that modifies the physicochemical properties of starch without destroying its granular structure. During annealing, starch granules in excess water (>65%) or at intermediate moisture levels (40–55% w/w) are incubated for a specific time at a temperature above the glass transition temperature (Tg) but below the onset temperature of gelatinization (Hoover, 2010). ANN conditions (incubation temperature and time) facilitate the infusion of water into the amorphous layers and reversible, limited granule swelling (Jayakody & Hoover, 2008). The annealing phenomena can be monitored easily with differential scanning calorimetry (DSC). Native starches often show a broad melting peak, usually attributed to the distribution of the thickness of the crystalline lamellae and to crystal defects. After annealing, the DSC endotherm associated with the gelatinization of amylopectin shifts to a higher temperature and becomes narrower and deeper. This modification reflects the decrease in the heterogeneity within the starch crystallites caused by the annealing as well as the improvement in the organization of the chains within the crystalline lamellae (Genkina, Wasserman, Noda, Tester, & Yuryev, 2004; Genkina, Wikman, Bertoft, & Yuryev, 2007; Jacobs et al., 1998; Jayakody & Hoover, 2008; Kohyama & Sasaki, 2006; Tester & Debon, 2000). Although annealing has been studied extensively, the molecular mechanisms that underlie annealing are only somewhat understood, and many questions remain unanswered with respect to the annealing-induced structural changes in starches from different botanical sources.

The relationship between the internal structure of amylopectin and the thermal properties of starches (Vamadevan, Bertoft, & Seetharaman, 2013) from four different types of amylopectin (Bertoft et al., 2008, 2012) suggests that the alignment of external chains within the crystalline lamellae depends on the flexibility of the spacers within or between the building blocks in the amorphous lamellae. Unfavorable combinations of the spacer links, which limit the parallel packing of double helices within the cluster; dangling chains, which are too short to participate in the formation of double helices (Gerard, Colonna, Buleon, & Planchot, 2002); amylose tie chains, which are amylose molecules that pass through both the crystalline and amorphous layers (Koroteeva et al., 2007; Yuryev et al., 2004); and the long B chains that are interspersed into the crystalline lamellae (Genkina et al., 2007) can be considered as potential molecular defects. It has been postulated that molecular defects influence the optimum registration of double helices within the crystalline lamellae (Kiseleva et al., 2005). According to a more recent modification of the backbone model (Bertoft, Laohaphatanalert, Piyachomkwan, & Sriroth, 2010), amylopectin double helices are attached to an amorphous backbone, which influences the alignment of chains within the crystalline lamellae. Thus, a worthwhile investigation would be to examine how the improvements in structural organization produced by the annealing of starches are related to the internal organization of amylopectin chains. The work described herein has explored this area, has served as validation of the models proposed in our previous study (Vamadevan et al., 2013), and has offered a means of visualizing the organization of chains in the amylopectin molecule.

2. Experimental procedure

2.1. Materials

The following 16 starch samples were selected from the series used in previous studies (Bertoft et al., 2008, 2012), in which their origin and structural details were described: Andean yam bean (*Pachyrhizu sahipa*, AYS), rye (*Secale cereale*, RS), oat (*Avena sativa*, OS), normal barley (*Hordeum vulgare*, NBS), waxy barley (*Hordeum vulgare* WBS), waxy maize (*Zea mays*, WMS), rice containing medium amylose (*Oryza sativa*, MRS), waxy rice (*Oryza sativa*, WRS), sago (*Metroxylon sagu*, SS), mung bean (*Vigna radiata*, MBS), West Indian arrowroot (*Maranta arundinacea*, AS), tapioca (*Manihot esculenta*, TS), edible canna (or Queensland arrowroot, *Canna edulis*, CS), normal potato (*Solanum tuberosum*, NPS), waxy potato (*Solanum tuberosum*, PAPS), and lesser yam starch (*Dioscorea esculenta*, YS). Selected data related to the structure of the samples are provided in supplementary data (Table S1).

2.2. Differential scanning calorimetry

The gelatinization parameters of the starches were measured using a TA Instruments, Q2000 differential scanning calorimeter (DSC) equipped with a thermal analysis data station and data recording software (TA Instruments, Universal Analysis 2000). Starch dispersions in water (1:3) were equilibrated for 3 h at room temperature before DSC analysis. The scanning temperature range and the heating rates were 10–110 °C and 2 °C/min, respectively. In all measurements, the thermogram was recorded with deionized water as a reference. The transition temperatures reported are the onset (To), peak (Tm), and conclusion (Tc) temperatures. The enthalpy of gelatinization (ΔH) was estimated by integrating the area between the thermogram and a base line under the peak and was expressed as J/g of dry starch. The ratio of $\Delta H/(Tm - To)$ was described as the peak height index (PHI).

2.3. Annealing

Starch samples were incubated in excess water (80% by weight) for 3 h or 24 h at 6 °C lower than the onset gelatinization temperature of native samples. The annealing temperature was chosen as a function of the onset gelatinization temperature of untreated starch in order to avoid the melting of the crystallites in the starches. The samples were then centrifuged at 2000 × *g* for 15 min at 20 °C, and the supernatant was decanted. The annealed starches were washed twice with distilled water and acetone, and recovered by centrifugation (2000 × *g*, 15 min). The samples were then air-dried for 24 h.

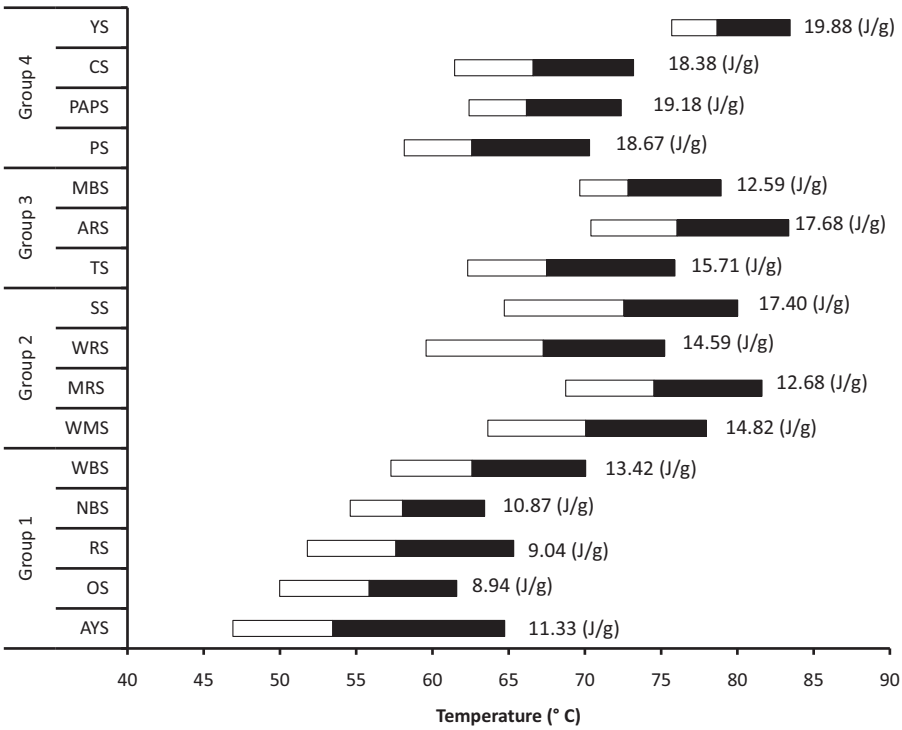

Fig. 1. Gelatinization transition temperatures and gelatinization enthalpy (ΔH) of native starches with four structural types of amylopectin. Bars indicate the temperature range of gelatinization (To – Tc) and border verifies the position of Tm. AYS – Andean yam starch, OS – oat starch, RS – rye starch, NBS – normal barley starch, WBS – waxy barley starch, WMS – waxy maize starch, MRS – medium amylose containing rice starch, WRS – waxy rice starch, SS – sago starch, TS – tapioca starch, ARS – arrowroot starch, MBS – mung bean starch, PS – potato starch, PAPS – potato amylopectin starch, CS – canna starch, YS – lesser yam starch.

2.4. Statistical analysis

Statistical analysis was performed with the use of SPSS statistical software (IBM SPSS Statistics 20). All determinations were replicated three times, and the mean values and standard deviations were reported. Using the analysis of variance (ANOVA) technique, the means of the gelatinization parameters were compared based on Tukey's test to a 5% significance level. Pearson correlation analysis was also conducted in order to study the correlation between internal chain length distribution and thermal properties.

