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# Impact of molecular structure of amylopectin and amylose on amylose chain association during cooling

Hyun-Jung Chung, Qiang Liu *

*Guelph Food Research Centre, Agriculture and Agri-Food Canada, 93 Stone Road West, Guelph, ON, Canada N1G 5C9*

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ABSTRACT

The effect of amylose molecular size and amylopectin branch chain length on thermal behavior of native starch, defatted starch and isolated amylose during cooling was studied. Six starches from corn, amylomaize, rice, potato, bean and pea with varying amylose content (20–70%) were heated to 180 °C at 10 °C/min and subsequently cooled at 10 °C/min to 5 °C in a differential scanning calorimeter (DSC) in excess water. On cooling of native corn and amylomaize starches, two distinct exotherms were observed at around 70 and 30 °C, which were attributed to amylose–lipid complex formation and amylose chain association, respectively. After defatting, a larger exotherm at a higher temperature during cooling was observed for corn and amylomaize starches, whereas bean and pea starches showed a smaller exotherm at a lower temperature. Amylomaize, with a large amount of lipid and longer amylopectin branch chains, exhibited the lowest onset temperature of amylose chain association in native and defatted starches among the tested starches, indicating the lipid and long branch chains in amylopectin restrict amylose chain association. The enthalpy of amylose chain association in defatted starch was positively correlated with apparent amylose content ($R^2 = 0.999$). In amylose isolated from each starch, a larger exotherm with a peak temperature around 60 °C was observed on cooling. Potato amylose, which had a long average chain length ($\overline{\mathrm{DP}}_n = 4915$), showed the lowest temperature and enthalpy of amylose chain association. The peak temperature of amylose chain association increased with increasing solid concentration of isolated amylose, whereas enthalpy reached a maximum at a concentration of 20–30% (w/w) solid.

## 1. Introduction

Starch consists of two polysaccharides, amylose and amylopectin. Amylose is essentially long linear chains composed of α-(1 → 4)-linked D-glucose units with a few branches (Hizukuri, Takeda, Yasuda, & Suzuki, 1981). Amylopectin has a larger molecular weight and much shorter chains of α-(1 → 4)-linked D-glucan which is highly branched through additional α-(1 → 6)-linked D-glucose linkages (Hizukuri, 1986). Most starches contain 20–30% amylose and 70–80% amylopectin, but the ratio varies with the botanical source of the starch. Starch granules have a semi-crystalline structure, comprised of crystalline and amorphous regions. The crystalline regions are believed to be densely packed structures, which are thought to involve the regions of amylopectin branches. Amylose is considered to be present predominantly in the amorphous regions of starch granules (Gidley & Bulpin, 1989).

Resistant starch (RS) has been defined as the fraction of starch not digested in the small intestine (Englyst, Kingman, & Cummings, 1992). RS is classified into four types according to the mechanism that prevents its enzymatic digestion. From these four types, RS type III is of particular interest since it preserves its nutritional functionality during most normal cooking processes, which enables its use as an ingredient in a wide variety of conventional foods (Haralampu, 2000). It is generally accepted that RS type III contains mainly retrograded amylose although retrograded amylopectin after debranching may also reduce enzyme susceptibility (Eerlingen & Delcour, 1995; Sievert, Czuchajowska, & Pomeranz, 1991). In the formation of RS type III, the starch granule is irreversibly destroyed and amylose is leached from granules into solution as a random coil polymer. Upon cooling, the polymer chains begin to reassociate as double helices. Upon further retrogradation, the double helices aggregate to form three-dimensional crystalline structure (Eerlingen & Delcour, 1995; Haralampu, 2000; Shamai, Bianco-Peled, & Shimoni, 2003; Sievert et al., 1991). This crystalline structure is very stable and shows a melting endotherm around 150 °C when heated in the differential scanning calorimeter in excess water (Ring, Colonna, et al., 1987; Sievert & Pomeranz, 1989; Sievert & Pomeranz, 1990).

In another study, Ziegler and colleagues reported that amylose spherulites was formed upon cooling when starch dispersion were heated to temperature >170 °C (Creek, Ziegler, & Runt, 2006; Nordmark & Ziegler, 2002; Ziegler, Creek, & Runt, 2005; Ziegler, Nordmark, & Woodling, 2003). The spherulites are semi-crystalline

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* Corresponding author. Tel.: +1 519 780 8030; fax: +1 519 826 2600.
*E-mail address:* liuq@agr.gc.ca (Q. Liu).

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entities with some degree of radial symmetry displaying 'Malteses cross' extinction pattern when viewed between crossed polarizer (Ziegler et al., 2003). The melting of spherulites has been observed by DSC thermograms around 60–100 °C in various starches (Ziegler et al., 2003) and 120–140 °C in isolated amylose (Creek et al., 2006). Spherulite formation has been favored in starches containing a greater percentage of linear material and exhibiting B-type X-ray diffraction patterns. The degree of crystallinity increases with amylose content (Creek et al., 2006; Ziegler et al., 2005).

Sievert and Wursch (1993a) observed that following melting of the amylose crystalline structure, an exothermic transition appeared at 40–50 °C during controlled cooling of potato amylose and RS type III starch. They suggested that the exothermic transition during cooling reflects the process of amylose chain association, which may be related to amylose aggregation and gelation. The exothermic transition during cooling has been studied using DSC (Boltz & Thompson, 1999; Sievert & Wursch, 1993a; Sievert & Wursch, 1993b). Sievert and Wursch (1993a) found that when the amylose/amylopectin mixtures in the range 0–95% were heated and cooled, amylose chain association was restricted by the presence of amylopectin. Sievert and Wursch (1993b) reported that the addition of lipids led to decreased amylose chain association and the interaction between amylose chains was reduced at cooling rate of greater 10 °C/min. Boltz and Thompson (1999) reported that when four high amylose maize starches were heated from 120 to 180 °C and cooled, a broad exotherm at around 95 °C was observed during the cooling for initial heating to 120 or 140 °C, whereas for initial heating to 160 and 180 °C, a sharper exotherm appeared below 55 °C. They also suggested that the lipid content and amylose content could influence amylose chain association. However, the effects of free and bound lipids in starch granule and the impact of molecular structure of amylose and amylopectin in various starches such as cereal, tuber and legume on amylose chain association during cooling have not been examined.

