Initial Heating Temperature and Native Lipid Affects Ordering of Amylose During Cooling of High-Amylose Starches

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ABSTRACT

Formation of ordered structures from disordered amylose is practically important. The thermal behavior of high-amylose maize starches was studied during cooling, following heating, and during subsequent reheating. Four commercial high-amylose genotype maize starches with varying amylose contents (*ae du, ae su2,* and *ae* [nominally both 50 and 70% amylose]) were heated to either 120, 140, 160, or 180°C, cooled to 5°C, and reheated to 180°C in a differential scanning calorimeter. Each starch was studied with its native lipid, as well as in reduced-lipid and lipid-free form. On cooling of lipid-containing starches, two distinct exotherms were observed and attributed to amylose-lipid complex formation and to amylose chain association. A distinct exotherm at ≈75°C was attributed to amylose-lipid complex formation. The exotherm attributed to amylose chain association on cooling varied according to the initial heat treatment, lipid level, and starch type. Starches with higher amylose contents showed larger exotherms on cooling. For initial heat treatments to 120 or 140°C, a broad exotherm beginning at ≈95°C was observed on cooling. In contrast, for initial heat treatments to 160 and 180°C, a sharper exotherm with a peak temperature below ≈55°C was observed. Upon reheating, samples that had been initially heated to 120 or 140°C showed a peak at >140°C that was attributed to the melting of ordered amylose. Starches initially heated to 160 or 180°C did not show this peak. This work illustrates that initial heating temperature, as well as lipid content and amylose content, all affect amylose chain association during cooling. Thus, this work suggests strategies for controlling ordering of amylose during processing.

Granular high-amylose maize starch cannot be completely cooked out in water at atmospheric pressures. However, thermal behavior during gelatinization of this material may be conveniently studied by differential scanning calorimetry (DSC) in a sealed pan. The complex DSC gelatinization endotherm appears to have two components: one at <95°C and one at >95°C. Because the latter endotherm is evident on an immediate rescan, and for different starches the enthalpy is roughly proportional to the lipid content, it is generally attributed to melting of a lipid-amylose complex (Kugimiya and Donovan 1981, Daniels and Thompson 1995). Swelling behavior of high-amylose maize starch granules is largely unstudied because of the methodological difficulty of working under controlled conditions at high pressures (Daniels and Thompson 1995).

Under certain conditions, cooling of cooked high-amylose starch leads to formation of a new physical state of the amylose, as observed by an endotherm at 140–160°C, an endotherm which is not observed before cooking the original granular starch (Sievert and Pomeranz 1989). Native high-amylose starch is known to be high in type II resistant starch (Berry 1986), which is defined as starch in its native granular state that is resistant to digestion in the small intestine (Englyst et al 1992). After cooking and cooling, high-amylose starch gives high yields of type III resistant starch (Berry 1986, Sievert and Pomeranz 1989), which is defined as retrograded starch that resists digestion (Englyst et al 1992). Sievert and Pomeranz (1989) observed a broad endothermic transition with a peak at ≈155°C when their resistant starch preparation from amylomaize VII was heated. They ascribed this transition to melting of amylose crystallites (Sievert and Pomeranz 1989, 1990). Sievert and Wursch (1993a) attributed exothermic transitions during controlled cooling of isolated potato amylose fractions and various starches to amylose chain association. The formation of resistant starch likewise has been attributed to the ordering of amylose chains (Sievert et al 1991). Based on previous studies of amylose behavior (Gidley 1989, Gidley and Bulpin 1989), Sievert and Wursch (1993b) suggested that the exotherms observed during the cooling of either amylose or a thermally treated resistant starch preparation reflect chain association, which may involve amylose aggregation and gelation dominated by formation and subsequent lateral aggregation of B-type double helices in crystalline arrays.

Many combinations of time and temperature treatments have been used to make type III resistant starch from various sources of native starch. Even for starches with normal amylose levels, it is recognized that cooking at >100°C can increase the yield of type III resistant starch. The temperature treatments have included autoclaving the starch at 110°C (Berry 1986), at 121°C (Berry 1986, Bjorck et al 1987, Sievert and Pomeranz 1989, Sievert and Wursch 1993b), at 127°C (Berry 1986, Bjorck et al 1987), at 134°C (Berry 1986, Bjorck et al 1987, Russell et al 1989, Sievert and Pomeranz 1989), or at 148°C (Sievert and Pomeranz 1989) for periods ranging from 30 min to 1 hr. Autoclaving-cooling cycles have also been repeated as many as 20 times (Sievert and Pomeranz 1989). Cooling and storage of the heat-treated starches has included cooling to room temperature and overnight storage at 4°C (Bjorck et al 1987, Sievert and Pomeranz 1989, Sievert and Wursch 1993b), cooling to room temperature followed by two-day storage at 4°C (Berry 1986), and drying at 80°C (Russell et al 1989). In work designed based on polymer crystallization theory, Eerlingen et al (1993a) cooked normal wheat starch to 121°C and then cooled to various holding temperatures (0, 68, and 100°C) with the goal of obtaining a maximum yield of resistant starch. They suggested that nucleation was high at 0°C and limiting at 100°C. In contrast, working with pea amylose, Vallera et al (1994) observed extremely rapid gelation, and they could not observe a difference in gel structure between slowly cooled (0.5°C/min) and rapidly cooled (quench cooled from 90°C to room temperature under running water) amylose at 2–8% amylose concentration. Though these results may aid in understanding the retrogradation behavior of amylose, it is not obvious that this work would apply to high-amylose maize starches. Optimal design of processes for creating resistant starch from high-amylose maize starches demands a better understanding of the thermal behavior of these starches.