3. Results

In this study, 16 starch samples from four amylopectin types were examined by DSC before and after ANN. The samples represented a broad range of starch sources from different types of plants and with varied crystalline structures. Type 1 included starches from Andean yam bean, oat, rye, normal barley, and waxy barley; type 2 was comprised of rice with medium amylose content, waxy rice, waxy maize, and sago; type 3 contained tapioca, mung bean, and arrowroot; and type 4 consisted of B-crystalline starches: potato, waxy potato, canna, and yam.

3.1. Melting parameters of native starches

Starches from the four different amylopectin types differed with respect to their gelatinization temperatures (To, Tm, Tc), melting temperature range (Tc – To), and ΔH, as shown in Fig. 1, and with respect to their peak height index (PHI) (Fig. 2). The Tm of all 16 starches followed the order YS > ARS > MRS > SS > MBS > WMS > TS > WRS > CS > PAPS > PS > WBS > NBS > RS > OS > AYS. The differences observed in the gelatinization temperatures and the ΔH within and between the amylopectin types were discussed in detail by Vamadevan et al. (2013).

PHI represents the quantitative evaluation [ΔH/(Tm – To)] of variations in the shape of the endotherm, which reflects the degree of homogeneity or perfection of the crystallites (Krueger, Knutson, Inglett, & Walker, 1987). The PHI of starches from the four different types followed the order type 4 ≥ type 3 > type 2 > type 1. However, the barley starches (NBS and WBS), which structurally belong to type 1, exhibited a higher PHI than did any of the other starches in either type 1 or type 2.

3.2. Melting parameters of annealed starches

All of the starches were annealed for 3 h or 24 h so that the influence of the internal organization of amylopectin on the annealing process could be examined. The gelatinization parameters of native and ANN starches from type 1, type 2, type 3, and type 4 are shown in Tables 1a–1d, respectively. AYS was omitted from the following discussion because partial gelatinization was noticed after ANN. It is relevant to note that AYS possesses a greater quantity of A_{fp}-chains (DP 6–8) than any other starch used in this experiment (Table S1), and that the asymmetry of its calorimetric peak was the most pronounced (Fig. 1). For every starch, regardless of the source, compared with its native counterpart, annealing (3 h and 24 h) delayed the To and narrowed the gelatinization endotherm. However, the extent of the change differed between and within types (Tables 1a–1d). The Tc of the starches in type 2 (Table 1b) and in type 3 (except for MBS) (Table 1c) remained unchanged after annealing. The increase in gelatinization temperatures accompanied by a narrowing of the gelatinization endotherm after ANN has been reported in many studies (Jacobs et al., 1998; Jayakody & Hoover, 2008; Kiseleva et al., 2004; Kohyama & Sasaki, 2006; Tester & Debon, 2000).

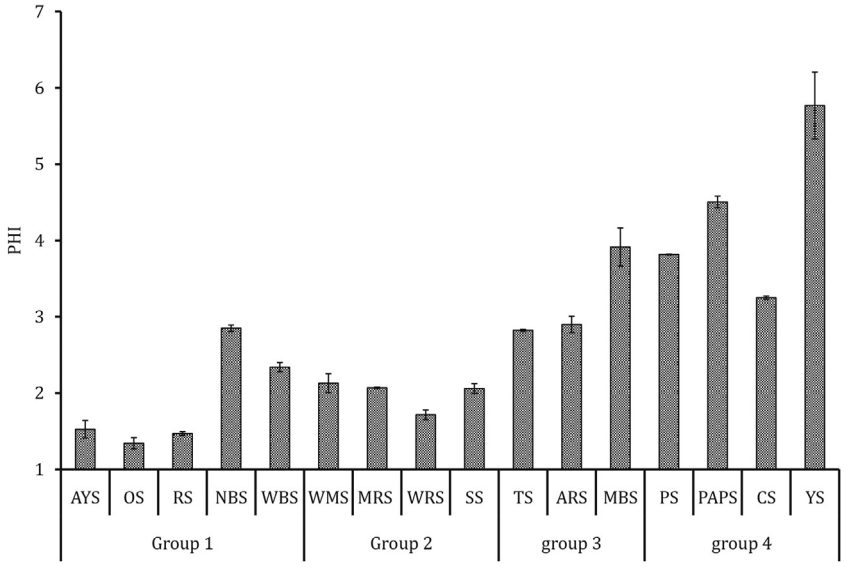

Fig. 2. Peak height index of the starches from four different types of amylopectin. AYS – Andean yam starch, OS – oat starch, RS – rye starch, NBS – normal barley starch, WBS – waxy barley starch, WMS – waxy maize starch, MRS – medium amylose containing rice starch, WRS – waxy rice starch, SS – sago starch, TS – tapioca starch, ARS – arrowroot starch, MBS – mung bean starch, PS – potato starch, PAPS – potato amylopectin starch, CS – canna starch, YS – lesser yam starch.

3.3. Effects of 3 h annealing on melting parameters

The increase in the To and Tm after 3 h ANN followed the order type 1 > type 3 ≥ type 4 > type 2 (Tables 1a–1d). However, SS from type 2 (Table 1b) showed a greater increase in To than did starches from either type 3 (Table 1c) or type 4 (Table 1d). Starches from type 2 (WMS, MRS, WRS, SS) and ARS from type 3 showed no significant difference in Tm after 3 h of annealing (Table 1b). The Tc of OS, RS, and NBS from type 1 (Table 1a) increased significantly, but the Tc of other starches (Tables 1b–1d) remained unchanged after 3 h ANN. However, in type 1, the To increased to a more pronounced extent than did the Tc (Table 1a). A slight increase in ΔH was observed in RS (Table 1a), MRS (Table 1b), type 3 (except for TS; Table 1c), and type 4 (Table 1d) starches. However, ARS (Table 1c) showed a very significant increase in ΔH after 3 h ANN.

3.4. Effects of 24 h annealing on melting parameters

The extent of the increase in the To and the Tm after 24 h ANN followed the same order observed after 3 h ANN: type 1 > type 3 ≥ type 4 > type 2 (Tables 1a–1d). However, type 2 starches showed a significant increase in Tm after 24 h annealing (Table 1b). As well, a slight increase in Tc after 24 h annealing was observed in starches from type 1 (Table 1a) and type 4 (Table 1d).

Annealing for 24 h significantly increased the ΔH of most of the starches. However, the ΔH of OS (Table 1a), WMS, and WRS (Table 1b) starches remained unchanged. Higher ΔH values were observed in starches from type 3 (Table 1c) and type 4 (Table 1d). As shown in Fig. 3, IB-CL correlated positively ($r = 0.93$, $p < 0.01$) with ΔH. Yam starch is likely highly ordered as evidenced by its narrow endotherm and high Tm in its native state, and therefore only

Table 1a
Gelatinization parameters of native, annealed type 1 starches[p].