The objective of this research was to understand the effect of amylose/amylopectin ratio and lipids (free and bound) in various starches (normal corn, high amylose corn, rice, potato, bean and pea) on amylose chain association as observed during cooling, and to investigate the impact of amylose molecular size and amylopectin branch chain length on amylose chain association with native and defatted starches, and isolated amylose. We anticipate that this research work could be useful to determine optimal processing condition for creating RS type III from various starch sources for functional food products.

## 2. Materials and methods

### 2.1. Materials

Normal corn starch (Cat. No. S-4126), potato starch (Cat. No. S-4251), and rice starch (Cat. No. S-7260) were purchased from Sigma Chemical Co. (St. Louis, MO). High amylose maize starch (70% amylose, Hylon VII) was obtained from National Starch and Chemical Co. (Bridgewater, NJ). Bean (*Phaseolus vulgaris* L.: cultivar, *Majesty*) and pea (*Pisum sativum* L.: cultivar, *1674-13)* were obtained from the Department of Plant Agriculture, University of Guelph, and the Crop Development Centre, University of Saskatchewan, respectively. Bean and pea starches were extracted from seeds using the procedure of Chung et al. (2008).

### 2.2. Lipid content and defatted starch

Surface and bound lipid contents of native starch were determined by extraction at room temperature with chloroform–methanol (2:1 v/v) under vigorous agitation, and at elevated temperature (100 °C) with *n*-propanol-water (3:1 v/v), respectively, according to the procedure of Vasanthan and Hoover (1992).

Defatted starch was obtained by dispersion of starch in dimethyl sulfoxide (DMSO), followed by ethanol precipitation according to the procedure of Boltz and Thompson (1999). The starch sample in 90% DMSO (1.0% w/v) was heated in a boiling water bath for 1 h to gelatinize completely. The starch was precipitated using ethanol and centrifuged at 6000*g* for 10 min. The supernatant was decanted. The precipitate was washed with ethanol and acetone, and dried overnight in a 50 °C forced air oven.

### 2.3. Apparent amylose content

Apparent amylose content of native starch was measured by a concanavalin A (con A) method (Gibson, Solah, & McCleary, 1997) using an amylose/amylopectin assay kit (Megazyme International Ireland Ltd., Bray, Ireland).

### 2.4. Amylose isolation and characteristics of isolated amylose

The isolation of amylose from native starch was carried out following the procedure of Jane and Chen (1992). The average degree of polymerization ($\overline{\mathrm{DP}}_n$) of isolated amylose was determined by dividing total carbohydrate content by its reducing residue. Total carbohydrate content was analyzed by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The reducing residue was measured by a modified Park-Johnson procedure (Hizukuri et al., 1981). Iodine affinity of isolated amylose was measured using an automatic potentiometric titrator (DL 50, Mettler-Toledo Inc., Columbus, OH) at 25 °C (Schoch, 1964).

### 2.5. Amylopectin chain length distribution

The chain length distribution of amylopectin after debranching whole starch with isoamylase (Crystal form, Hayashibara Biochemical Lab. Inc., Okayama, Japan) was determined by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Dionex, Sunnyvale, CA) following the procedure of Liu, Gu, Donner, Tetlow, and Emes (2007).

### 2.6. Thermal analysis

Thermal analysis was carried out on native and defatted starches, and isolated amylose using a differential scanning calorimeter (2920 Modulated DSC, TA Instruments, New Castle, DE) equipped with a refrigerated cooling system. The sample (12 mg) and distilled water (28 μl, 70% moisture content) were added to a high-volume pan and immediately sealed. The sealed pan was allowed to equilibrate overnight at room temperature. The sample pan was heated from 5 to 180 °C at 10 °C/min and then cooled to 5 °C at 10 °C/min. The sample pan was immediately reheated to 180 °C at 10 °C/min. An empty pan was used as a reference.

### 2.7. 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 software SPSS V. 8.2 (SPSS Institute Inc., Cary, NC).

## 3. Results and discussion

### 3.1. Starch lipid content

The free and bound lipid contents of native starches were in the range 23–163 and 71–1021 mg/100 g, respectively (Table 1). The

**Table 1**
Lipid content of native starches (mg of lipid/100 g of dry starch) [A].

| Starch | Free lipid | Bound lipid |
|---|---|---|
| Corn | 61 ± 1$^{c}$ | 567 ± 9$^{c}$ |
| Amylomaize | 163 ± 17$^{a}$ | 1021 ± 31$^{a}$ |
| Rice | 86 ± 0$^{b}$ | 722 ± 3$^{b}$ |
| Potato | 25 ± 3$^{e}$ | 71 ± 1$^{d}$ |
| Bean | 23 ± 0$^{e}$ | 104 ± 5$^{d}$ |
| Pea | 36 ± 1$^{d}$ | 88 ± 1$^{d}$ |

[A] Values followed by a different superscript in each column are significantly different ($P < 0.05$).

free lipid content followed the order: amylomaize > rice > corn > pea > potato ≈ bean. The bound lipid content followed the order: amylomaize > rice > corn > bean > pea > potato. These results are in agreement with those reported by Vasanthan and Hoover (1992) for cereal, legume, tuber and root starches, and by Boltz and Thompson (1999) for corn starches.

### 3.2. Characteristics of amylose

The apparent amylose content of native starch determined by concanavalin A method followed the order: amylomaize (68.9%) > pea (34.5%) > bean (33.4%) > corn (26.9%) > rice (21.5%) > potato (20.6%) (Table 2). Iodine affinities of isolated amylose were in the range 19.4–20.6, which indicated a high purity of isolated amylose, since theoretical iodine affinity for pure amylose was assigned as 20 (Jane & Chen, 1992). The average degree of polymerization ($\overline{\mathrm{DP}}_n$) of isolated amylose followed the order: potato (4915) > pea (1410) > bean (1244) > corn (1041) > rice (964) > amylomaize (850). These results were comparable to those reported as 4920 by Takeda, Shirasaka, and Hizukuri (1984), 1400 by Biliaderis, Grant, and Vose (1981), 1300 by Biliaderis et al. (1981), 990 by Takeda, Shitaozono, and Hizukuri (1988), 1100 by Takeda, Hizukuri, and Juliano (1986), and 690 by Takeda, Takeda, and Hizukuri (1993) for potato, pea, bean, corn, rice and amylomaize, respectively.

