# Annealing of Maize Starches at Elevated Temperatures

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## ABSTRACT

Maize starches were annealed in excess water at temperatures from 50 to 70°C by a multistep process, which allowed higher annealing temperatures than could be obtained by single-step annealing, without gelatinizing starch granules. Gelatinization of the annealed samples was then studied by differential scanning calorimetry. Annealing of normal and waxy maize starches increased the onset and peak temperature, with relatively little change in gelatinization enthalpy. Amylomaizes increased dramatically in enthalpy, and the endotherms became more distinct as annealing temperature increased. This change in gelatinization characteristics is interpreted as evidence that interaction between amylose and amylopectin occurs in amylomaize granules during annealing. These differences in interaction are consistent with differences in crystal structure; A-type crystals of normal and waxy starch are relatively stable, and amylose and amylopectin domains appear to be separate. Amylomaize starches contain B-type crystallites, which are energetically more likely to reform, possibly to give A-type structures. The B-type starches are considered to have a higher degree of intermingling of amylose and amylopectin, which allows more extended interaction during annealing, resulting in increased gelatinization enthalpy.

The study of the crystalline nature of starch and of the events that occur during starch gelatinization are important, both for their fundamental aspects and from a practical point of view, as they apply to starch processing in the wet-milling industry and to use of starch products in foods. Although a great deal is known about starch crystallinity and starch gelatinization, many questions still remain. The complexity of starch granule composition and structure, and the multiplicity of events that can occur before and during gelatinization, present an enduring challenge.

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Early studies of gelatinization relied primarily upon detection of the birefringence end-point temperature when viewing starch granules in a polarizing microscope equipped with a hot stage. This approach is limited by the subjective nature of the observations and by the fact that temperature is the only quantitative information obtained. In spite of these limitations, Lelievre (1973) accomplished some sophisticated interpretation of gelatinization phenomena by applying the Flory-Huggins equation for analysis of polymer melting in presence of diluents.

Calorimetric techniques have greatly enhanced our understanding of the dynamics of starch gelatinization and the crystalline nature of starch. In addition to providing measurement of onset, peak, and final temperatures for starch gelatinization, it is possible to measure gelatinization enthalpy to obtain a quantitative estimation of crystallinity and order in the starch granule. Zobel et al (1965) first reported the use of differential thermal analysis

for measurement of starch gelatinization, using differential scanning calorimetry (DSC). DSC endotherms for cereal starches were also reported by Stevens and Elton (1971). DSC data were interpreted in terms of polymer crystallite melting by Donovan (1979), Donovan and Mapes (1980), and Kugimiya et al (1980), by applying the Flory-Huggins equation for melting of polymer crystallites. This approach has been questioned by Slade and Levine (1984) and by Maurice et al (1985) because of the nonequilibrium nature of starch gelatinization. Biliaderis et al (1986) used DSC together with thermomechanical analysis to provide information into the crystalline nature of the starch granule, the dynamics of the melting process during granule gelatinization, and the possible role of amylose in stabilizing granule swelling during gelatinization. On the basis of their studies and by comparison with other polymer systems, they proposed that three separate domains exist within the starch granule: a bulk amorphous region, consisting primarily of amylose; a crystalline region, consisting of amylopectin; and a nonordered intercrystalline region, also primarily amylopectin. Gelatinization was described as an intricate process involving melting, recrystallization, annealing, and final melting of the amylopectin crystallites. Water acts as a plasticizer to lower the glass transition temperature of the amorphous regions, thus facilitating melting of the starch crystallites; water contents of 30% or more were required to achieve the full plasticizing effect. These researchers contended that the melting process is essentially irreversible, so that the classic Flory-Huggins equation is therefore not applicable to starch gelatinization.

The effect of annealing on starch gelatinization was first demonstrated by Gough and Pybus (1971), who annealed wheat starch by heating it in water at 50°C, then determined the birefringence end-point temperature. They found that annealing raises the initial temperature of gelatinization and narrows the gelatinization range. These effects were interpreted as being the result of changes in the internal structure of the starch granule, but no qualitative changes in X-ray diffraction patterns were detected. Marchant and Blanshard (1978) interpreted the annealing effect as a partial melting of some crystallites with a general realignment of the polymer chains within the granule. These early studies of starch annealing presented no quantitative data on thermal changes during annealing.