Sample	Treatment	To[q]	Tm[q]	Tc[q]	Tc – To[r]	ΔH[s]	PHI[t]
AYS	Native	46.9 ± 0.2[a]	53.5 ± 0.0[a]	64.7 ± 0.1[a]	17.8 ± 0.0[a]	11.3 ± 0.1[a]	1.5 ± 0.1[a]
	ANN[u] 3 h	51.7 ± 0.0[b]	55.6 ± 0.2[b]	64.1 ± 0.3[a]	12.4 ± 0.4[b]	10.4 ± 0.2[a]	2.5 ± 0.0[b]
	ANN 24 h	55.1 ± 0.1[c]	58.0 ± 0.1[c]	64.3 ± 0.0[a]	9.2 ± 0.1[c]	10.9 ± 0.4[a]	3.6 ± 0.0[c]
OS	Native	50.0 ± 0.1[a]	55.9 ± 0.1[a]	61.6 ± 0.0[a]	11.6 ± 0.1[a]	8.9 ± 0.5[a]	1.3 ± 0.1[a]
	ANN 3 h	55.9 ± 0.0[b]	59.3 ± 0.0[b]	64.1 ± 0.1[b]	8.2 ± 0.1[b]	8.9 ± 0.1[a]	2.4 ± 0.0[b]
	ANN 24 h	58.6 ± 0.1[c]	61.8 ± 0.1[c]	66.6 ± 0.1[c]	8.0 ± 0.0[c]	9.4 ± 0.1[a]	2.7 ± 0.0[c]
RS	Native	51.9 ± 0.1[a]	57.6 ± 0.1[a]	65.3 ± 0.2[a]	13.5 ± 0.1[a]	9.0 ± 0.1[a]	1.5 ± 0.0[a]
	ANN 3 h	57.4 ± 0.1[b]	61.1 ± 0.2[b]	67.1 ± 0.2[b]	9.7 ± 0.1[b]	10.5 ± 0.0[b]	2.5 ± 0.1[b]
	ANN 24 h	59.7 ± 0.0[c]	63.2 ± .0.0[c]	69.2 ± 0.0[c]	9.4 ± 0.0[c]	10.6 ± 0.1[b]	2.9 ± 0.0[c]
NBS	Native	54.6 ± 0.1[a]	58.0 ± 0.0[a]	63.4 ± 0.1[a]	8.8 ± 0.1[a]	10.9 ± 0.1[a]	2.9 ± 0.0[a]
	ANN 3 h	59.2 ± 0.0[b]	61.3 ± 0.0[b]	65.7 ± 0.1[b]	6.5 ± 0.1[b]	10.9 ± 0.4[a]	4.8 ± 0.2[b]
	ANN 24 h	62.1 ± 0.2[c]	63.7 ± 0.2[c]	67.8 ± 0.2[c]	5.7 ± 0.0[c]	12.0 ± 0.2[b]	6.6 ± 0.1[c]
WBS	Native	57.3 ± 0.1[a]	62.6 ± 0.0[a]	70.1 ± 0.1[a]	12.1 ± 0.8[a]	13.4 ± 0.1[a]	2.3 ± 0.1[a]
	ANN 3 h	61.8 ± 0.3[b]	65.1 ± 0.3[b]	70.8 ± 0.1[a]	9.1 ± 0.2[b]	13.4 ± 0.2[a]	3.7 ± 0.2[b]
	ANN 24 h	64.3 ± 0.0[c]	66.9 ± 0.1[c]	72.1 ± 0.1[b]	7.9 ± 0.0[c]	15.3 ± 0.5[b]	5.5 ± 0.4[c]

AYS – Andean yam starch, OS – oat starch, RS – rye starch, NBS – normal barley starch, WBS – waxy barley starch.

[p] All data reported on dry basis. Means within each column with different superscripts for their annealed (3 h, 24 h) and native counterparts are significantly different ($P < 0.05$) by Tukey's test.

[q] To, Tm, Tc represent the onset, mid-point and conclusion temperatures respectively.

[r] (Tc – To) represents the gelatinization temperature range.

[s] Gelatinization enthalpy of starch expressed in J/g of dry starch.

[t] PHI represents the peak height index (ΔH/(Tm – To)).

[u] ANN – annealing.

Table 1b
Gelatinization parameters of native, annealed type 2 starches[p].

Sample	Treatment	To[q]	Tm[q]	Tc[q]	Tc – To[r]	ΔH[s]	PHI[t]
WMS	Native	63.6 ± 0.0[a]	70.1 ± 0.0[a]	78.0 ± 0.3[a]	14.3 ± 0.3[a]	14.8 ± 0.1[a]	2.1 ± 0.1[a]
	ANN[u] 3 h	65.9 ± 0.1[b]	70.6 ± 0.1[a]	77.5 ± 0.2[a]	11.7 ± 0.3[b]	15.3 ± 1.0[a]	3.1 ± 0.2[b]
	ANN 24 h	68.1 ± 0.0[c]	72.2 ± 0.1[b]	78.3 ± 0.2[a]	10.3 ± 0.1[c]	16.3 ± 0.9[a]	3.8 ± 0.2[c]
MRS	Native	68.7 ± 0.1[a]	74.5 ± 0.0[a]	81.6 ± 0.1[a]	12.9 ± 0.2[a]	12.7 ± 0.3[a]	2.1 ± 0.0[a]
	ANN 3 h	70.5 ± 0.0[b]	74.7 ± 0.0[a]	80.5 ± 0.1[a]	9.9 ± 0.1[b]	13.9 ± 0.0[b]	3.1 ± 0.0[b]
	ANN 24 h	72.7 ± 0.1[c]	76.4 ± 0.0[b]	81.8 ± 0.0[a]	9.1 ± 0.1[c]	15.0 ± 0.1[c]	3.8 ± 0.0[c]
WRS	Native	59.6 ± 0.0[a]	67.3 ± 0.2[a]	75.2 ± 0.1[a]	15.7 ± 0.1a	14.6 ± 0.5[a]	1.7 ± 0.1[a]
	ANN 3 h	62.0 ± 0.1[b]	67.3 ± 0.1[a]	74.1 ± 0.3[a]	12.2 ± 0.4b	14.7 ± 0.6[a]	2.6 ± 0.1[b]
	ANN 24 h	64.3 ± 0.1[c]	68.9 ± 0.1[b]	75.1 ± 0.1[a]	10.8 ± 0.0c	15.2 ± 0.0[a]	3.1 ± 0.0[c]
SS	Native	64.7 ± 0.0[a]	72.6 ± 0.2[a]	79.0 ± 0.2[a]	15.3 ± 0.1[a]	17.4 ± 0.3[a]	2.1 ± 0.1[a]
	ANN 3 h	70.0 ± 0.1[b]	72.7 ± 0.1[a]	78.8 ± 0.0[a]	8.8 ± 0.0[b]	17.6 ± 0.4[a]	6.0 ± 0.1[b]
	ANN 24 h	72.3 ± 0.0[c]	74.3 ± 0.0[b]	79.5 ± 0.2[a]	7.3 ± 0.2[c]	20.2 ± 0.3[b]	9.2 ± 0.1[c]

WMS – waxy maize starch, MRS – medium amylose containing rice starch, WRS – waxy rice starch, SS – sago starch.
[p] All data reported on dry basis. Means within each column with different superscripts for their annealed (3 h, 24 h) and native counterparts are significantly different ($P < 0.05$) by Tukey's test.
[q] To, Tm, Tc represent the onset, mid-point and conclusion temperatures respectively.
[r] (Tc – To) represents the gelatinization temperature range.
[s] Gelatinization enthalpy of starch expressed in J/g of dry starch.
[t] PHI represents the peak height index (ΔH/(Tm – To)).
[u] ANN – annealing.

Table 1c
Gelatinization parameters of native, annealed type 3 starches[p].

Sample	Treatment	To[q]	Tm[q]	Tc[q]	Tc – To[r]	ΔH[s]	PHI[t]
TS	Native	62.3 ± 0.2[a]	67.5 ± 0.1[a]	75.9 ± 0.1[a]	13.6 ± 0.1[a]	15.7 ± 0.0[a]	2.8 ± 0.0[a]
	ANN[u] 3 h	66.0 ± 0.0[b]	68.8 ± 0.0[b]	75.5 ± 0.1[a]	9.5 ± 0.0[b]	15.8 ± 0.1[a]	5.4 ± 0.1[b]
	ANN 24 h	68.5 ± 0.1[c]	70.8 ± 0.1[c]	76.7 ± 0.0[a]	8.2 ± 0.1[c]	18.8 ± 1.1[b]	7.2 ± 0.1[c]
ARS	Native	70.4 ± 0.3[a]	76.1 ± 0.3[a]	83.4 ± 0.1[a]	13.0 ± 0.2[a]	17.7 ± 0.2[a]	2.9 ± 0.1[a]
	ANN 3 h	74.5 ± 0.0[b]	76.7 ± 0.0[a]	82.2 ± 0.2[a]	7.7 ± 0.2[b]	20.6 ± 0.0[b]	9.5 ± 0.2[b]
	ANN 24 h	77.0 ± 0.1[c]	79.0 ± 0.1[b]	83.7 ± 0.1[a]	6.6 ± 0.1[c]	20.9 ± 0.9[b]	10.4 ± 0.2[c]
MBS	Native	69.7 ± 0.2[a]	72.8 ± 0.4[a]	78.9 ± 0.5[a]	9.3 ± 0.3[a]	12.6 ± 1.0[a]	3.9 ± 0.2[a]
	ANN 3 h	74.2 ± 0.0[b]	75.6 ± 0.0[b]	80.7 ± 0.0[a]	6.5 ± 0.0[b]	14.3 ± 0.0[a,b]	9.6 ± 0.4[b]
	ANN 24 h	76.7 ± 0.1[c]	78.0 ± 0.2[c]	83.0 ± 0.2[b]	6.3 ± 0.1[b]	15.5 ± 0.3[b]	10.6 ± 0.9[c]

TS – tapioca starch, ARS – arrowroot starch, MBS – mung bean starch.
[p] All data reported on dry basis. Means within each column with different superscripts for their annealed (3 h, 24 h) and native counterparts are significantly different ($P < 0.05$) by Tukey's test.
[q] To, Tm, Tc represent the onset, mid-point and conclusion temperatures respectively.
[r] (Tc – To) represents the gelatinization temperature range.
[s] Gelatinization enthalpy of starch expressed in J/g of dry starch.
[t] PHI represents the peak height index (ΔH/(Tm – To)).
[u] ANN – annealing.