### 3.3. Amylopectin chain length distribution

The amylopectin chain length distribution of native starches is presented in Table 3. The average chain length of amylopectin followed the order: B-type starch [amylomaize (23.1) > potato (21.7)] > C-type starch [pea (20.7) > bean (20.4)] > A-type starch [corn (19.8) > rice (18.2)]. Jane et al. (1999) also reported that B-type starch had longer chains than A-type starch. The rice and corn starches (A-type starch) had a greater proportion of short chains (DP 6–12) and a smaller proportion of long chains (DP ⩾ 37) than potato and amylomaize starches (B-type starch). The pea and bean starches (C-type starch) had intermediate amounts of both short and long branch chains. These results are in agreement with those reported by Jane et al. (1999).

**Table 2**
Apparent amylose content of native starches and iodine affinity and average degree polymerization ($\overline{\mathrm{DP}}_n$) of isolated amylose[A].

| Starch | Apparent amylose content (%) | Isolated amylose | |
|---|---|---|---|
| | | IA[B] | $\overline{\mathrm{DP}}_n$[C] |
| Corn | 26.9 ± 0.1$^{d}$ | 19.5 ± 0.2$^{bc}$ | 1041 ± 32$^{d}$ |
| Amylomaize | 68.9 ± 0.6$^{a}$ | 20.6 ± 0.2$^{a}$ | 850 ± 12$^{f}$ |
| Rice | 21.5 ± 0.1$^{e}$ | 19.7 ± 0.5$^{bc}$ | 964 ± 14$^{e}$ |
| Potato | 20.6 ± 0.3$^{f}$ | 19.4 ± 0.1$^{c}$ | 4915 ± 64$^{a}$ |
| Bean | 33.4 ± 0.4$^{c}$ | 19.9 ± 0.2$^{b}$ | 1244 ± 1$^{c}$ |
| Pea | 34.5 ± 0.2$^{b}$ | 19.9 ± 0.1$^{b}$ | 1410 ± 16$^{b}$ |

[A] Values followed by a different superscript in each column are significantly different ($P < 0.05$).
[B] Iodine affinity.
[C] Average degree of polymerization ($\overline{\mathrm{DP}}_n$).

### 3.4. Thermal characteristics of native starch

DSC heating thermograms of native starch showed dual endothermic peaks for corn, amylomaize, and rice starches, and a single endothermic peak for potato, bean, and pea starches (Fig. 1A). The low (60–80 °C) and high (90–110 °C) temperature endotherms were attributed to starch gelatinization and melting of the amylose–lipid complex, respectively.

On cooling of corn and amylomaize starches, two exothermic peaks were observed (Fig. 1B). The exotherm with a peak temperature around 75 °C was attributed to the formation of amylose–lipid complex (Boltz & Thompson, 1999; Sievert & Wursch, 1993a; Sievert & Wursch, 1993b). A second exotherm at 30 °C could be assigned to amylose chain association based on previous studies (Sievert & Wursch, 1993a; Sievert & Wursch, 1993b). Rice starch showed a single exotherm during cooling with a peak temperature around 75 °C, which was attributed to the formation of amylose–lipid complex. The absence of an exotherm at low temperature (around 30 °C) in rice starch could be due to its low apparent amylose content (Table 2). The enthalpy of formation of the amylose–lipid complex during cooling was 0.5, 1.9, and 1.0 J/g in corn, amylomaize, and rice, respectively (Fig. 1B). These enthalpies are positively correlated with free and bound lipid contents (Table 1). Potato starch exhibited no exotherm on cooling, in agreement with results of Sievert and Wursch (1993b). This was probably due to low lipid (Table 1) and apparent amylose content (Table 2). Bean and pea starches showed a single exotherm around 45 °C with absence of formation of the amylose–lipid complex due to low lipid content (Table 1).

The $T_o$, $T_p$ and $\Delta H$ of the second exotherm (30–50 °C), which reflected amylose chain association, are presented in Table 4. The $T_o$ and $T_p$ of amylose chain association in native starch followed the order: bean ⩾ pea > corn > amylomaize. This order is negatively correlated with lipid content of native starches (Table 1), which is consistent with other studies that show amylose chain association is hindered by lipid in starch (Boltz & Thompson, 1999; Czuchajowska, Sievert, & Pomeranz, 1991; Eerlingen, Cillen, & Delcour, 1994; Szczodrak & Pomeranz, 1992). During the initial heating to 180 °C, the ordered amylose structure and amylose–lipid complexation were disrupted (Eliasson, 1986). Subsequent cooling could induce the reordering of amylose and lipid. Starches with native lipid form amylose–lipid complexes preferentially over aggregates of double helical linear chain segments (amylose chain association) since the amylose–lipid complexation was kinetically favored (Czuchajowska et al., 1991). This amylose–lipid complex might sterically interfere with association of remaining amylose, resulting in a decrease in $T_p$ of the lower temperature exotherm. The $\Delta$H of the exothermic transition of amylose chain association was high for amylomaize (5.8 J/g) and lower for corn (1.0 J/g). This result confirmed that this exotherm during cooling was related to amylose content in native starch.

Subsequent reheating after cooling of corn, amylomaize and rice starches displayed a melting endotherm with a temperature of 90–120 °C (Fig. 1C). As observed initial heating curve, this endothermic transition could be attributed to the melting of amylose–lipid complexes because the enthalpy of endotherm was positively related to the lipid content (Table 1 vs. Fig. 1C). For corn and amylomaize starches, broad endotherm in the range of 120–160 °C was also observed. During cooling, the linear amylose chain could form a single or double helical structure, which could then contribute the double helical linear chain segments arranged into aggregates.