The effects of lipids on the association of amylose and on the formation of resistant starch have also been investigated (Czuchajowski et al 1991, Szczodrak and Pomeranz 1992, Eerlingen et al 1994). Defatting of wheat and high-amylose maize starch increased resistant starch yields, and sodium dodecyl sulfate decreased resistant starch yields when added to defatted starches (Eerlingen et al 1994). Czuchajowski et al (1991), Eerlingen et al (1994), and Szczodrak and Pomeranz (1992) found that the addition of lipids led to amylose-lipid complex formation, and decreased resistant starch yields. Szczodrak and Pomeranz (1992) and Czucha-

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jowska et al (1991) concluded that amylose-amylose association was hindered by lipid addition, and that amylose-lipid complexation was kinetically favored. Eerlingen et al (1994) hypothesized that regions of an amylose molecule engaged in amylose-lipid complexation could hinder amylose-amylose interactions of neighboring segments of the molecule. The possibility that amylose-lipid complexes might serve as a nucleation site for amylose chain association has not yet been considered.

Sievert and Holm (1993) found that when isolated potato amylose with added lysophosphatidylcholine was initially heated to temperatures from 120 to 180°C, the melting enthalpy of amylose-lysophosphatidylcholine complexes during reheating was greater as initial heating temperature increased. On reheating of samples, a higher temperature and broad endotherm (occurring from ≈145 to 170°C with peak temperature [T_p] ≈155°C) was observed for samples initially heated to 100, 121, or 140°C, but not for samples initially heated to 160 or 180°C. Initial heating to 140°C produced the largest endotherm during reheating. Sievert and Holm (1993) stated that endotherms in the higher temperature range (T_p ≈ 155°C) that appeared during reheating in the thermograms of amylose samples initially heated to ≤140°C might be attributed to the melting of nonlipid complexed amylose structures. They suggested that if the initial heating was not sufficient to disorder these structures, the amylose would not be available to complex with the lipid. These authors did not address the nature of the structures that melt at a broad range of high temperatures (T_p ≈ 155°C). These structures may have been unique to the amylose preparation used and may have been present initially but not have melted during initial heating to <140°C. The authors did not comment on why these broad, high-temperature endotherms appear more pronounced after initial heating to 140°C than after initial heating to 121°C.

Doublier et al (1992) showed that 1–12% amylose dispersions formed gels after being dispersed to optical clarity by heating to <160°C, but that the dispersions formed precipitates after heating to higher temperatures. Retrogradation of waxy-type starches also is related to the initial heating temperatures, with a difference in retrogradation behavior observed for initial heating to 160°C compared to 180°C (Fisher and Thompson 1997). We hypothesized that retrogradation of high-amylose maize starches will also be affected by initial heating temperatures. The objective of this research is to understand the effects of initial heating temperature on amylose chain association as observed during cooling, on amylose-lipid complex formation as observed during cooling, and on the endotherms observed during subsequent reheating.

MATERIALS

Commercial maize samples obtained included: *ae* genotype reputed to contain 50% amylose (*ae*-V; Hylon V, National Starch and Chemical Co.), *ae* genotype reputed to contain 70% amylose (*ae*-VII; Hylon VII, National Starch and Chemical Co.), *ae su2* genotype (ARD 2634, American Maize Co., now Cerestar USA), *ae du* genotype (Amaizo 2568F, American Maize Co., now Cerestar USA), *wx* genotype (waxy maize [WM], Amioca, National Starch and Chemical Co.), and normal genotype (common corn starch, [CCS], Melojel, National Starch and Chemical Co.). Starches that were not treated to reduce native lipid are designated "native starch." In some samples, the lipid levels were reduced either partially or completely, and those samples are referred to as "reduced-lipid" or "lipid-free."

METHODS

Preparation of Reduced-Lipid and Lipid-Free Starches

Reduced-lipid forms of each starch sample were obtained by water-saturated butanol extraction at room temperature according to the procedure of Kim and Hill (1984). When the change in enthalpy (ΔH) for gelatinization of native starches was compared to the ΔH of the reduced-lipid samples, and the latter corrected for ΔH of lost amylose-lipid complexation, values were in reasonable agreement (within 7% for *ae su2*, *ae*-V, and *ae du;* within 22% for *ae*-VII) (data not shown).

Total lipids were determined after gelatinization and acid hydrolysis (Morrison et al 1980). Starch was gelatinized in boiling water and then boiled in concentrated HCl, and lipids were extracted using diethyl ether/hexane (Daniels and Thompson 1995). The results of this lipid analysis are shown in Table I.

Lipid-free starch was obtained by dispersion of starch in dimethyl sulfoxide (DMSO), followed by ethanol precipitation of the dispersion. Samples in 90% DMSO were heated in a boiling water bath for 2 hr. The starch was precipitated with 80% ethanol, centrifuged at 6,000 × *g* for 15 min, and the ethanol supernatant was decanted. The precipitate was washed twice by resuspending it in 80% ethanol and centrifuging. The precipitate was resuspended in acetone, centrifuged at 6,000 × *g* for 15 min, and dried overnight in a 50°C oven. Lipid-free, DMSO-dispersed material prepared in this manner has been termed nongranular starch (Banks and Greenwood 1975).

Both reduced-lipid and lipid-free forms of the starch samples were ground with a mortar and pestle to obtain a fine powder suitable for analysis by DSC.

DSC

Thermal analysis was performed using a differential scanning calorimeter (DSC 7, Perkin Elmer Corp., Norwalk, CT) equipped with a thermal analysis data station (Perkin Elmer) Indium was used as a calibration standard. The reference cell contained a sealed, empty, stainless steel pan. Starch samples (≈18.0 mg, dwb) were weighed into preweighed stainless steel pans (Perkin Elmer) All starches were assumed to contain 10% moisture. Deionized water was added to make ≈30% (w/w) starch suspensions, and samples were stirred with a needle. Pans were sealed, the total weights were determined, and the suspensions were stored overnight at 21°C. All samples were heated at 10°C/min. For initial thermal treatment, samples were heated from 5°C to either 120, 140, 160, or 180°C. Samples were then cooled at 10°C/min to 5°C. Samples were immediately reheated to 180°C at 10°C/min. Analyses were performed in duplicate, and results are presented as the mean.