Table 1d
Gelatinization parameters of native, annealed type 4 starches[p].

Sample	Treatment	To[q]	Tm[q]	Tc[q]	Tc – To[r]	ΔH[s]	PHI[t]
PS	Native	58.2 ± 0.0[a]	62.6 ± 0.0[a]	70.3 ± 0.1[a]	12.2 ± 0.1[a]	18.7 ± 0.3[a]	3.8 ± 0.1[a]
	ANN[u] 3 h	60.9 ± 0.0[b]	63.8 ± 0.1[b]	70.1 ± 0.2[a]	9.2 ± 0.2[b]	20.1 ± 0.8[a,b]	6.4 ± 0.2[b]
	ANN 24 h	64.2 ± 0.1[c]	66.6 ± 0.1[c]	72.2 ± 0.2[b]	8.0 ± 0.3[c]	21.7 ± 0.3[b]	8.4 ± 0.0[c]
PAPS	Native	62.4 ± 0.1a	66.2 ± 0.0[a]	72.4 ± 0.3[a]	10.0 ± 0.3[a]	19.2 ± 0.0[a]	4.5 ± 0.0[a]
	ANN 3 h	65.6 ± 0.0b	68.4 ± 0.0[b]	73.4 ± 0.1[a]	7.8 ± 0.1[b]	20.6 ± 0.2[b]	7.0 ± 0.0[b]
	ANN 24 h	66.8 ± 0.0c	69.6 ± 0.0[c]	74.8 ± 0.1[b]	8.0 ± 0.1[b]	20.7 ± 0.3[b]	6.9 ± 0.3[c]
CS	Native	61.5 ± 0.0[a]	66.6 ± 0.0[a]	73.2 ± 0.0[a]	11.7 ± 0.0[a]	18.4 ± 0.1[a]	3.3 ± 0.0[a]
	ANN 3 h	64.5 ± 0.0[b]	67.5 ± 0.0[b]	73.2 ± 0.2[a]	8.7 ± 0.2[b]	20.6 ± 0.8[b]	6.8 ± 0.0[b]
	ANN 24 h	67.9 ± 0.0[c]	70.3 ± 0.0[c]	75.1 ± 0.1[b]	7.2 ± 0.1[c]	22.7 ± 0.2[c]	8.7 ± 0.0[c]
YS	Native	75.7 ± 0.1[a]	78.7 ± 0.2[a]	83.5 ± 0.3[a]	7.8 ± 0.2[a]	19.9 ± 0.1[a]	5.8 ± 0.4[a]
	ANN 3 h	78.2 ± 0.0[b]	80.1 ± 0.0[b]	82.6 ± 0.1[a]	4.4 ± 0.1[b]	21.0 ± 0.5[a,b]	10.6 ± 0.5[b]
	ANN 24 h	80.8 ± 0.0[c]	82.4 ± 0.0[c]	84.4 ± 0.1[b]	3.6 ± 0.0[c]	22.2 ± 0.1[b]	12.8 ± 0.2[c]

PS – potato starch, PAPS – potato amylopectin starch, CS – canna starch, YS – lesser yam starch.
[p] All data reported on dry basis. Means within each column with different superscripts for their annealed (3 h, 24 h) and native counterparts are significantly different ($P < 0.05$) by Tukey's test.
[q] To, Tm, Tc represent the onset, mid-point and conclusion temperatures respectively.
[r] (Tc – To) represents the gelatinization temperature range.
[s] Gelatinization enthalpy of starch expressed in J/g of dry starch.
[t] PHI represents the peak height index (ΔH/(Tm – To)).
[u] ANN – annealing.

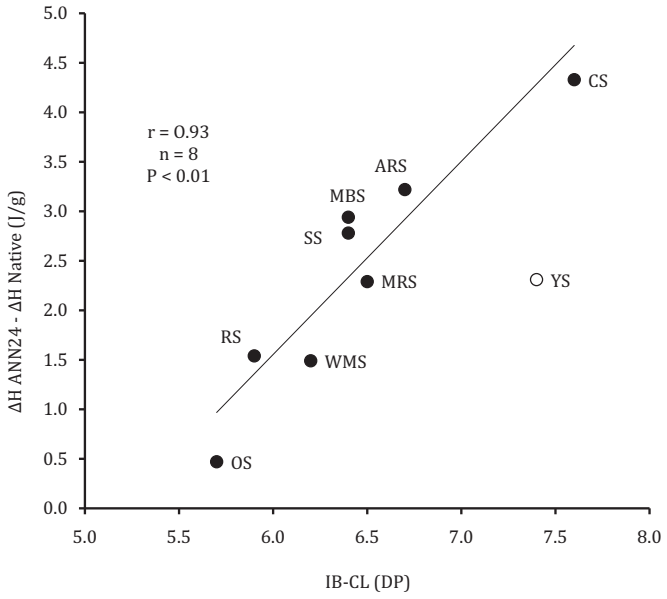

Fig. 3. Correlation coefficient (r) for the relationship IB-CL and increase in gelatinization enthalpy after 24 h annealing. The r-value does not include yam starch. Filled circles indicate samples from types 1 to 3. Open circle indicates yam.

canna starch was included from type 4 starches in this correlation analysis.

3.5. Relationship between melting parameters before and after annealing

Interesting relationships between the increase in the gelatinization parameters obtained by annealing (ANN3 h ΔTo, ANN3 h ΔTm, ANN24 h ΔTo, ANN24 h ΔTm) and the melting parameters of the native starches (To and Tm) were observed between the cereal starches from types 1 and 2 (Fig. 4). After 3 h and 24 h ANN, increases in To (Fig. 4a and b) and Tm (Fig. 4c and d) of these A-type polymorph cereal starches correlated negatively with their native gelatinization parameters (ANN3 h ΔTo: $r = -0.95$; ANN3 h ΔTm: $r = -0.95$; ANN24 h ΔTo: $r = -0.95$; ANN24 h ΔTm: $r = -0.94$, $p < 0.01$, $n = 7$). In other words, the higher the To or Tm, the lower the increase in these parameters. However, the gelatinization temperatures of native starches from type 3 and type 4 (as well as the SS from type 2) showed no correlation with temperature differences after annealing.

4. Discussion

In this study, the two-state model (native and molten) was used to describe the melting process of crystalline lamellae. Melting process was considered as quasi-equilibrium (heating rate: 2 °C/min) and DSC thermograms were rather symmetrical. The differences observed in the gelatinization parameters of native starches from