**Table 3**
Amylopectin branch chain length distributions of native starches[A].

| Starch | Average chain length | % Distribution | | | |
|---|---|---|---|---|---|
| | | DP [B] 6–12 | DP 13–24 | DP 25–36 | DP ⩾ 37 |
| Corn | 19.8 ± 0.1$^{e}$ | 24.2 ± 0.6$^{b}$ | 52.3 ± 0.3$^{b}$ | 14.5 ± 0.3$^{cd}$ | 9.1 ± 0.0$^{d}$ |
| Amylomaize | 23.1 ± 0.0$^{a}$ | 14.7 ± 0.3$^{e}$ | 48.3 ± 0.9$^{d}$ | 21.9 ± 0.8$^{a}$ | 15.1 ± 0.2$^{a}$ |
| Rice | 18.2 ± 0.2$^{f}$ | 31.4 ± 0.2$^{a}$ | 49.5 ± 0.4$^{c}$ | 11.5 ± 0.0$^{e}$ | 7.6 ± 0.6$^{e}$ |
| Potato | 21.7 ± 0.1$^{b}$ | 19.3 ± 0.1$^{d}$ | 52.2 ± 0.2$^{b}$ | 14.1 ± 0.1$^{d}$ | 14.5 ± 0.2$^{a}$ |
| Bean | 20.4 ± 0.0$^{d}$ | 20.2 ± 0.3$^{c}$ | 54.2 ± 0.1$^{a}$ | 16.1 ± 0.2$^{b}$ | 9.5 ± 0.0$^{c}$ |
| Pea | 20.7 ± 0.0$^{c}$ | 19.5 ± 0.2$^{cd}$ | 55.0 ± 0.2$^{a}$ | 15.1 ± 0.2$^{c}$ | 10.5 ± 0.2$^{b}$ |

[A] Values followed by a different superscript in each column are significantly different ($P < 0.05$).
[B] DP, degree of polymerization.

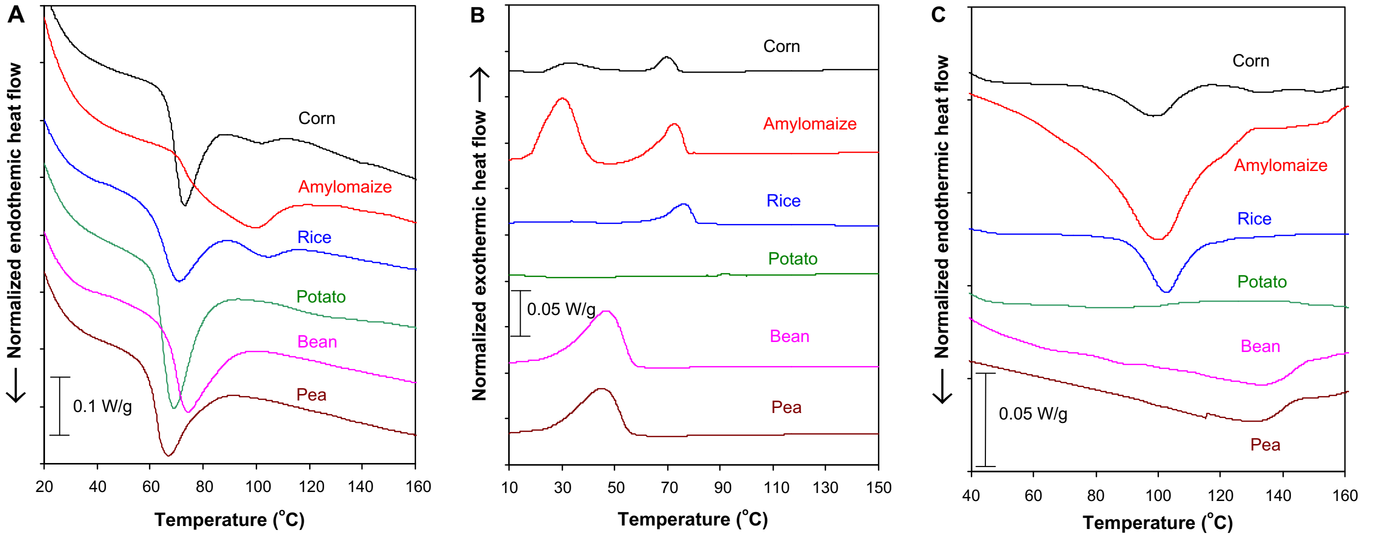

**Fig. 1.** DSC initial heating (A), subsequent cooling (B), and reheating (C) curves of native starches from corn, amylomaize, rice, potato, bean and pea.

**Table 4**
Thermal analysis of cooling curves of native and defatted starches and isolated amylose[A].

| Type | Starch | Amylose chain association | | |
|---|---|---|---|---|
| | | $T_o$ (°C) [B] | $T_p$ (°C) [B] | $\Delta H$ (J/g) [C] |
| Native starch | Corn | 47.5 ± 0.2$^{c}$ | 34.0 ± 1.8$^{b}$ | 1.0 ± 0.1$^{d}$ |
| | Amylomaize | 40.5 ± 0.6$^{d}$ | 29.8 ± 0.4$^{c}$ | 5.8 ± 0.1$^{a}$ |
| | Rice | nd[D] | nd | nd |
| | Potato | nd | nd | nd |
| | Bean | 56.8 ± 0.1$^{a}$ | 47.5 ± 0.1$^{a}$ | 4.8 ± 0.1$^{b}$ |
| | Pea | 54.4 ± 0.6$^{b}$ | 46.2 ± 0.9$^{a}$ | 3.8 ± 0.2$^{c}$ |
| Defatted starch | Corn | 56.7 ± 0.6$^{a}$ | 38.8 ± 1.3$^{a}$ | 1.8 ± 0.2$^{c}$ |
| | Amylomaize | 47.8 ± 0.6$^{c}$ | 39.0 ± 0.8$^{a}$ | 7.3 ± 0.3$^{a}$ |
| | Rice | nd | nd | nd |
| | Potato | nd | nd | nd |
| | Bean | 50.2 ± 0.9$^{b}$ | 39.9 ± 1.3$^{a}$ | 2.5 ± 0.2$^{b}$ |
| | Pea | 49.6 ± 0.5$^{b}$ | 37.4 ± 0.0$^{a}$ | 2.7 ± 0.3$^{b}$ |
| Isolated amylose | Corn | 68.5 ± 1.6$^{ab}$ | 60.1 ± 0.6$^{ab}$ | 13.3 ± 0.4$^{cd}$ |
| | Amylomaize | 67.0 ± 0.8$^{b}$ | 59.3 ± 0.6$^{b}$ | 16.9 ± 0.2$^{b}$ |
| | Rice | 66.1 ± 0.8$^{b}$ | 57.4 ± 0.3$^{c}$ | 13.9 ± 0.4$^{c}$ |
| | Potato | 62.6 ± 0.6$^{c}$ | 56.1 ± 0.4$^{d}$ | 13.1 ± 0.3$^{d}$ |
| | Bean | 69.8 ± 0.8$^{a}$ | 61.9 ± 1.2$^{a}$ | 17.8 ± 0.2$^{a}$ |
| | Pea | 67.7 ± 0.8$^{ab}$ | 58.6 ± 0.1$^{b}$ | 16.8 ± 0.1$^{b}$ |