RESULTS

Initial Heating

Initial heating of high-amylose maize starch samples is shown in Fig. 1.

Cooling

Common and waxy corn starch. Samples of CCS only showed one exotherm during cooling when lipid was present, and no exotherm was seen with lipid-free samples (data not shown). The exotherm apparent during cooling of lipid-containing CCS samples was attributed to the formation of amylose-lipid complexes. Samples of WM showed no exotherms during cooling (data not shown).

High-amylose corn starches. During cooling, all native starch and reduced-lipid starches showed an exotherm with a peak at ≈75°C (Fig. 2). Based on extensive literature (Biliaderis et al 1985, Eliasson and Krog 1985, Sievert and Wursch 1993b) and the fact that lipid-free samples did not show this exotherm during cooling, this exotherm was assigned to formation of amylose-lipid complexes. A second exotherm was also observed for many of the high-amylose samples, with location and magnitude varying according to heat treatments.

Lipid-free, high-amylose starch samples that were initially heated to either 120 or 140°C have a broad exotherm beginning at ≈95°C on cooling. Consistent with previous authors, we attribute this event to amylose chain association (Sievert and Wursch 1993a,b). Lipid-containing samples showed this broad exotherm as well as an exotherm with a peak at ≈75°C (Fig. 2). The ΔH values for the

amylose-lipid complex alone are presented in Table II. The ΔH values for amylose association were then calculated by subtracting the ΔH of the amylose-lipid complex formation from the ΔH of both events during cooling as illustrated by the *ae su2* genotype thermograms B3 and C3 in Fig. 2.

During cooling of lipid-free, high-amylose samples initially heated to 160°C, only one exotherm was observed, which occurred at a lower T_o than the exotherms of lipid-free samples initially heated to 120 or 140°C. This event is also attributed to amylose chain association (Sievert and Wursch 1993a,b). During cooling of the *ae*-VII and *ae su2* native starches from 160°C, the amylose association exotherm ΔH was reduced as compared to lipid-free starches. During cooling of the *ae*-V and *ae du* native starches from 160°C, the amylose chain association exotherm was barely discernible (Fig. 2). The reduced-lipid *ae su2* and *ae*-VII starches cooled from 160°C had both a larger amylose association ΔH than native or lipid-free starches and the exotherm was narrower (based on peak width at half-height) relative to lipid-free samples but with a peak at approximately the same temperature (Fig. 2). The reduced-lipid starches of *ae*-V and *ae du* cooled from 160°C had a lower amylose chain association exotherm enthalpy than lipid-free samples of those genotypes.

Lipid-free, high-amylose starches initially heated to 180°C had one exotherm during cooling, similar to that observed when initially heated to 160°C. However, this amylose association exotherm was at a lower temperature than the exotherm for samples initially heated to 160°C. The ΔH values of the exotherms were larger for samples initially heated to 180°C than for samples initially heated to 160°C (Table II). For the *ae su*2 and *ae*-VII genotypes, the enthalpy of the amylose-association exotherm during cooling from 180°C was lower for native lipid samples as compared to reduced-lipid or lipid-free samples. For *ae du* native starch initially heated to 180°C, there was only a suggestion of an exotherm at ≈20°C. For native *ae*-V starch initially heated to 180°C, there was no indication of an amylose association exotherm during cooling.

TABLE I
Lipid Content of Maize Starches (mg of lipid/100 g of dry starch)

Starch	Native	Reduced-Lipid
ae su2	1,030 ± 97	392 ± 54
ae-VII	1,079 ± 78	606 ± 69
ae-V	1,016 ± 83	453 ± 63
ae du	1,165 ± 60	687 ± 49
Common corn starch	539 ± 38	286 ± 81

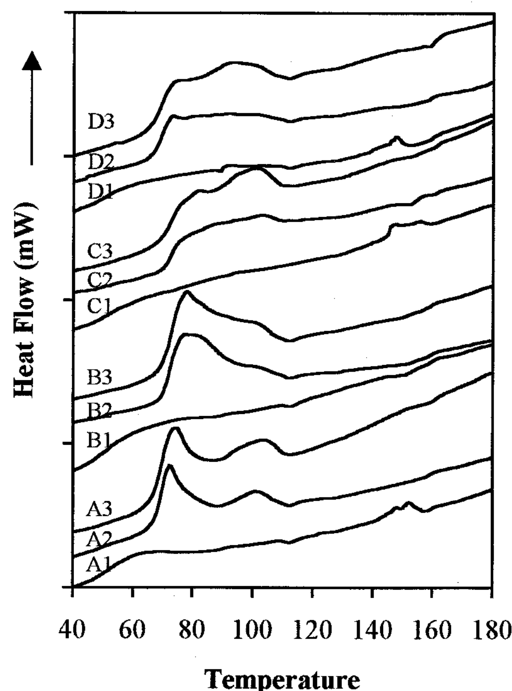

Fig. 1. Initial heating of starches. Each tick mark on the y-axis corresponds to 5 mW. Letters A–D: *ae du*, *ae*-V, *ae*-VII, and *ae su2* starches, respectively. 1–3: Lipid-free, reduced-lipid, and native starches, respectively.

The effect of lipid-amylose interactions on amylose association was strongly influenced by starch genotype. In *ae*-VII starch initially heated to 160 or 180°C, the amylose association exotherm T_p decreased as the lipid content increased from lipid-free to reduced-lipid to native starch (Fig. 2). In native *ae su2* starch initially heated to either 160 or 180°C, the T_p for amylose association during cooling was lower than the T_p for either reduced-lipid or lipid-free *ae su2* starches (which were similar). In contrast, on cooling of *ae du* starch initially heated to 180°C, the amylose association exotherm T_p was lower in lipid-free samples than in reduced-lipid samples. In native *ae*-V samples, no amylose association exotherm was apparent during cooling.