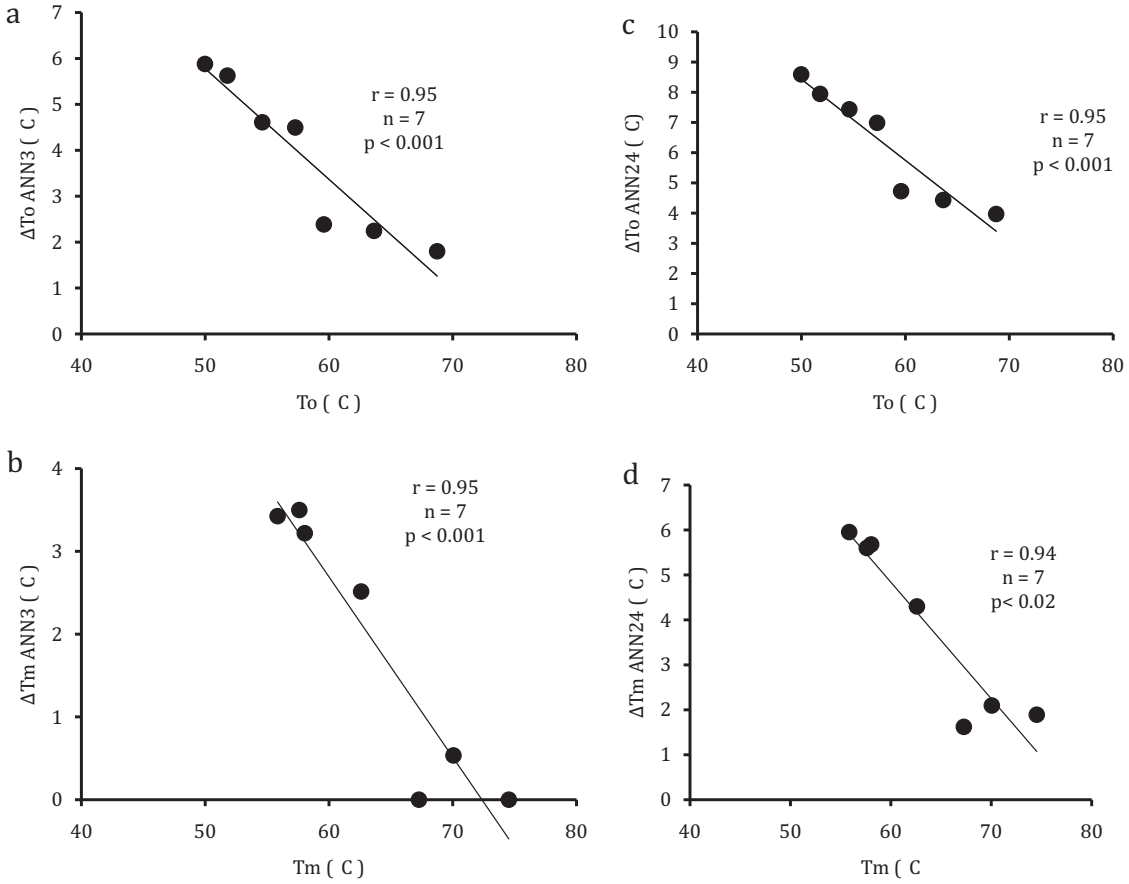

Fig. 4. Difference between gelatinization temperatures (annealed – native) versus native gelatinization temperatures: (a) ΔTo (annealed 3 h – native) vs. native To, (b) ΔTm (annealed 3 h – native) vs. native Tm, (c) ΔTo (annealed 24 h – native) vs. native To and (d) ΔTm (annealed 24 h – native) vs. native Tm.

four different amylopectin types have been shown to be influenced by molecular order (internal organization and alignment of the double helices), length of the external chains and amount of A_{fp}-chains (Vamadevan et al., 2013). Therefore, the higher melting temperatures (Tm) observed in yam (B-type), arrowroot (C-type) and rice (A-type) strongly suggest that the Tm of starch granules is not governed by polymorph type but is determined primarily by the internal structure of the amylopectin, which dictates the optimal packing of the double helices within the crystalline lamellae. This explanation seems logical, given the following context. In terms of the Thompson–Gibbs equation, the difference in the melting temperatures of semi-crystalline polymers can be expressed according to the crystal density (type of polymorph), the thickness of the crystalline lamellae, and the free surface energy of the crystal face (Wunderlich, 1980). Free surface energy is signified by the crystal defects (Bershtein & Egorov, 1994). Since starch granules are considered to be semi-crystalline biopolymers, the above three variables can be used to describe the changes in the melting temperature of the crystalline lamellae (Yuryev, Kozlov, Noda, Bertoft, & Blennow, 2007). However, previous studies have reported that decreases in crystal density accompany accumulating molecular defects (Vermeylen, Goderis, Reynaers, & Delcour, 2004; Yuryev et al., 2007), an effect that suggests that crystal density does not depend solely on the type of polymorph. An additional suggestion is that the water trapped within the A-polymorphs has very low mobility, whereas B-polymorphs have both trapped water with low mobility and weakly bound water that fills the central channel within the crystallites (Bogracheva et al., 2002). However, using proton NMR techniques, Tang, Godward, and Hills (2000) demonstrated the existence of three water populations in B-type starch:water systems, which are assigned to water molecules in the amorphous growth ring; the semi-crystalline lamellae; and the central channel. It has been postulated that accumulated defects in native granules act as a canal that can be easily filled by water (Kiseleva et al., 2003). The assumption would therefore be that the water population assigned (weakly bound) in the semi-crystalline lamellae (Tang et al., 2000) is possibly influenced by the quantity and type of defects in the crystalline lamellae. Such weakly bound water would increase plasticization during heating and destabilize the crystal at lower temperatures. The relatively very low Tm observed in Andean yam starch (Table 1a) in type 1 probably corresponded to the largest number of A_{fp}-chains (Table S1). This correlation seems plausible since lintnerized waxy maize and waxy potato showed similar gelatinization temperatures after ANN, even though waxy potato has a larger number of dangling chains than does waxy maize (Genkina et al., 2007). The authors came to the same conclusion: physical defects in the crystals have a major impact on starch gelatinization temperature irrespective of the polymorphic pattern.

The assembly of crystalline lamellae has also been considered to be negatively influenced by (1) long amylopectin chains (DP > 40) that are interspersed into the crystalline lamellae (Sanderson et al., 2006), (2) double helices between the A-chains and long B-chains, and by (3) amylose tie chains (Jenkins & Donald, 1995; Sanderson et al., 2006; Yuryev et al., 2004).

However, the formation of higher-order starch granular structure during biosynthesis is determined not only by the molecular structure, but also by the crystallization conditions, such as the crystallization temperature or the growth temperature. Many researchers have studied the effects of growth temperature on structural features and physicochemical properties, and they have found that increased growth temperatures decrease crystal defects (Kiseleva et al., 2003; Protserov et al., 2002; Tester, 1997; Tester & Karkalas, 2001; Tester, South, Morrison, & Ellis, 1991). This phenomenon has been described as in vivo annealing (Tester, Debon, & Karkalas, 1998).

4.1. General impact of annealing on the granular structure of starch

It has been proposed that the Tm represents a measure of crystalline perfection (Tester, 1997; Tester & Morrison, 1990). Since no polymorphic change has been reported after annealing (Jayakody & Hoover, 2008), based on the Thompson–Gibbs equation, the increased Tm and narrowed gelatinization endotherm can be attributed to decreased crystal defects and to increased thickness of the crystallites.

In a broad sense, defects accumulated during biosynthesis can be categorized as either molecular or structural defects (Genkina et al., 2007). Non-equilibrium conditions during biosynthesis probably cause structural defects, such as unpacked double helices within crystalline lamellae, unordered ends of double helices, and untwisted ends of double helices (Koroteeva et al., 2007). Based on the relationship observed between the internal structure and the melting parameters of native starches, it has been hypothesized that the packing of the double helix register is influenced primarily by molecular order (IB-CL, NBbl). However, crystallization conditions likely influence the number of unpacked double helices. Since amylopectin chain length distribution (covalent bonds) is unaffected by annealing conditions, ANN cannot eliminate the molecular defects, but equilibrium conditions during ANN can facilitate the energy that promotes conformations and reorientations. The decrease in crystal defects after annealing can diminish the free surface energy of the crystal face.

The increase of crystallite thickness has been postulated to be due to (1) an improvement in double helix registration (Genkina et al., 2004a, 2007; Kiseleva et al., 2004); (2) a lengthening of double helices through the twisting of unordered ends in pre-existing double helices (Genkina, Wasserman, & Yuryev, 2004); and (3) an ordering of the unordered ends of double helices (Genkina et al., 2007; Kozlov et al., 2007). A schematic model depicting the structural parameters and proposed crystal defects is shown in Fig. 5.