The relationship between annealing and the glass transition temperature in starch granules was studied by Hoseney et al (1986) and by Yost and Hoseney (1986). Those studies demonstrated that annealing occurs only when starch is heated to a temperature above its glass transition point, but not high enough to cause irreversible melting, or gelatinization. Neither those studies, nor the work of Biliaderis et al (1986), attempted to isolate annealing effects from other events that might occur immediately before, or during, final melting of starch crystallites. Furthermore, in all of those experiments, temperature increases during gelatinization measurements were very rapid relative to the time required for the annealing process to reach equilibrium.

Previous studies at this laboratory by Krueger et al (1987a,b) determined changes in heat of gelatinization that occur as a result of annealing, demonstrated that partial annealing of starch granules occurs during the wet-milling process for corn starch, and measured the effect of the annealing process on corn starches with various amylose/amylopectin ratios.

In two recent reviews, Zobel (1988a,b) extensively discussed the crystalline nature of starch, the organization of the starch granule, and the dynamics of the melting process. Zobel et al (1988) reported an X-ray diffraction study of starch gelatinization. At moisture contents of greater than 30%, their X-ray studies confirmed that the endothermic transitions observed by DSC are related to loss of crystalline structure. Degree of crystalline order and polymer-solvent (plasticizer) interactions are major factors in determining melting behavior of starch, analogous to other natural and synthetic polymers. The wide range of melting temperature was attributed to variation in degree of crystalline perfection within the granule and to variable interaction with granule moisture. For normal maize starch, heating caused formation of crystalline V-type complexes of amylose with fatty

acids present in the granule. This was interpreted to partially explain how the differences that have been reported for heat of gelatinization values of lipid-bearing starches could be caused by competing melting and crystallizing effects. X-ray analysis failed to confirm DSC evidence of starch melting in samples at moisture contents lower than about 30%. A glass transition was postulated to account for DSC endotherms in such samples. Contrary to the interpretation of Biliaderis et al (1986), annealing and recrystallization were not found to be factors in gelatinization experiments on potato starch.

Russell (1987a,b) has questioned the concept proposed by Biliaderis et al (1986) that starch crystallinity is solely due to amylopectin, and has proposed that amylose makes some contribution, either by cocrystallization with amylopectin or by short-range ordering. In his view, short-range ordering involves both bulk amorphous regions and intercrystalline areas, permitting double helix formation between amylose and amylopectin, providing entities that contribute to the DSC melt endotherms. Russell interprets the double endotherms observed in limited water to be the melting of crystallites (the M1 endotherm in Donovan's terminology) and the disruption of the double helices to be associated with short-range order (the G endotherm in Donovan's terminology). He predicts from his model of the granule and its environment that total G+M1 enthalpy should be proportional to amylopectin content; any special factors such as incorporation of amylose into amylopectin crystals or increased short-range ordering would cause endotherms larger than those expected on the basis of amylopectin content. It seems apparent that if short-range ordering can occur between amylose and amylopectin regions, the opportunity for such ordering may be enhanced during annealing, when the molecules are more mobile.

Work previously done in our laboratory (Krueger et al 1987b) lends support to Russell's theory regarding the contribution of short-range ordering or partial incorporation of amylose into amylopectin crystallites to gelatinization behavior. Starches containing amylose were found to have higher gelatinization enthalpies than would be expected on the basis of their amylopectin content alone; i.e., enthalpy of gelatinization was greater for normal starch granules than for waxy maize starch granules, per unit weight of amylopectin in the sample. Annealing at 50°C had no effect on the gelatinization enthalpy of waxy maize starch but increased the enthalpy of amylose-containing starches. The extent of the change varied with the composition of the starch.

Additionally, our previous work showed that measurable effects of annealing occurred only in an excess of water and required relatively long times to develop and to reach equilibrium. Effects of annealing occurred more rapidly at higher annealing temperatures; however, the study was limited by onset of gelatinization.