[A] Values followed by a different superscript in each column among each type are significantly different ($P < 0.05$).
[B] $T_o$ and $T_p$ indicate the temperatures of the onset and peak of chain association, respectively.
[C] Enthalpy of chain association.
[D] Not detected.

These aggregates may be the structure (amylose crystallites) that is disrupted during reheating, resulted in the endothermic peak at 120–160 °C (Sievert & Pomeranz, 1989; Sievert & Pomeranz, 1990). Bean and pea starches also exhibited broad endotherm around 120–160 °C which could be attributed to the melting of amylose crystallites as observed in corn and amylomaize starch. Due to low lipid and amylose contents, potato starch showed no endotherm as observed in cooling curve.

### *3.5. Thermal characteristics of defatting in starch*

Native starch was defatted to avoid the formation of amylose–lipid complexes which could compete and interfere with amylose chain association. The defatted corn, amylomaize, bean and pea starches exhibited a distinct single exotherm during cooling, whereas the defatted potato and rice starches showed no exotherm, as seen for those native starches (Fig. 2A). This provided evidence that a single exotherm in rice starch (Fig. 1B) reflected the formation of amylose–lipid complexes.

It was interesting to observe that the $T_p$ of the exotherm of defatted starches was not significantly different among tested starch samples (Table 4). However, different result was reported by Sievert and Wursch (1993b) who found that the $T_p$ of the exotherm in defatted amylomaize, pea, and corn starches was 27.7, 36.4, and 19.4 °C, respectively. This discrepancy could be due to different defatting methods used for the analyses. Sievert and Wursch (1993b) removed the lipid with hot solvent under reflux. However, the disruption of granule structure should be required for the efficient removal of lipids from starch granules (Morrison & Conventry, 1985). It seems plausible that the solvent extraction could not properly deplete the lipid in native granular starch and thus the residual lipid could influence the amylose chain association in the above study. In the present work, lipid-free starch was prepared by gelatinization in DMSO. It can be suggested that the amylose chain association of lipid-free starch may occur at a similar peak temperature regardless of starch source.

The $T_o$ of the exotherm on cooling of defatted starch followed the order: corn (56.7 °C) > bean (50.2 °C) ≈ pea (49.6 °C) > amylomaize (47.8 °C). This was in reversed order with average chain length as well as proportion of long branch chains (DP $\geqslant$ 37) in amylopectin. This indicated that a greater proportion of long amylopectin chains in starch may influence the $T_o$ of amylose chain association on cooling. Boltz and Thompson (1999) suggested that the interaction between longer amylopectin chains and amylose might produce chain association during cooling and could contribute to the exotherm. However, amylomaize, which had a greater average chain length and higher proportion of long amylopectin chains (DP $\geqslant$ 37) (Table 3), showed the lowest $T_o$ of the exotherm among the defatted starches. Although the more branched amylopectin could contribute to the exotherm by self-association and interactions with amylose, we could not observe these contributions in the present work. Rather, the longer branch chain in amylopectin would serve as steric interference to amylose chain association, allowing the exotherm to occur at a low temperature on cooling.

The $T_o$, $T_p$ and $\Delta H$ of defatted corn and amylomaize starches increased compared to their native starches (Table 4). The extent of the increase in $T_p$ and $\Delta H$ of amylomaize (9.2 °C and 1.5 J/g) was higher than that of corn (4.4 °C and 0.8 J/g), possibly due to much higher lipid content in amylomaize starch. As mentioned above, the lipid in starch would hinder amylose chain association, and thus amylose in defatted starch could have a greater ability to associate during cooling, which would explain higher $T_o$, $T_p$ and $\Delta H$ in defatted starch. However, we observed much lower $\Delta H$ and exothermic transition temperatures ($T_o$ and $T_p$) of amylose chain association in defatted legume starch (bean and pea) compared to native starch (Table 4). Although the total lipid content of bean (127 mg/100 g) and pea (124 mg/100 g) starches was much lower that that of corn and amylomaize starches, some amylose could be involved in amylose–lipid complexes (Table 1). Thus, the amount of available amylose to form new single or double helices during cooling might be much greater after defatting pea and bean starches. Boltz and Thompson (1999) also reported a similar trend that the reduced-lipid high amylose corn starch had a narrower exotherm and an increase in exotherm enthalpy of amylose chain association when compared to its lipid-free starch. Lipid associated with starch has been found to be different among starch source (Vasanthan & Hoover, 1992). The ratio of bound lipid to total (bound + free) lipid was much higher in cereal starch than in legume starch (Vasanthan & Hoover, 1992), which is consistent with the present work (90%, 86%, and 89% in corn, amylomaize, and rice