Reheating

Common corn and waxy maize starch. WM starch showed no endotherms during immediate reheating from any of the initial heating temperatures. For all initial heating temperatures, CCS showed endotherms at ≈100°C during reheating (data not shown). This endotherm is attributed to the melting of amylose-lipid complexes. CCS that was initially heated to 140°C and cooled also showed a very small endotherm (estimated to be ≈1.1 J/g) at ≈160°C during reheating (data not shown).

High amylose corn starches. During reheating of native lipid and reduced-lipid high-amylose starches, an endotherm was observed at ≈100°C corresponding to the melting of the amylose-lipid complex (Fig. 3). Lipid-free samples initially heated to 120°C had a broad endotherm with a peak at ≈150°C during reheating (Table II). A broad endothermic event with a peak at ≈160°C was observed for all starches initially heated to 140°C. In starches initially heated to 140°C, the ΔH of this event at ≈160°C decreased as more lipid was present in the samples. The endotherm at ≈160°C was much reduced or absent for starches initially heated to 160°C, and was totally absent for all starches initially heated to 180°C (with the exception of a minor, but reproducible, response for native *ae su2* starch).

DISCUSSION

We suggest that the amylose association exotherm observed during cooling of lipid-free samples (Fig. 2) initially heated to 120 or 140°C is very broad because these initial heating temperatures did not disorder all ordered amylose present, and thus nucleation sites remained. If some nucleation sites were not melted by heating to 120 or 140°C, then these nucleation sites might contribute to the observed higher temperatures (up to 95°C) of amylose chain association on cooling. This concept is in agreement with work by Doublier et al (1992), who suggested that initial heating of amylose dispersions to <160°C would leave a large number of nuclei present. Higher temperature amylose chain association resulting from original nucleation sites would properly be considered to result from a type of annealing. According to the definition of Wunderlich (1976), annealing includes recrystallization in the sense that residual crystallinity is used as a substrate for the crystallization of partially molten or dissolved material. We suggest that when this annealed amylose is reheated, the melting is observed as the endotherm at >140°C. This endotherm was not present during the initial heating of samples to 160°C (data not shown) or 180°C (Fig. 1; also Sievert and Pomeranz 1989), nor on reheating of starches initially heated to these temperatures. Thus, the initial heating led to the endotherm, and partial melting and annealing is a plausible explanation for the new endotherm at ≈155°C on reheating. Repeated autoclaving and cooling has increased the B-type crystallinity and lead to enhancement of the 155°C endotherm (Sievert et al 1991).

Repeated heating to a temperature above the melting of the amylose-lipid complexes but <155°C (Sievert et al 1991) might be considered an annealing process as with each cycle additional amylose would be available for amylose association. Three processes might be occurring in each heating-cooling cycle: melting of amylose-lipid complexes, amylose double helix formation due to increased amylose mobility, and increased packing of amylose double helices.

In contrast to the onset at ≈95°C of amylose association exotherms during cooling of samples initially heated to 120 or 140°C, the amylose association exotherms of samples initially heated to 160 or 180°C ocurred at lower temperatures during cooling. In the case of samples initially heated to 160 or 180°C, we suggest that the lower T_0 of the exotherm during cooling is due to the melting of most or all nucleation sites contributed by ordered amylose during the initial heat treatment. This hypothesis is in agreement with the work of Doublier et al (1992), who suggested that complete melting of amylose crystals occurred during initial heating of amylose dipersions to >160°C, but not for initial heating to <160°C. We believe that any residual ordered amylose would serve as nucleation sites, allowing exothermic ordering to occur at higher temperatures on cooling. When the heat treatment is sufficient to eliminate these residual nuclei, the lack of nucleation sites could explain why the amylose chains would need to reach a lower temperature before they could associate, as these lower temperatures could be required for nucleation.

Despite the large amylose association exotherm observed during cooling of samples initially heated to 160 or 180°C, no corresponding endotherm was observed during reheating of these samples. This lack of an endotherm remains a puzzle. Working with a 95:5 amylose-to-amylopectin ratio, Sievert and Wursch (1993a) observed a much larger cooling exotherm than the subsequent endotherm on reheating. We speculate that the amylose melting may be occurring over such a broad temperature range during reheating that an endotherm is not readily apparent.

With mixtures of amylose and amylopectin heated to 180°C, the size and location of the exotherm during cooling has been shown to vary according to the proportion of amylose (Sievert and Wursch 1993a). When monoacyl lipid is present, we may assume that during cooling of high-amylose maize starch DSC monitors the formation of amylose double helices and amylose-lipid single helices. The total amount of amylose that is ordering during cooling may therefore be estimated based on the sum of the ΔH of amylose