The present study was undertaken as an attempt to circumvent the temperature limit for annealing to provide further understanding of starch crystallinity and the relative contributions of amylose and amylopectin. Our earlier work indicated that higher annealing temperatures might be attained by a multistep annealing procedure, whereby the sample is first annealed to equilibrium at one temperature, then reannealed at a slightly higher temperature. Because the initial annealing raises the onset temperature, the sample could then accommodate a higher annealing temperature without gelatinizing. This step could be repeated until further annealing caused no increase in the onset temperature. At this point, the sample would have presumably achieved its maximum attainable degree of crystallinity, and further increase in annealing temperature would result in gelatinization. In this way information could be obtained on the extent of possible annealing prior to gelatinization, the effect of such annealing on granule crystallinity, and the extent of amylose-amylopectin interactions in different types of starch granules with various crystal structures.

Maize starches were used for the study because samples are available with widely varying proportions of amylose and amylopectin, from 100% amylopectin to 70% amylose. In addition, the maize starches provide examples of both A- and B-type crystal structures; normal and waxy starches contain A-type crystals,

whereas amylomaize starches with more than 49% amylose have B structures (Zobel 1988b).

## MATERIALS AND METHODS

### Starch Samples

Starches were commercial samples from American Maize-Products Co., Hammond, IN. Samples used were from waxy and normal maizes and from class V (50% amylose) and class VII (70% amylose) amylomaizes. Actual values for apparent amylose content measured in our laboratory (Knutson 1986) were 0, 24.3, 51.4, and 66.1%, respectively. Samples were used as received, without defatting, to avoid the possibility of annealing or otherwise altering granule structure by the defatting procedure.

### Annealing Procedure

Starch samples were placed in three volumes of water and heated for times ranging from 2 to 72 hr at temperatures of 50, 55, 60, 65, or 70° C. Samples were then centrifuged and supernatant water was decanted. Starches were treated twice with acetone and centrifuged, then allowed to air-dry. For multistep annealing the same procedure was followed, starting with starch that had previously been annealed at each of the lower 5° C temperature intervals. After each heating interval the starch was centrifuged, treated with acetone, dried, and then annealed again at the next temperature. As the study progressed, it became apparent that multistep annealing effects were still observed at 70° C in waxy and high-amylose starches. Therefore, annealing of these two starches was continued to 75 and 80° C.

### Compositional Analysis

Carbohydrate content of the samples was measured by the phenol-sulfuric acid method (Dubois et al 1956). Sample weights for DSC were corrected accordingly to compensate for moisture content of the starches. Samples had a mean value of 85% starch based on wet weight. Supernatants from annealing procedures were also analyzed by this method to check for soluble carbohydrates leached from the granules. Amylose content of annealed samples was measured by the simplified colorimetric procedure for maize starches (Knutson 1986) to determine whether any amylose was being leached from the granules.

### Calorimetry

DSC analysis was done with a Perkin-Elmer DSC-2 calorimeter equipped with a thermal analysis data station (TADS). Accurately weighed samples of approximately 3 mg were placed in sealable pans with 10 $\mu$l of water, sealed and allowed to equilibrate overnight at room temperature. The pan was placed in the sample cell of the instrument, and a pan containing an equivalent weight of gelatinized starch was placed in the reference cell. Samples were heated from room temperature to 120° C at a heating rate of 10° C per minute. Enthalpy, onset temperature, and peak temperature were calculated automatically with the Perkin-Elmer "TADS" software programs. Samples with sharp, clearly defined endotherms were run in duplicate; samples with less clearly defined endotherms were run at least in quadruplicate. Standard deviations for onset and peak temperatures were approximately 0.5° C for normal and waxy maize starches, and 1.5° C for high-amylose starches. Standard deviations for enthalpy values were 0.3 cal/g for normal and waxy starches and 0.7 cal/g for high-amylose starches.

## RESULTS

### Effect of Annealing on Leaching of Starch from Granules

No soluble carbohydrate was detected in supernatant from annealing procedures, nor was there any detectable change in the amylose content of any of the starches.

### Normal and Waxy Maize Starch

*Long-term annealing.* Onset temperature, peak temperature, and enthalpy for normal and waxy maize starch samples annealed 24 hr are given in Table I. Endotherms for normal starch are shown in Figure 1 and for waxy starch in Figure 2. In addition to the usual gelatinization endotherm, a second, minor endotherm was observed prior to annealing in both normal and waxy maize starch. This endotherm in the control sample is presumed to be an artifact, caused by effects of the wet-milling procedure; e.g., a partial annealing as previously described by Krueger et al (1987a). The magnitude of this endotherm was quite small in the control; as such, it could have been ignored. However, the annealing process caused the appearance of larger endothermic transitions in this region, which in some instances merged with the primary endotherm as the onset and peak temperatures of the primary endotherm increased. Therefore, in order to accurately evaluate effects of annealing on gelatinization enthalpy it was necessary to calculate enthalpy values of both the primary and secondary endotherms in the control samples.