4.2. Impact of annealing on starches from different amylopectin types

The following explanation can account for the greater increase after 3 h ANN observed in the Tm of type 1 starches relative to that in the starches in the other types. Model 1 (Vamadevan et al., 2013) shows that unfavorable spacer arms/short IB-CLs increase the number of non-parallel double helices during biosynthesis, with a greater effect on type 1 starches than on those of the other types. Since type 1 starches are characterized by large cluster sizes and a greater number of building blocks (Bertoft et al., 2012), a reasonable assumption would be that the number of disoriented (out of the crystalline register) double helices in type 1 starches is higher than in the other types (Fig. 6). Disoriented (unpacked) double helices have been considered to be located within the crystalline lamellae but not to participate in the formation of crystallites (Kozlov et al., 2007). As discussed earlier, annealing cannot change the length of the spacer arms, which, when short, do not promote the parallel packing of double helices. ANN therefore cannot improve the parallel packing within the crystalline lamellae of type 1 starches. However, equilibrium conditions during annealing could facilitate the reorientation of unpacked double helices within the crystalline register, which possibly decreased the heterogeneity of the crystallites as indicated by the narrowed endotherm (increased PHI) (Table 1a) and increased the Tm of the crystallites (Table 1a) by increasing the crystallite thickness (Fig. 6).

Next to type 1, type 3 and type 4 starches showed the highest response to annealing. It is relevant to note that the interblock segments of type 3 and type 4 starches are relatively longer than those of type 1 or type 2 (Bertoft et al., 2012) (Table S1). Longer IB-CL

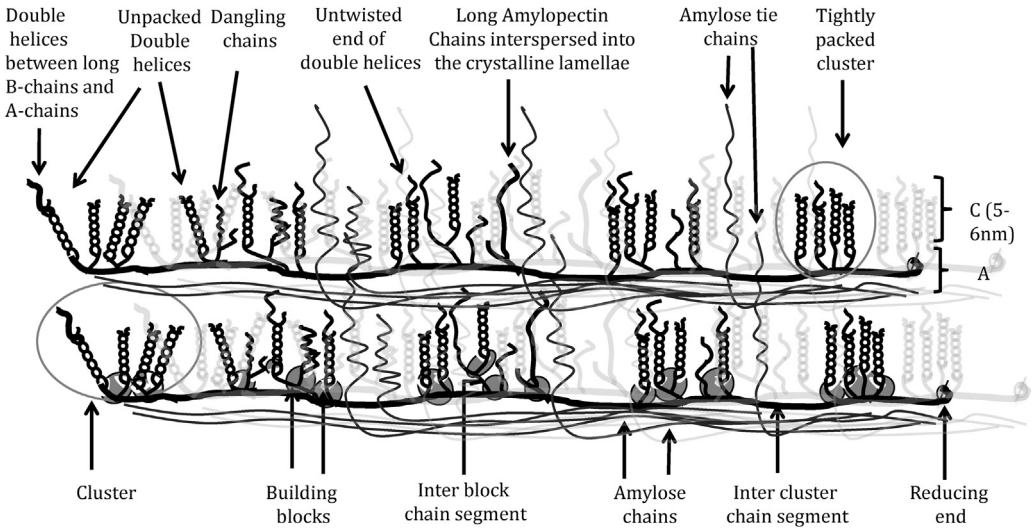

Fig. 5. Schematic model of the backbone structure of amylopectin highlighting specific internal structures and proposed crystal defects. C: crystalline lamella; A: amorphous lamella. Gray indicates another amylopectin molecule participating in the semi-crystalline arrangement in the starch granule.

may provide more room for either the reorganization of unpacked double helices or the parallel packing of chains within a cluster during ANN. On the other hand, the presence of longer external chains in type 3 and type 4 may increase the possibility of holding (1) a higher number of disordered ends, which may be associated with amorphous lamellae; (2) a larger number of untwisted ends of double helices; and (3) splayed double helices, which are not paralleled packed but are packed within the crystalline register (Fig. 7).

The absence of any observed change in the Tm of type 2 starches after ANN (Table 1b) indicates that crystallite thickness was unaffected by annealing. This observation agrees with the results presented in other published studies of waxy maize starch (Genkina et al., 2007), although the annealing conditions described were different from the ones employed in this work. However, the narrowed endotherm (increased PHI) observed in type 2 starches after ANN suggests that they may have fewer unpacked double helices (Table 1b). Sanderson et al. (2006) used SAXS modeling to demonstrate that waxy maize starches contain fewer splayed mesogens (double helices tilted away from a preferred orientation) than do C-type and B-type starches. Based on this observation, the authors postulated that the degree of double helix splay is related to the length of the mesogen (double helix) unit.

A decrease in the heterogeneity of types 3 and 4 starches following 3 h ANN, as reflected by lower PHI values, therefore, primarily indicates either reorientation of unpacked double helices or improved parallel packing of double helices (aligning of the splayed double helices) within the crystalline register. This theory seems plausible when contrasted with types 1 and 2 starches; (1) little or no increase in enthalpy (RS, MRS) was observed in type 1 or type 2 starches; (2) as evidenced by an increase in the PHI, in

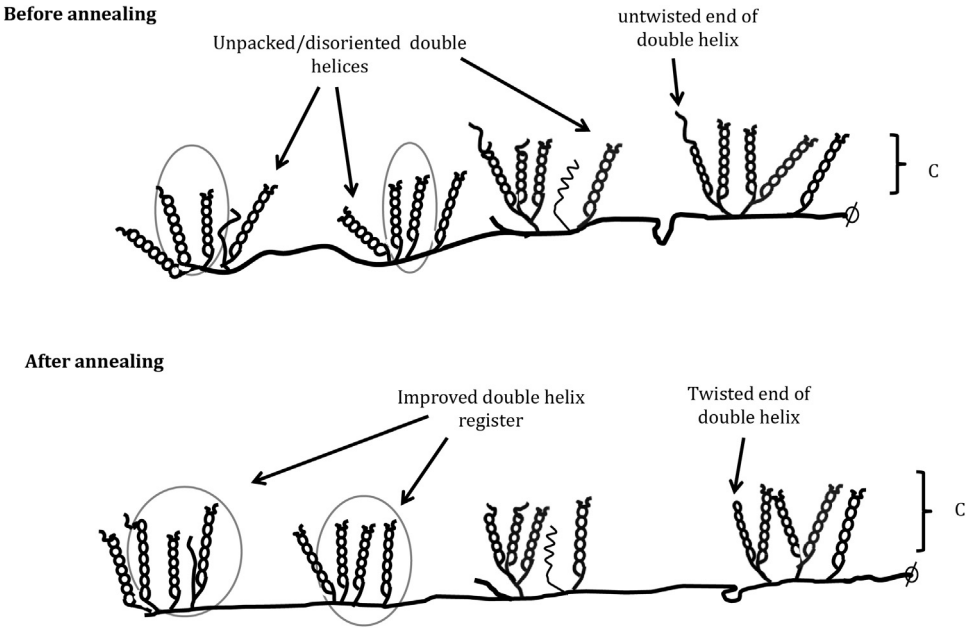

Fig. 6. Schematic diagram for organization of double helices in the crystalline lamella before and after annealing. Thick lines trace the backbone of amylopectin molecule – model for type 1 starches.

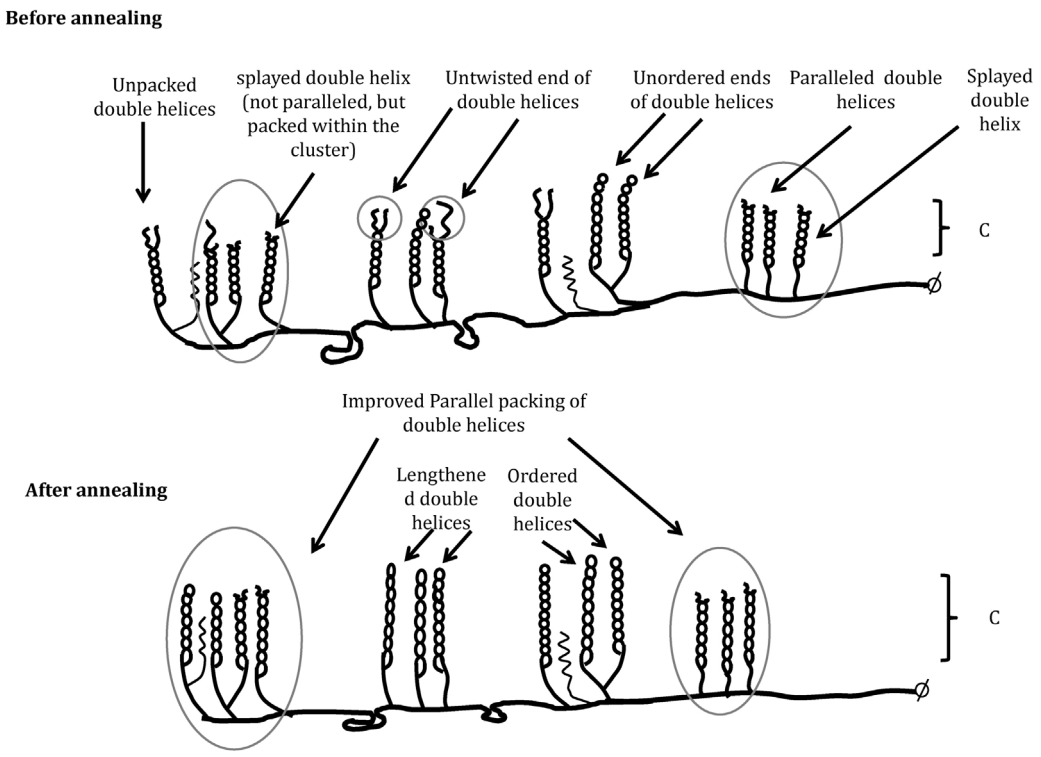

Fig. 7. Schematic diagram for organization of double helices in the crystalline lamella before and after annealing. Thick lines trace the backbone of amylopectin molecule – model for type 4 starches.