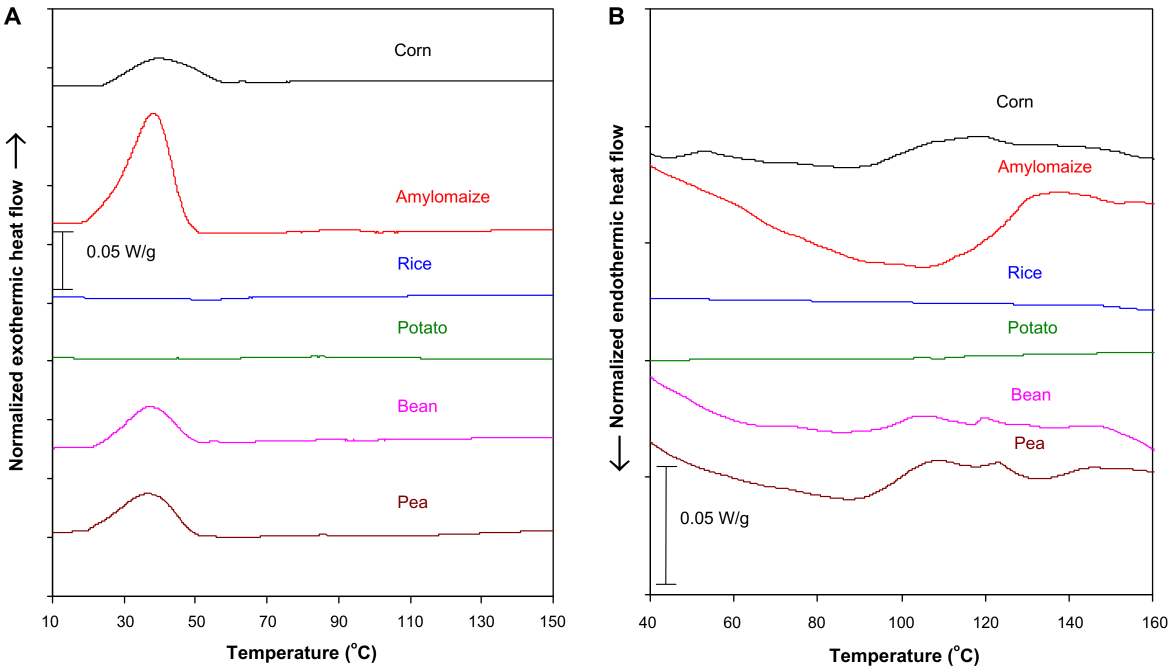

**Fig. 2.** DSC cooling (A) and subsequent reheating (B) curves of defatted starches from corn, amylomaize, rice, potato, bean and pea.

starches vs. 82% and 71% in bean and pea starches, which were calculated from Table 1). Furthermore, the monoacyl lipid content, which interacts most strongly with amylose helices, was greater in cereal starch than legume starch (Vasanthan & Hoover, 1992). Thus, the lipid in legume starch might not readily complex with amylose helices, and thus less amylose–lipid complex was present. Besides, the complexation of amylose–lipid in legume starch is loosely packed when compared to cereal starch. We assume that this amylose–lipid complexation in legume starch might rather serve as a nucleation site for amylose chain association than interfere with ordering of amylose. The nucleation site could be used as a substrate for the ordering of new double helices in amylose chains, resulting in the exotherm appearing at a higher temperature and increased amylose association. This seems plausible, since the amylose chain association of legume starch occurred at a lower temperature after removal of residual lipid, which means the absence of nucleation sites.

It is also interesting to observe that the correlation coefficient between amylose chain association enthalpy and amylose content was greater in defatted starch ($R^2 = 0.999$) than in native starch ($R^2 = 0.571$). We strongly suggest that the lipid in native starch substantially influenced the formation of amylose ordering during cooling, either by inhibition (corn and amylomaize) or promotion (bean and pea), which could result in a weaker relationship between amylose chain association enthalpy and amylose content in native starch. After removal of lipid, a positive correlation close to 1 was obtained, which is strong evidence that the exotherm at 30–60 °C during cooling could be attributed to the association of amylose chains.

No endothermic transition was observed during reheating of defatted rice and potato starches as shown in reheating profile of its native starch in DSC (Figs. 2B and 1C). For defatted corn, amylomaize, bean and pea starches, a broad endotherm with a temperature range of 60–120 °C was observed although the enthalpy was a lower than that observed in its native starch (Fig. 1C). The broad endotherm around 60–120 °C of amylomaize was larger than that of other starches (Fig. 2B). The defatted starch did not contain the amylose–lipid complexes, suggesting the endotherm observed around 60–120 °C could be due to the melting of spherulites. The formation of spherulites during cooling of gelatinized starch has been reported by several researches (Ring, Miles, Morris, Turner, & Colonna, 1987; Ziegler et al., 2003). Nordmark and Ziegler (2002) suggested that the melting endotherms of amylose–lipid complex and spherulites overlapped at 80–110 °C. Consequently, it can be suggested that the endothermic transition around 60–120 °C in reheating curve of native starches (Fig. 1C) was resulted from both melting of the amylose-complexes and spherulites, while the spherulites could be ruled out in endotherm (60–120 °C) of defatted starch (Fig. 2B). Ziegler et al. (2003) reported that spherulite formation was favored in starches with a higher percentage of linear polymers, which could support our result that the enthalpy of endotherm at 60–120 °C was positively correlated with amylose content (Fig. 2B). Ziegler et al. (2003) also postulated that starch exhibiting B or C-type crystallinity generally formed spherulites more easily than A-type starches since the B- or C-type starch contains more long linear chains or lightly branched structure. However, we have no evidence of spherulites formation of potato starch (B-type starch). This discrepancy in formation of spherulites in potato starch could be due to difference in sample condition. The sample were observed after 2 day storage at 20 °C following starch gelatinization (Ziegler et al., 2003), whereas our samples was subsequently reheated after cooling. This suggests that the potato starch, which contains relatively low amylose content but long branch chain in amylopectin, do not allow for spherulites formation due to limited time for diffusion and reorganization of linear fraction. For defatted corn, amylomaize, bean and pea starches, small endotherm with a temperature range of 120–160 °C was also present. This could be corresponded to melting amylose crystallites as discussed in native starches.