For normal starch (Fig. 1), onset and peak temperatures of the primary endotherm of single-step annealed samples increased with higher annealing temperature; the endotherm became taller and sharper up to a temperature of 60° C. At 65° C, the size of the primary endotherm decreased significantly, as is reflected in the decrease in enthalpy. By 70° C there was very little of the primary endotherm remaining, but the secondary endotherm was more prominent, indicating reordering of starch chains and higher

**TABLE I**
24-hr Annealing: Effect of Annealing Temperature (° C) on Gelatinization Characteristics of Normal and Waxy Maize Starch

| Starch/ Annealing Temperature | Primary Endotherm | | | | | | | | Secondary Enthalpy | | Total | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Onset Temperature[a] | | Peak Temperature | | $T_P - T_0$[b] | | Enthalpy | | | | | |
| | SS | MS | SS | MS | SS | MS | SS | MS | SS | MS | SS | MS |
| Waxy | | | | | | | | | | | | |
| Control | 67.2 | ... | 72.8 | ... | 5.6 | ... | 3.72 | ... | 0.49 | ... | 4.21 | ... |
| 50 | 68.4 | ... | 73.5 | ... | 5.1 | ... | 3.69 | ... | 1.49 | ... | 5.19 | ... |
| 55 | 72.4 | 72.0 | 76.3 | 75.9 | 3.9 | 3.9 | 3.96 | 3.82 | 1.15 | 0.75 | 5.11 | 4.57 |
| 60 | 75.3 | 74.6 | 78.3 | 78.1 | 3.0 | 3.5 | 3.98 | 3.85 | 0.61 | 1.01 | 4.59 | 4.86 |
| 65 | 76.4 | 77.5 | 81.3 | 80.2 | 4.9 | 2.7 | 1.54 | 3.51 | 0.85 | 0.15 | 2.38 | 3.67 |
| 70 | 73.6 | 79.9 | 84.1 | 82.9 | 10.5 | 3.1 | 0.84 | 1.59 | 0.58 | 1.63 | 1.42 | 3.21 |
| 75 | ... | 78.4 | ... | 81.5 | ... | 3.2 | ... | 1.39 | ... | 0.00 | ... | 1.39 |
| 80 | ... | 80.8 | ... | 86.5 | ... | 5.7 | ... | 1.72 | ... | 0.00 | ... | 1.72 |
| Normal | | | | | | | | | | | | |
| Control | 69.2 | ... | 73.2 | ... | 3.9 | ... | 3.28 | ... | 0.53 | ... | 3.80 | ... |
| 50 | 70.4 | ... | 74.0 | ... | 3.6 | ... | 3.22 | ... | 0.89 | ... | 4.11 | ... |
| 55 | 73.2 | 72.6 | 76.1 | 75.7 | 2.9 | 3.1 | 3.38 | 3.36 | 0.38 | 1.17 | 3.76 | 4.54 |
| 60 | 76.4 | 75.5 | 78.6 | 78.2 | 2.2 | 2.8 | 2.92 | 3.34 | 0.86 | 0.71 | 3.78 | 4.05 |
| 65 | 76.8 | 78.4 | 80.2 | 80.4 | 3.3 | 2.0 | 1.13 | 2.18 | 0.28 | 0.47 | 1.41 | 2.64 |
| 70 | 77.4 | 77.9 | 80.8 | 82.9 | 3.4 | 4.9 | 0.33 | 0.95 | 1.13 | 0.31 | 1.46 | 1.26 |

[a] SS = Single step; MS = multiple step.
[b] Peak minus onset temperature.

temperatures required for melting. For multistep annealing, the endotherm was still sharp at 65°C, then decreased at 70°C, with no evidence of the secondary endotherm other than the broadening of the base of the primary endotherm. Enthalpy changes shown in Table I give evidence of the degree of gelatinization caused by annealing. Initially, annealing caused a significant increase in total enthalpy. At 55°C, the first temperature at which samples were subjected to multistep annealing, primary gelatinization enthalpy was the same for both procedures, and was slightly increased above that of the control and the 50°C annealed sample. At higher annealing temperatures, primary enthalpy began to decrease. The decrease was much more rapid for samples subjected to single-step annealing, indicating that multistep annealing affords partial protection against gelatinization. Changes in enthalpy of the secondary endotherm with increased annealing temperature were somewhat erratic.