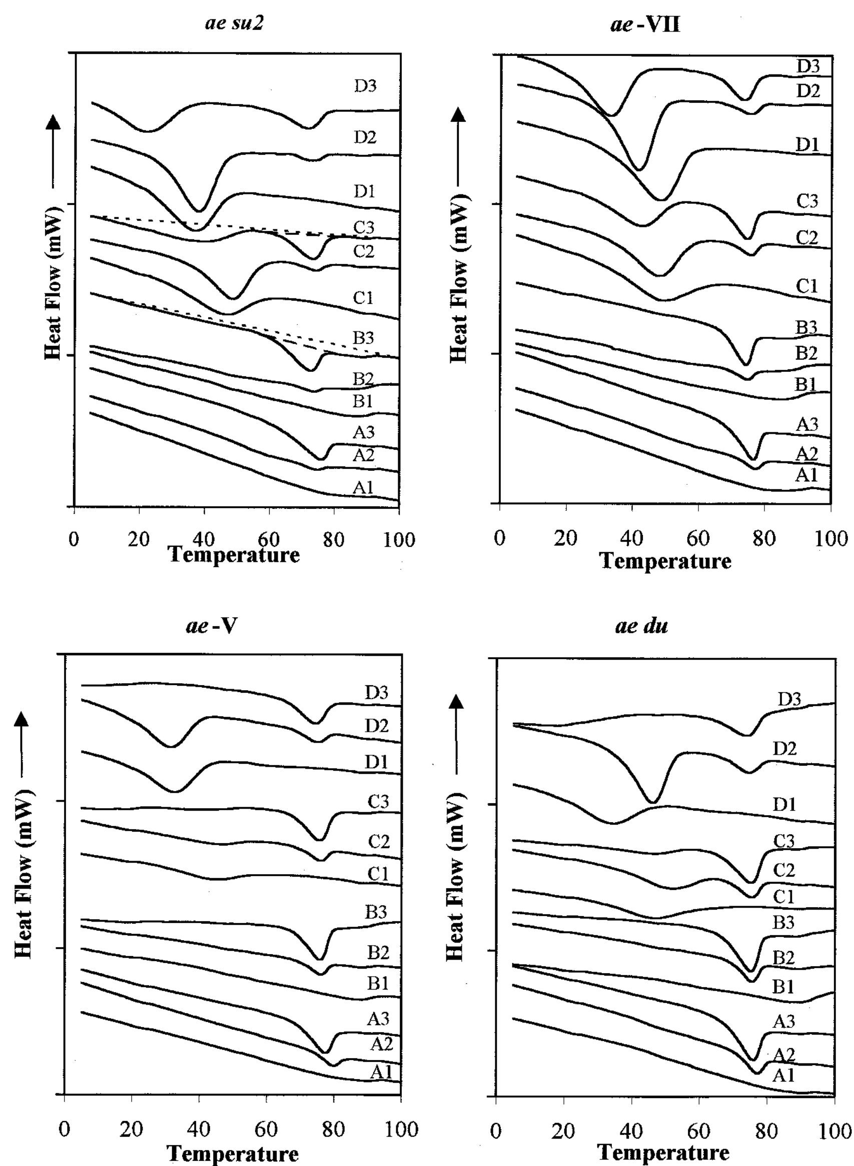

Fig. 2. Cooling of starches after initial heat treatment to various temperatures. Each tick mark on the y-axis corresponds to 5 mW. A–D: Initial heating to 120, 140, 160, and 180°C, respectively. 1–3: Lipid-free, reduced-lipid, and native starches, respectively. Thermograms B3 and C3 of *ae su2* starch both illustrate the method used to distinguish the enthalpy of amylose chain association (broad exotherm) and amylose-lipid complex formation (more narrow exotherm centered at ≈75°C).

association and amylose-lipid complex formation (Table III). To compute the amount of amylose that is ordering during cooling, values for the Δ*H* for fully double helical starch and the maximum Δ*H* for amylose-lipid complexes are needed. Assuming that double helical order is the mechanism for the amylose chain association, then the Δ*H* observed for amylose association during cooling can be compared to the values for fully double-helical starch (Table III), as described by Cooke and Gidley (1992), to determine the amount of starch forming double helices. They used A-type debranched glycogen (≈20% solids) to estimate endotherm melting Δ*H* for fully double-helical starch as 35 J/g . The value of 35 J/g, used for calculations of percentage of starch as double helices (Table III), is reasonably consistent with more recent extrapolated values of ≈30 J/g for fully ordered enzyme-resistant retrograded *ae*-V and ≈39 J/g for fully ordered enzyme-resistant retrograded *ae*-VII (Gidley et al 1995). The amount of starch forming single helical amylose-lipid complexes can be estimated based on the Δ*H* for amylose-lysophosphatidylcholine complexes, which was reported as 27.7 J/g of amylose (Sievert and Holm 1993). This estimated total for ordering on cooling does not include amylose that retained its order after initial heat treatment, as may be the case for samples initially heated to 120 or 140°C.

By comparing the totals in Table III, it is evident that *ae*-VII starch produces the highest proportion of fully double-helical starch, as observed during cooling. For each initial heating temperature, *ae*-VII starch has the highest proportion of double-helical starch on cooling, with *ae su2* starch having the next highest values, and *ae*-V and *ae du* starches having relatively low levels of double helical order. The amount of starch that orders on cooling can also be expressed as the proportion of the total amylose that orders on cooling. Based on estimates of amylose content obtained by differential alcohol precipitation (Klucinec and Thompson 1998), we esti-

TABLE II
Thermal Analysis of Starches After Heating to Various Initial Temperatures[a]