### 3.6. Thermal characteristics of purified amylose

The DSC cooling curves of isolated amylose from various starches are shown in Fig. 3A. The exotherm occurred between 30 and 70 °C with an enthalpy range of 13.1–17.8 J/g. These results were greater than those for defatted starch. Similar results have been reported by Sievert and Wursch (1993b) for potato amylose and various defatted starches (amylomaize, pea, maize and wheat). They claimed that amylopectin restricted the amylose chain association, possibly due to a dilution effect and steric hindrance, which was a result of the immiscibility of amylose and amylopectin in the aqueous solution. In the present study, the substantial increase in enthalpy and temperature of the exotherm when cooling isolated amylose compared to its counterpart of defatted starch could support the assumption described by Sievert and Wursch (1993b).

Potato amylose exhibited the lowest temperatures (62.6 °C for $T_o$ and 56.1 °C for $T_p$) of exotherm and the lowest enthalpy (13.1 J/g) among the isolated amyloses tested. The ordering of amylose chains is influenced by the amylose chain length (Gidley & Bulpin, 1989; Lu, Jane, & Keeling, 1997; Sievert & Wursch, 1993a). Lu et al. (1997) found that when the different molecular sized amylose subfactions prepared by enzyme hydrolysis were incubated at various temperatures, small molecular sized potato amylose had a higher retrogradation tendency. Gidley and Bulpin (1989) claimed that the rate of amylose association was substantially dependent on the chain length (degree of polymerization). Among the amyloses tested, the chain length of potato amylose ($\overline{\mathrm{DP}}_n = 4915$) was significantly long, while the $\overline{\mathrm{DP}}_n$ of other amyloses ranged between 850 and 1410. This longer potato amylose could reduce mobility during cooling, which might retard and restrict amylose chain association, resulting in lower temperature and enthalpy of the exothermic transition in the thermogram. Another possible explanation for the low temperature and enthalpy of potato amylose is the purity of isolated amylose from native starch. The isolated potato amylose had the lowest iodine affinity value of the tested amylose fractions (Table 2). This meant lower purity during isolation of amylose, and the presence of a small amount of amylopectin in isolated amylose. The residual amylopectin could inhibit amylose chain association in potato amylose as described previously.

The amylose from corn, amylomaize, rice, bean and pea exhibited marginal differences in $T_o$ and $T_p$ of the exotherm (Table 4). There was no significant relationship between amylose chain association and $\overline{\mathrm{DP}}_n$ of amylose. We assume that the differences in $\overline{\mathrm{DP}}_n$ of amylose from various starches, with the exception of potato amylose, could not lead to substantial changes in chain association. The $\Delta H$ of amylose chain association in amylomaize, bean and pea was over 16 J/g, whereas that from corn, rice, and potato was below 14 J/g. This could be explained by the iodine affinity, which is related to amylose purity, as mentioned above. The former three amyloses had an iodine affinity greater than 19.9, while the latter three had an iodine affinity less than 19.7.

Subsequent reheating after cooling resulted in the appearance of a broad endothermic peak around 130–160 °C in all amylose samples (Fig. 3B). Creek et al. (2006) showed that the melting temperature (120–150 °C) of spherulites in isolated amylose from maize starch was higher than those observed for native maize starch (60–100 °C) prepared under the same conditions (Ziegler et al., 2003), suggesting the greater linearity of the leached amylose and higher overall amylose content in isolated amylose from

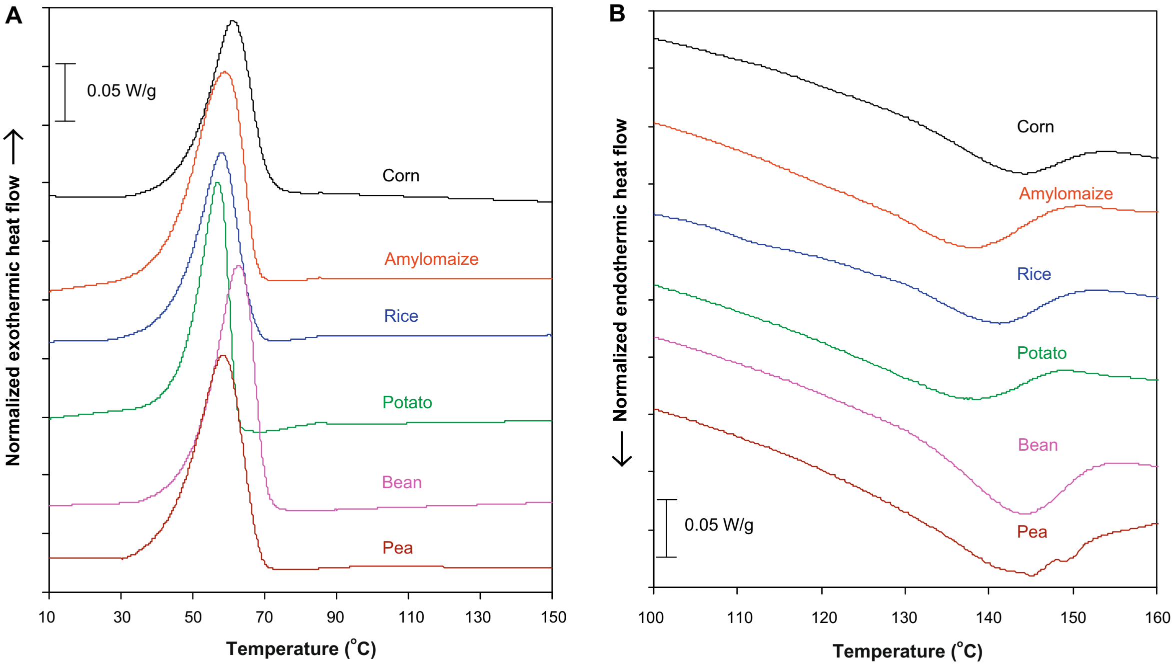

**Fig. 3.** DSC cooling (A) and subsequent reheating (B) curves of isolated amyloses from corn, amylomaize, rice, potato, bean and pea starches.

maize starch. The melting temperature of spherulites in isolated amylose from different starch sources in this study was higher than those observed in native or defatted starches (Fig. 3B vs. Fig. 1C or Fig. 2B) as observed by Creek et al. (2006). Thus, we assume that the melting endotherm of spherulites could be overlapped with melting of amylose crystallites, which usually observed at 140–160 °C. Consequently, a broad endotherm of isolated amylose around 130–160 °C in DSC profile of subsequent reheating after cooling could be attributed to both melting of spherulites and amylose crystallites.