Endotherms for waxy starch (Fig. 2) were comparable to those for normal dent starch; they were somewhat broader and with higher enthalpy values under comparable conditions, but generally similar until the multistep annealing temperature of 70°C was reached. At that point, the waxy maize still showed a portion of the primary endotherm quite sharply. Multistep annealing of waxy maize starch therefore was continued to 75 and 80°C to see how far gelatinization could still be observed. A significant amount of starch remained ungelatinized at these annealing temperatures, as indicated by the enthalpy of 1.7 cal/g required to gelatinize the 80°C example.

*Effect of annealing time.* When starch samples were annealed at temperatures near the gelatinization range, DSC data indicated that enthalpies decreased with increased annealing time (Table II). The normal starch samples annealed at 60 and 65°C in particular show this effect, indicating that there is a degree of time dependence during gelatinization. The same trend was observed at 55°C, but the decrease was not statistically significant. In all cases, onset and peak temperatures tended to increase with increased annealing time.

### Class V Amylomaize Starch

Prior to annealing, the endotherm for the class V amylomaize starch granules, containing 50% amylose, showed a typical amylomaize endotherm, namely, a steep rise at the beginning, but with a lower peak than for normal and waxy maize starches, followed by a gradual decline, which reached to a temperature of 100°C or more (Fig. 3, Table III). After annealing, the onset and peak temperatures both increased, and the tailing edge became less gradual. In the single-step annealing procedure, the onset temperature, peak temperature, and gelatinization enthalpy all increased until annealing temperature reached 70°C; when annealing was carried to 75°C, enthalpy decreased and the endotherm was noticeably smaller, indicative of gelatinization during annealing. Samples annealed at 80°C were nearly completely gelatinized as evidenced by the very low gelatinization enthalpy. For multistep annealing, maximum enthalpy was reached at 60°C, and it decreased steadily with higher annealing temperature. How-
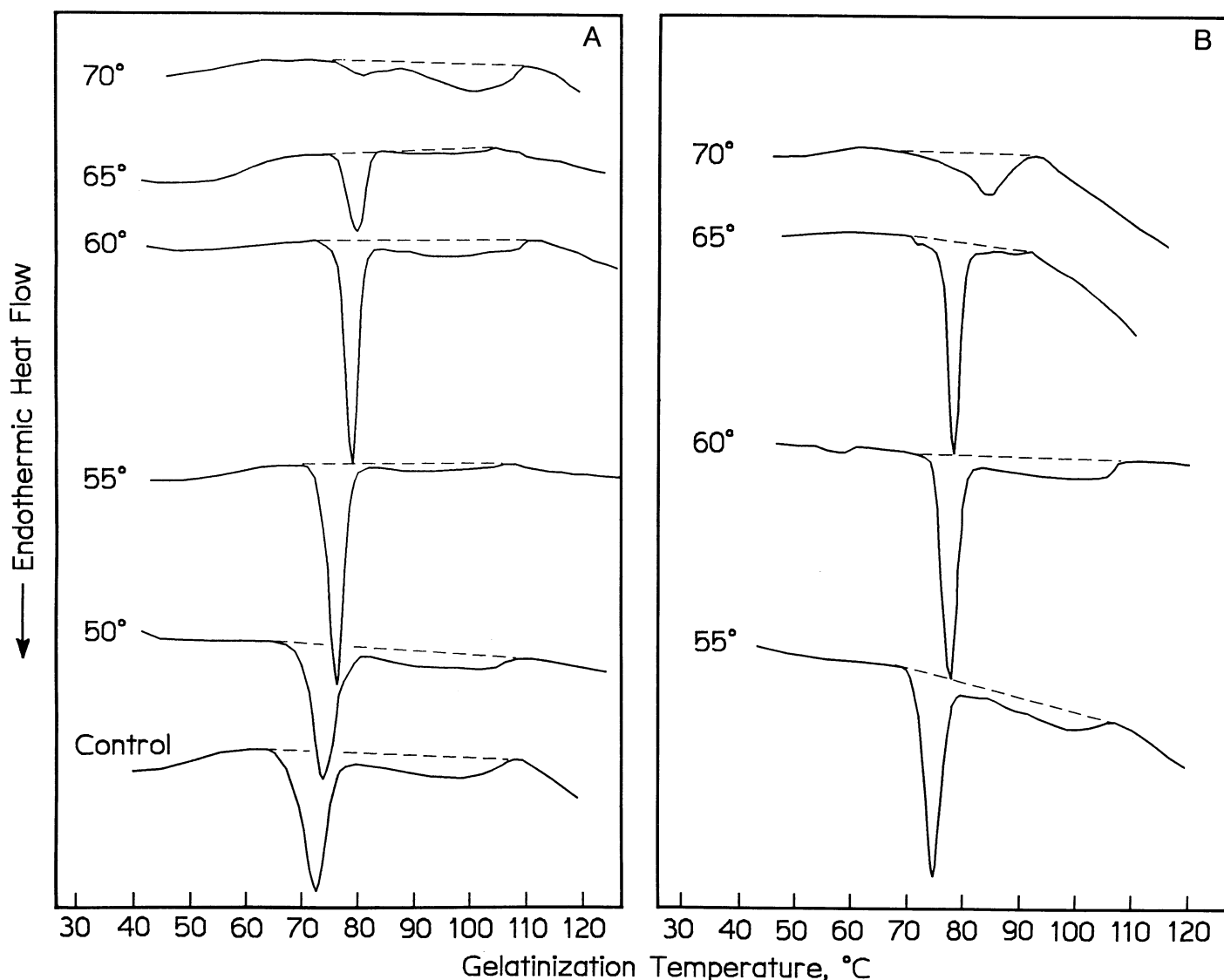