type 2 starches, the gelatinization endotherm narrowed without a change in the Tm. Furthermore, the narrowing of the gelatinization endotherm (increase in PHI) in type 3 and type 4 starches was accompanied by increasing enthalpy. The enthalpy of gelatinization represents the rupture of the hydrogen bonds that stabilize amylopectin double helices (Cooke & Gidley, 1992). No difference in the gelatinization enthalpy indicates that no change has occurred in either the double helical order or in the number of hydrogen bond links. Increased gelatinization enthalpy after annealing could be explained by (1) an increase in the number of double helices, (2) an increased number of stabilizing hydrogen bonds or intermolecular interactions between double helices (Kiseleva et al., 2005; Tester et al., 1998), and (3) a lengthening of the double helices through the optimization of the intrahelical bonds (Kiseleva et al., 2004; Tester, Debon, & Sommerville, 2000). However, most studies have shown that the number of double helices remained constant after annealing (Kiseleva et al., 2005; Tester et al., 1998; Tester & Debon, 2000). An increase in the Tm that is unaccompanied by a change in the gelatinization enthalpy could therefore be related to the optimization of the packing of the double helices that were disoriented (unpacked) during biosynthesis, and the ordering of the ends of existing double helices that were unpacked into crystallites (Kozlov et al., 2007). Interestingly, 3 h ANN radically increased the ΔH and PHI of ARS (type 3) without changing the Tm. This result suggests that the crystallite thickness of ARS was unaffected by 3 h ANN, and hence the insignificant change in the Tm possibly reflects a small number of structural defects within the crystalline lamellae, whereas the increased ΔH could be related to the formation of new stabilizing H-bonds between improved parallel double helices. This finding was not surprising because ARS has a C-type crystal pattern, coupled with longer IB-CLs compared to type 2 starches and the lowest number of A_{fp}-chains (Table S1). This combination of characteristics probably improved the close packing of the double helices within specific areas of the crystalline lamellae.

When the incubation time was increased to 24 h, the same general trend was identified with respect to changes in the gelatinization temperatures. However, 24 h ANN significantly increased the Tm and the gelatinization enthalpy of all starches, with the exception of OS. The increase in the Tm accompanied by the significant increase in gelatinization enthalpy after 24 h ANN indicates that the longer incubation likely increases the mobility of the double helices within the crystallite to a greater extent. However, it was interesting to observe that the amount of change in gelatinization enthalpy differed significantly among the amylopectin types. Greater changes were observed in types 3 and 4 starches than in types 1 and 2 cereal starches. However, yam and waxy potato starches exhibited smaller changes than other starches from type 3 or type 4. It should be noted that in their native states, yam and waxy potato starches have a higher PHI than any other starches. Since the PHI reflects the degree of homogeneity, the change observed suggests that yam and waxy potato starches are highly ordered in nature. With respect to the degree of change in enthalpy, the greater difference between starches in types 1 and 2, on the one hand, and those in types 3 and 4, on the other, was not surprising. As shown in Fig. 3, IB-CL correlated positively ($r = 0.93$, $p < 0.01$) with the change in enthalpy. As discussed earlier, annealing cannot improve the parallel packing of double helices in type 1 starches to a more significant extent because of the physical strain caused by the large number of building blocks and the shorter IB-CL. As noted above, type 2 starches may have a greater structural order in their native state, since few or no changes were observed after ANN. Greater improvement within clusters would therefore be expected in starches from type 3 and type 4. Improved parallel packing would increase the connections between the double helices provided by the H-bonds. However, the extent of the increase would depend on the existing level of crystalline perfection. The increased enthalpy after annealing could thus be related to the lengthening and alignment of the double helices within the cluster.

The negative correlation observed between the melting parameters (To, Tm) of native starches from type 1 and type 2 (A-type polymorphs) and the extent of the increase in these parameters caused by annealing indicates that the extent of change depends on the existing crystalline order (degree of crystalline perfection) and on the type of defects present. Tester et al. (1998) postulated that the least perfect crystallites in starches have the greatest potential to be reordered. According to the proposed model, in type 1 starches, the structural improvement took place primarily between clusters through improvement in the alignment of unpacked double helices (Fig. 6). However, the gelatinization temperatures of native starches from type 3 and type 4 showed no correlation with the amount of change after annealing. This suggests that in types 3 and 4 starches, structural improvement occurred within the cluster through improvement in the alignment of splayed double helices and in the ordering the ends of untwisted and unordered double helices (Fig. 7).

5. Conclusion

A short IB-CL (unfavorable spacer arm) has a negative impact on double helix registration because of the increased number of unpacked double helices within the crystalline register. The increases in Tm and PHI and the absence of change in enthalpy after ANN suggest that, in their native state, type 1 starches are characterized by a greater number of double helices out of the crystalline register. The smaller impact of annealing observed in type 2 starches indicates that they possess fewer diminishable crystal defects. This possibility suggests that the DP of the IB-CL and the external chains of type 2 starches in their native state are optimal for the assembly of crystalline lamellae with fewer structural defects. The flexibility of the longer IB-CL and external chains probably also contributes to the greater increase in enthalpy in types 3 and 4 starches. Overall, the results show that annealing can be used as a probe for the exploration of the dynamic molecular organization of native starches.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.07.006.

References

Alvani, K., Qi, X., & Tester, R. F. (2012). Gelatinisation properties of native and annealed potato starches. *Starch – Stärke*, *64*(4), 297–303.

Bertoft, E. (2004). On the nature of categories of chains in amylopectin and their connection to the super helix model. *Carbohydrate Polymers*, *57*(2), 211–224.

Bertoft, E., Koch, K., & Aman, P. (2012). Building block organisation of clusters in amylopectin from different structural types. *International Journal of Biological Macromolecules*, *50*(5), 1212–1223.

Bertoft, E., Laohaphatanalert, K., Piyachomkwan, K., & Sriroth, K. (2010). The fine structure of cassava starch amylopectin. Part 2: Building block structure of clusters. *International Journal of Biological Macromolecules*, *47*(3), 325–335.

Bertoft, E., Piyachomkwan, K., Chatakanonda, P., & Sriroth, K. (2008). Internal unit chain composition in amylopectins. *Carbohydrate Polymers*, *74*(3), 527–543.

Bogracheva, T. Y., Wang, Y. L., Wang, T. L., & Hedley, C. L. (2002). Structural studies of starches with different water contents. *Biopolymers*, *64*(5), 268–281.

Bershtein, V. A., & Egorov, V. M. (1994). In T. J. Kemp (Ed.), *Differential Scanning Calorimetry of Polymers. Physics, Chemistry, Analysis Technology* (p. 253). New York/London/Toronto/Sydney/Singapore: Ellis Horwood.

Chauhan, F., & Seetharaman, K. (2013). On the organization of chains in amylopectin. *Starch – Stärke*, *65*(3–4), 191–199.

Cooke, D., & Gidley, M. J. (1992). Loss of crystalline and molecular order during starch gelatinisation: origin of the enthalpic transition. *Carbohydrate Research*, *227*(0), 103–112.

French, D. (1972). Fine Structure of Starch and its Relationship to the Organization of Starch Granules. *Journal of the Japanese Society of Starch Science*, *19*(1), 8–25.