Genotype	Initial Heating Temperature (°C)	Lipid Level	Cooling: Amylose-Lipid Complex Δ*H*	Cooling: Amylose-Lipid Complex T_p	Cooling: Amylose Chain Association Δ*H*	Cooling: Amylose Chain Association T_p	Reheating[b] Δ*H*	Reheating[b] T_p
ae su2	120	Native	2.93	74.4	5.31	nd[c]	1.63	142.7
		Reduced-lipid	0.31	74.0	8.74	nd	2.63	145.6
		Lipid-free	nd	nd	8.37	71.8	3.51	141.5
	140	Native	2.39	72.2	5.26	nd	2.33	155.3
		Reduced-lipid	0.31	73.1	8.82	nd	3.37	154.9
		Lipid-free	nd	nd	7.88	78.6	5.22	158.0
	160	Native	2.60	73.2	6.65	37.4	nd	nd
		Reduced-lipid	0.49	74.3	11.31	48.5	nd	nd
		Lipid-free	nd	nd	9.56	48.0	0.60	170.1
	180	Native	2.50	71.8	4.95	21.7	0.20	143.6
		Reduced-lipid	0.48	73.1	13.71	39.0	nd	nd
		Lipid-free	nd	nd	11.43	38.1	nd	nd
ae-VII	120	Native	2.63	75.6	6.26	nd	2.42	142.6
		Reduced-lipid	0.75	76.1	8.94	nd	2.81	140.8
		Lipid-free	nd	nd	10.20	70.9	4.65	155.6
	140	Native	2.39	75.4	7.69	nd	3.69	154.7
		Reduced-lipid	0.57	75.9	9.22	nd	3.59	155.2
		Lipid-free	nd	nd	10.02	78.8	5.85	159.5
	160	Native	2.53	75.8	9.61	44.4	nd	nd
		Reduced-lipid	0.75	77.0	12.62	50.2	nd	nd
		Lipid-free	nd	nd	11.76	50.6	0.67	157.2
	180	Native	2.43	75.3	12.55	34.7	nd	nd
		Reduced-lipid	0.87	76.7	15.14	42.5	nd	nd
		Lipid-free	nd	nd	16.88	47.1	nd	nd
ae-V	120	Native	2.49	77.0	5.58	nd	0.30	142.7
		Reduced-lipid	0.85	78.3	6.15	nd	1.48	155.3
		Lipid-free	nd	nd	6.06	75.9	3.10	153.9
	140	Native	2.69	75.9	3.75	nd	1.95	157.2
		Reduced-lipid	0.87	76.2	3.54	nd	2.34	125.9
		Lipid-free	nd	nd	4.19	84.9	4.21	161.2
	160	Native	2.76	75.4	0.31	nd	0.69	169.8
		Reduced-lipid	0.98	75.9	3.33	43.6	0.55	161.6
		Lipid-free	nd	nd	4.25	43.9	0.99	165.9
	180	Native	2.35	74.2	nd	nd	nd	nd
		Reduced-lipid	0.90	73.8	7.14	30.9	nd	nd
		Lipid-free	nd	nd	7.00	32.3	nd	nd
ae du	120	Native	3.42	77.6	7.61	nd	1.13	160.5
		Reduced-lipid	1.23	76.9	6.78	nd	1.40	152.9
		Lipid-free	nd	nd	4.90	77.8	2.99	147.9
	140	Native	3.29	76.7	4.20	nd	2.17	162.1
		Reduced-lipid	1.27	76.4	4.78	nd	2.87	161.8
		Lipid-free	nd	nd	6.02	86.0	5.50	164.3
	160	Native	3.31	76.3	5.01	nd	nd	nd
		Reduced-lipid	1.22	76.6	6.98	52.6	0.88	165.0
		Lipid-free	nd	nd	7.62	48.8	1.49	169.9
	180	Native	3.45	75.1	1.94	20.3	nd	nd
		Reduced-lipid	1.40	75.7	10.86	46.4	nd	nd
		Lipid-free	nd	nd	7.13	35.6	nd	nd

[a] Mean of two observations. Δ*H* = enthalpy in J/g of starch, T_p = peak temperature in °C.
[b] High temperature endotherms were determined using a baseline that started at 125°C (amylose-lipid melting endotherms were not included).
[c] Not detected.

mate that nearly all of the amylose in *ae*-VII orders on cooling from 180°C, whereas far less orders on cooling from lower initial heating temperatures (Table III). Starch structural differences among the high-amylose genotypes might explain the differences we observed in the ability of amylose to complex lipid and to self-associate. Besides increasing the amylose content of starches, the *ae* gene is also known to produce amylopectin molecules with longer chain lengths (Yun and Matheson 1993). Although amylopectin molecules with longer chains might be expected to readily self-associate into helices and contribute to the exotherm, no exotherm was observed for cooling of gelatinized native *ae wx* starch (data not shown), which also has long constituent chains (Yuan et al 1993, Shi and Seib 1995). Although the amylopectin itself may not be involved, we cannot rule out possible interaction between amylose and more branched molecules in the high-amylose starches.

The much lower amylose association ΔH of native starches (when compared to lipid-free samples) is consistent with the idea that when more lipid interacts with amylose to form amylose-lipid complexes, less amylose is available to self-associate (Czuchajowski et al 1991, Eerlingen et al 1994). With a stoichiometric excess of lipid levels, the lipid could effectively compete for all amylose. At native lipid levels, amylose-lipid complexes might interfere with association of remaining amylose. Competition could also explain why native starches heated to 180°C had a lower T_p and ΔH of amylose association as compared to lipid-free samples. Perhaps once a sufficient percentage of the amylose is ordered as amylose-lipid complexes, association of remaining amylose is inhibited. The amylose association exotherm of starches of the *ae*-VII genotype initially heated to 160 or 180°C decreased in T_p as lipid content increased from lipid-free to reduced-lipid to native starch. This decrease in T_p could be due to steric interference by amylose-lipid complexes. It might also result from the most readily complexable amylose for amylose association also being the most susceptible to forming amylose-lipid complexes, thus leaving the less readily complexable amylose to self-associate at lower temperatures.

Preferential complexation of amylose with lipid has been exploited to develop methods to quantitate amylose by DSC (Kugimiya and Donovan 1981, Sievert and Holm 1993, Mestres et al 1996). These methods involve heating the amylose with added lipid, forming complexes on cooling, and then measuring ΔH of the amylose-lipid complex on reheating. Sievert and Holm (1993) noted that the greatest ΔH of complexation was observed for initial heating to 180°C.