### 3.7. Effect of concentration

To study the effect of concentration on amylose chain association, different solid concentrations of amylomaize amylose, which had the highest iodine affinity value, indicating high purity, were heated to 180 °C and subsequently cooled to 5 °C. An exothermic peak was observed as shown in DSC cooling curves (Fig. 4A). The enthalpy and temperature of amylose chain association during cooling as a function of amylose solid content are shown in Fig. 4B. With increasing solid concentration, we observed a shift of the exotherm of amylose chain association to a higher temperature. However, the enthalpy of the exotherm increased with increasing amount of solid up to 30%, and thereafter decreased. Amylose chain association during cooling is related to the mechanism of amylose precipitation or aggregation (Sievert & Wursch, 1993a). On cooling, the paste is transformed into an opaque elastic gel. The gelation involves initial phase separation into polymer-rich and polymer-deficient regions. This phase separation allows molecular entanglement which establishes gel junction zones in polymer-rich regions. The junction zones could adopt the double helix structure, followed by helix–helix aggregation (development of the network structure), indicating slow crystallization during gelation of amylose (Doublier & Choplin, 1989; Gidley, 1989; Miles, Morris, & Ring, 1985). Based on the process of gelation, at high concentration, densely packed polymer-rich regions might be formed during cooling. These polymer-rich regions might be expected to readily associate into double helices, which contributed to the appearance of the exotherm at an elevated temperature. This would explain the positive linear relationship between temperature of the exotherm and solid concentration. The increase in the enthalpy of the exotherm up to 30% solid concentration is consistent with the above densely packed polymer-rich regions. However, beyond 30% solid concentration of amylose, we assume that the polymer-rich regions could not achieve continuous junction zones due to deficient water content. This means that although the high concentration of amylose might form the densely packed polymer-rich regions, less amylose was adopted in established double helices. Therefore, low enthalpy of the exotherm at high concentration (>30%) reflects less self-association of amylose, possibly due to non-homogeneous polymer-rich regions during the amylose gelation process.

## 4. Conclusions

The amylose chain association that occurred during cooling was affected by lipid content, amylose/amylopectin ratio, chain length of amylose and amylopectin, and solid concentration. The lipid in corn and amylomaize starches engaged preferentially with amylose, which could sterically hinder amylose aggregation. In contrast, a limited amount of lipid in native legume starch (bean and pea) enhanced amylose chain association, because a small amount of amylose–lipid complex might have acted as a nucleation site. The enthalpy of amylose chain association was substantially correlated to amylose content after removal of lipid from native starch. Long chains of amylopectin in amylomaize starch and the long chain length of potato amylose resulted in the amylose chain association exotherm occurring at the lowest temperature among tested samples. This suggests that a long branch chain structure, either in amylose or amylopectin impedes amylose chain association. At a solid concentration of 20–30% (w/w) of amylose, a larger exotherm during cooling was observed, possibly a result of production of homogeneous and densely packed polymer-rich phase during amylose gelation. This observation may be useful in determining the optimum concentration required to produce resistant starch type III.

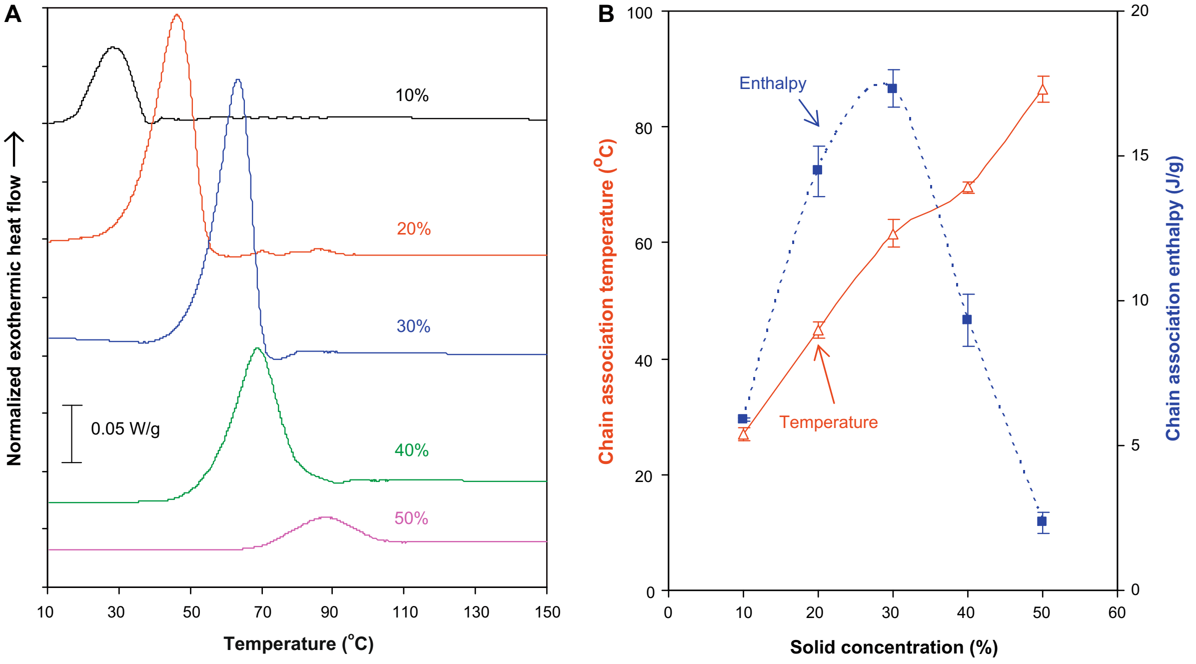

**Fig. 4.** DSC cooling curves (A) of isolated amylose from amylomaize at various concentrations (10–50% w/w) and correlation (B) between solid concentration and temperature ($T_p$) and enthalpy on amylose chain association.

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