French, D. (1984). Organization of starch granules. In R. L. Whistler, J. N. BeMiller, & E. F. Paschall (Eds.), *Starch: Chemistry and technology* (2nd ed., pp. 183–207). San Diego: Academic Press.

Genkina, N. K., Wasserman, L. A., Noda, T., Tester, R. F., & Yuryev, V. P. (2004). Effects of annealing on the polymorphic structure of starches from sweet potatoes (Ayamurasaki and Sunnyred cultivars) grown at various soil temperatures. *Carbohydrate Research*, *339*(6), 1093–1098.

Genkina, N. K., Wasserman, L. A., & Yuryev, V. P. (2004). Annealing of starches from potato tubers grown at different environmental temperatures. Effect of heating duration. *Carbohydrate Polymers*, *56*(3), 367–370.

Genkina, N. K., Wikman, J., Bertoft, E., & Yuryev, V. P. (2007). Effects of structural imperfection on gelatinization characteristics of amylopectin starches with A- and B-type crystallinity. *Biomacromolecules*, *8*(7), 2329–2335.

Gerard, C., Colonna, P., Buleon, A., & Planchot, V. (2002). Order in maize mutant starches revealed by mild acid hydrolysis. *Carbohydrate Polymers*, *48*(2), 131–141.

Gernat, C., Radosta, S., Anger, H., & Damaschun, G. (1993). Crystalline parts of three different conformations detected in native and enzymatically degraded starches. *Starch – Stärke*, *45*(9), 309–314.

Hizukuri, S. (1986). Plolymodal distribution of the chain lengths of amylopectins, and its significance. *Carbohydrate Research*, *147*(2), 342–347.

Hoover, R. (2010). The impact of heat-moisture treatment on molecular structures and properties of starches isolated from different botanical sources. *Critical Reviews in Food Science and Nutrition*, *50*(9), 835–847.

Jacobs, H., Mischenko, N., Koch, M. H. J., Eerlingen, R. C., Delcour, J. A., & Reynaers, H. (1998). Evaluation of the impact of annealing on gelatinisation at intermediate water content of wheat and potato starches: A differential scanning calorimetry and small angle X-ray scattering study. *Carbohydrate Research*, *306*(1–2), 1–10.

Jayakody, L., & Hoover, R. (2008). Effect of annealing on the molecular structure and physicochemical properties of starches from different botanical origins – A review. *Carbohydrate Polymers*, *74*(3), 691–703.

Jenkins, P. J., Cameron, R. E., & Donald, A. M. (1993). A universal feature in the structure of starch granules from different botanical sources. *Starch – Stärke*, *45*(12), 417–420.

Jenkins, P. J., & Donald, A. M. (1995). The influence of amylose on starch granule structure. *International Journal of Biological Macromolecules*, *17*(6), 315–321.

Kiseleva, V. I., Genkina, N. K., Tester, R., Wasserman, L. A., Popov, A. A., & Yuryev, V. P. (2004). Annealing of normal, low and high amylose starches extracted from barley cultivars grown under different environmental conditions. *Carbohydrate Polymers*, *56*(2), 157–168.

Kiseleva, V. I., Krivandin, A. V., Fornal, J., Blaszczak, W., Jelinski, T., & Yuryev, V. P. (2005). Annealing of normal and mutant wheat starches. LM, SEM, DSC, and SAXS studies. *Carbohydrate Research*, *340*(1), 75–83.

Kiseleva, V. I., Tester, R. F., Wasserman, L. A., Krivandin, A. V., Popov, A. A., & Yuryev, V. P. (2003). Influence of growth temperature on the structure and thermodynamic parameters of barley starches. *Carbohydrate Polymers*, *51*(4), 407–415.

Kohyama, K., & Sasaki, T. (2006). Differential scanning calorimetry and a model calculation of starches annealed at 20 and 50 °C. *Carbohydrate polymers.*, *63*(1), 82–88.

Koroteeva, D. A., Kiseleva, V. I., Krivandin, A. V., Shatalova, O. V., Błaszczak, W., Bertoft, E., et al. (2007). Structural and thermodynamic properties of rice starches with different genetic background: Part 2. Defectiveness of different supramolecular structures in starch granules. *International Journal of Biological Macromolecules*, *41*(5), 534–547.

Kozlov, S. S., Krivandin, A. V., Shatalova, O., Noda, T., Bertoft, E., Fornal, J., et al. (2007). Structure of starches extracted from near-isogenic wheat lines. *Journal of Thermal Analysis and Calorimetry*, *87*(2), 575–584.

Krueger, B. R., Knutson, C. A., Inglett, G. E., & Walker, C. E. (1987). A differential scanning calorimetry study on the effect of annealing on gelatinization behavior of corn starch. *Journal of Food Science*, *52*(3), 715–718.

Nikuni, Z. (1978). Studies on starch granules. *Starke*, *30*(4), 105–111.

Pérez, S., & Bertoft, E. (2010). The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review. *Starch – Stärke*, *62*(8), 389–420.

Protserov, V. A., Wasserman, L. A., Tester, R. F., Debon, S. J. J., Ezernitskaja, M. G., & Yuryev, V. P. (2002). Thermodynamic and structural properties of starches extracted from potatoes grown at different environmental temperatures. *Carbohydrate Polymers*, *49*(3), 271–279.

Robin, J. P., Mercier, C., Charbonniere, R., & Guilbot, A. (1974). Lintnerized starches. Gel filtration and enzymatic studies of insoluble residues from prolonged acid treatment of potato starch. *Cereal Chemistry*, *51*(3), 389–406.

Sanderson, J. S., Daniels, R. D., Donald, A. M., Blennow, A., & Engelsen, S. B. (2006). Exploratory SAXS and HPAEC-PAD studies of starches from diverse plant genotypes. *Carbohydrate Polymers*, *64*(3), 433–443.

Tang, H. R., Godward, J., & Hills, B. (2000). The distribution of water in native starch granules – multinuclear NMR study. *Carbohydrate Polymers*, *43*(4), 375–387.

Tester, R. F. (1997). Influence of growth conditions on barley starch properties. *International Journal of Biological Macromolecules*, *21*(1–2), 37–45.

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

Tester, R. F., Debon, S. J. J., & Karkalas, J. (1998). Annealing of wheat starch. *Journal of Cereal Science*, *28*(3), 259–272.

Tester, R. F., Debon, S. J. J., & Sommerville, M. D. (2000). Annealing of maize starch. *Carbohydrate Polymers*, *42*(3), 287–299.

Tester, R. F., & Karkalas, J. (2001). The effects of environmental conditions on the structural features and physico-chemical properties of starches. *Starch – Stärke*, *53*(10), 513–519.

Tester, R. F., & Morrison, W. R. (1990). Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose, and lipids. *Cereal Chemistry*, *67*(6), 551–557.

Tester, R. F., South, J. B., Morrison, W. R., & Ellis, R. P. (1991). The effects of ambient temperature during the grain-filling period on the composition and properties of starch from four barley genotypes. *Journal of Cereal Science*, *13*(2), 113–127.

Vamadevan, V., Bertoft, E., & Seetharaman, K. (2013). On the importance of organization of glucan chains on thermal properties of starch. *Carbohydrate Polymers*, *92*(2), 1653–1659.

Vermeylen, R., Goderis, B., Reynaers, H., & Delcour, J. A. (2004). Amylopectin molecular structure reflected in macromolecular organization of granular starch. *Biomacromolecules*, *5*(5), 1775–1786.

Wunderlich, B. (1980). . *Macromoleculear physics* (Vol. 3) New York: Academic Press, Inc.

Yuryev, V. P., Kozlov, S. S., Noda, T., Bertoft, E., & Blennow, A. (2007). Influence of different GBSS 1 and GWD combinations on the amylose location within wheat and potato starch granules. In V. P. Yuryev, P. Tomasik, & E. Bertoft (Eds.), *Starch: Achievements in understanding of structure and functionality* (pp. 1–47). New York: Nova Science Publishers.

Yuryev, V. P., Krivandin, A. V., Kiseleva, V. I., Wasserman, L. A., Genkina, N. K., Fornal, J., et al. (2004). Structural parameters of amylopectin clusters and semi-crystalline growth rings in wheat starches with different amylose content. *Carbohydrate Research*, *339*(16), 2683–2691.