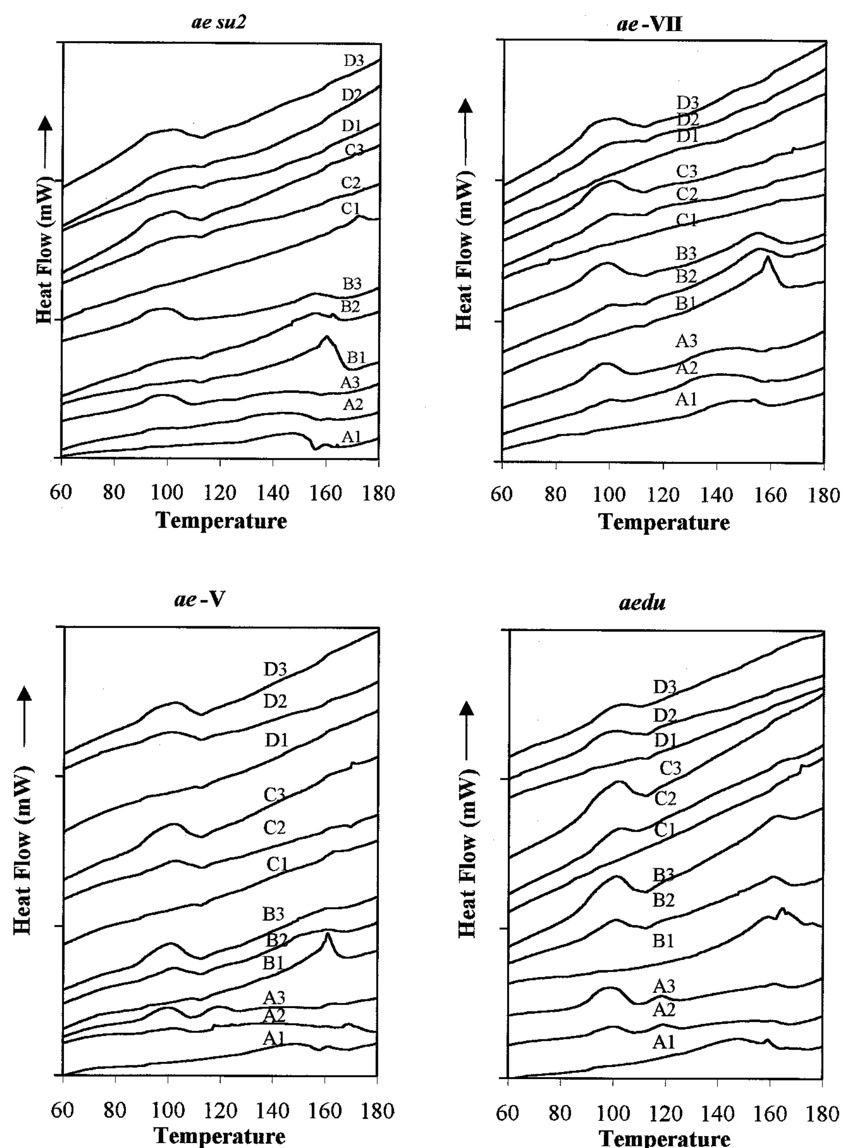

Fig. 3. Reheating of starches after initial heating to various temperatures and then cooling to 5°C. Each tick mark on the y-axis corresponds to 5 mW. A–D: Reheating after initial heating to 120, 140, 160, and 180°C, respectively. 1–3: Lipid-free, reduced-lipid, and native starches, respectively.

Under some circumstances, the presence of lipids enhanced amylose self-association. Reduced-lipid *ae su2* starch initially heated to 160 or 180°C had a narrower exotherm and an increase in the amylose chain association exotherm enthalpy as compared to lipid-free *ae su2* starch. The increased amylose association ΔH in the reduced-lipid *ae su2* starch is observed despite the fact that some amylose is involved in amylose-lipid complexes; thus the total amount of starch in ordered configurations is even higher than from amylose association alone (Table III). A narrower amylose association exotherm is also observed for reduced-lipid when compared to lipid-free *ae*-VII and *ae du* starches initially heated to 180°C, and the total order of *ae du* reduced-lipid starch initially heated to 180°C is much greater than the order of the lipid-free starch equivalent. For reduced-lipid *ae du* starch, the amylose association exotherm is at a higher temperature than for lipid-free starch, also suggesting enhanced amylose association kinetics.

Several structures could correspond to the amylose association exotherms observed during the cooling of these high-amylose maize starch samples (Eerlingen et al 1993b). The samples initially heated to 120 or 140°C could contain residual double helices after heating, which during exposure to the higher temperatures during heating and cooling, are ordered into double helical linear chain segments loosely arranged into aggregates (Gidley et al 1995) by an annealing process that enhances double helices present and forms new double helices. (With the DSC employed in this work, no useful data are recorded for approximately the first 20°C after the initiation of the cooling regime.) The exotherm (≤95°C) observed on continued cooling for the samples initially heated to 120 or 140°C probably

TABLE III
Calculated Proportions of Amylose in Helical Forms[a]

Genotype	Initial Heating Temperature (°C)	Lipid Level	Amylose Association[a] (ΔH)	Starch as Double Helices[b] (%)	Amylose-Lipid[c] (ΔH)	Starch as Amylose-Lipid Complexes[c] (%)	Starch as Double Helices and Amylose-Lipid Complexes (%)[d]	Amylose as Double Helices or Amylose-Lipid Complexes (%)[e]
*ae su*2	120	Native	5.31	15	2.93	11	26	54
		Reduced-lipid	8.74	25	0.31	1	26	54
		Lipid-free	8.37	24	0.00	0	24	50
	140	Native	5.26	15	2.39	9	24	49
		Reduced-lipid	8.82	25	0.31	1	26	55
		Lipid-free	7.88	23	0.00	0	23	47
	160	Native	6.65	19	2.60	9	28	59
		Reduced-lipid	11.31	32	0.49	2	34	71
		Lipid-free	9.56	27	0.00	0	27	57
	180	Native	4.95	14	2.50	9	23	48
		Reduced-lipid	13.71	39	0.48	2	41	85
		Lipid-free	11.43	33	0.00	0	33	68
ae-VII	120	Native	6.26	18	2.63	9	27	56
		Reduced-lipid	8.94	26	0.75	3	28	58
		Lipid-free	10.20	29	0.00	0	29	59
	140	Native	7.69	22	2.39	9	31	62
		Reduced-lipid	9.22	26	0.57	2	28	58
		Lipid-free	10.02	29	0.00	0	29	58
	160	Native	9.61	27	2.53	9	37	75
		Reduced-lipid	12.62	36	0.75	3	39	79
		Lipid-free	11.76	34	0.00	0	34	69
	180	Native	12.55	36	2.43	9	45	91
		Reduced-lipid	15.14	43	0.87	3	46	95
		Lipid-free	16.88	48	0.00	0	48	98
ae-V	120	Native	5.58	16	2.49	9	25	66
		Reduced-lipid	6.15	18	0.85	3	21	54
		Lipid-free	6.06	17	0.00	0	17	46
	140	Native	4.33	12	2.69	10	22	58
		Reduced-lipid	3.54	10	0.87	3	13	35
		Lipid-free	4.19	12	0.00	0	12	32
	160	Native	0.31	1	2.76	10	11	29
		Reduced-lipid	3.33	10	0.98	4	13	34
		Lipid-free	4.25	12	0.00	0	12	32
	180	Native	0.00	0	2.35	8	8	22
		Reduced-lipid	7.14	20	0.90	3	24	62
		Lipid-free	7.00	20	0.00	0	20	53
ae du	120	Native	7.61	22	3.42	12	34	83
		Reduced-lipid	6.78	19	1.23	4	24	58
		Lipid-free	4.90	14	0.00	0	14	34
	140	Native	4.20	12	3.29	12	24	58
		Reduced-lipid	4.78	14	1.27	5	18	45
		Lipid-free	6.02	17	0.00	0	17	42
	160	Native	5.01	14	3.31	12	26	64
		Reduced-lipid	6.98	20	1.22	4	24	59
		Lipid-free	7.62	22	0.00	0	22	53
	180	Native	1.94	6	3.45	12	18	44
		Reduced-lipid	10.86	31	1.40	5	36	88
		Lipid-free	7.13	20	0.00	0	20	50

[a] ΔH = enthalpy in J/g of starch.
[b] (Amylose association enthalpy)(35 J/g) × 100.
[c] (Amylose-lipid enthalpy)(27.7 J/g) × 100.
[d] (% of starch as double helices) + (% of starch as amylose-lipid complex).
[e] (% of starch as double helices and amylose-lipid complexes)(% amylose) × 100. Amylose values determined by the method of Klucinec and Thompson (1998) were 42.9, 50.6, 37.2, and 47.8%, for *ae su2*, *ae*-VII, *ae*-V, and *ae du*, respectively.

reflects the ordering of new double helices, which could then contribute to the double helical linear chain segments loosely arranged into aggregates. These loose aggregates of double helical linear chain segments may be the structures that cause the endotherms at >140°C during immediate reheating. Lipid-free samples showed the greatest ability to produce amylose ordering as observed on reheating (Fig. 2). Gidley et al (1995) proposed that structures present in resistant starch consist of double helical linear chain segments loosely packed into aggregates with approximate B-type packing geometry. Single chains were proposed to be incorporated as fringes and imperfections, together with some lipid inclusion complexes (Gidley et al 1995).

In the present work, the exothermic event observed during cooling of lipid-free samples initially heated to 160 or 180°C may be the amylose of these samples organizing into double helical linear chain segments. If these double helical chain segments were of variable length or were influenced by the presence of amylopectin, they could fail to produce a defined endotherm during reheating because they would dissociate over a broad temperature range. Alternatively, it is conceivable that the transition occurred at >180°C. Sievert and Pomeranz (1989) observed that after heating to 180°C, reheating of amylomaize preparations, and even of resistant starch residues, produced no transitions; thus these structures do not readily reform from these materials. In the present work, ordered structures (endotherms) were observed on reheating after heating high-amylose maize starch to 120 or 140°C but not to 180°C. Since none were observed on reheating after heating to 180°C, it may be that, once fully disordered, these ordered structures are kinetically difficult to obtain from these materials. This reasoning may account for why Sievert and Pomeranz (1989) considered the melting of resistant starch by heating to 180°C to be an irreversible transition.

Initial molecular or granular organization, such as amylose double helices, may serve as an important template for formation of the high-temperature endotherm seen during reheating after initial heating to 120 or 140°C, with the initial structures providing a basis for annealing. Lipid-free starches apparently have more initial structure (perhaps due to the DMSO and drying treatment used to remove lipid) to contribute to double helical formation than the granular samples, as the higher temperature endotherms on reheating are the largest for these samples (Fig. 3). Although native high-amylose maize starch contains type II resistant starch (Berry 1986), it shows no endotherms at >140°C (Sievert and Pomeranz 1989). If a high temperature (>140°C) endotherm that is generated by the thermal treatment can be related to type III resistant starch, then DSC may provide a means to differentiate between type II and type III resistant starch for high-amylose maize starches.

Native and reduced-lipid samples of *ae su2*, *ae*-V, and *ae du* that were initially heated to 120°C had, during reheating, two peaks in the range of 100–120°C, which may indicate the presence of both type I and type II lipid complexes (Karkalas et al 1995). For native and reduced-lipid samples of *ae*-V and *ae du* initially heated to 120°C, the lipid interferes with amylose association, and thus the endotherm at >140°C is reduced in ΔH compared to lipid-free samples.

In conclusion, we have found that for these four high-amylose maize starch genotypes, an endotherm at >140°C occurs only when the samples are initially heated to 120 or 140°C. This observation may be useful in designing processes for the manufacture of type III resistant starch from high-amylose starches. After cooling from 160 or 180°C, there was no endotherm at 140–180°C during reheating. These observations build on earlier work by Doublier et al (1992), which shows that whether amylose dispersions are heated at above ≈160°C can determine whether the amylose will gel or precipitate. The genotype of the high-amylose starch influences the extent of amylose association, probably as a result of differences in the proportion of amylose and in the molecular structures. Starches with native lipid form amylose-lipid complexes preferentially over amylose chain association. However, for some starches, a limited amount of lipid enhances amylose chain association.

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