Fig. 1. Gelatinization endotherms of 24-hr annealed normal maize starch: A, single-step annealing; B, multistep annealing.

ever, onset and peak temperatures continued to increase with annealing temperature up to 80°C.

### Class VII Amylomaize Starch

Gelatinization endotherms of the nonannealed class VII starch (70% amylose) granules were distinctly different from those of class V, with a gradual increase to a poorly defined maximum at about 100°C, followed by a more rapid decrease (Fig. 4, Table III). Annealing had a similar, but smaller, effect than that seen with class V starch. Because the enthalpy was relatively low and the endotherm shape more poorly defined than with class V starch, no attempt was made to anneal class VII starch beyond 70°C.

## DISCUSSION

Samples in this study were commercial starches, which are known to have undergone partial annealing in the wet-milling process (Krueger et al 1987a). They were thus partially protected from gelatinization at the lower annealing temperatures. The amount of gelatinization caused by annealing can be estimated from the decrease in gelatinization enthalpy. Thus, in single-step annealing of normal starch, the primary endotherm lost 11% of initial enthalpy at 60°C, 70% at 65°C, and was almost completely gelatinized at 70°C. In multistep annealing, there was no measurable loss of enthalpy at 60°C, 34% at 65°C, and 71% at 70°C.

The presence of the secondary endotherm in the waxy maize starch prior to annealing is presumed to be caused by partial annealing during the wet-milling process (Krueger et al 1987a). Kugimiya et al (1980) attributed the secondary endotherm in maize starch to formation of the amylose-lipid complex. However, the amylose content of waxy maize starch is negligible, so no opportunity for amylose-lipid complex formation exists. Furthermore, when gelatinized samples were allowed to cool and then reheated, no significant endothermic transition was observed.

Gelatinization is generally considered to be a function of temperature rather than time. Marchant and Blanshard (1978) showed that gelatinization at a given temperature may require 10–13 min to reach equilibrium; beyond that point, time is not thought to cause significant change. However, the data in Table II reveal that samples held at temperatures near their gelatinization range (i.e., >60°C) did undergo partial gelatinization with increased annealing time, as demonstrated by the decrease in gelatinization enthalpy. This loss of enthalpy was accompanied by an increase in onset and peak temperature ($T_O$ and $T_P$, respectively) and a decrease in the $T_P - T_O$ values in the residual crystallites. The decrease in total enthalpy is greater for normal starch than for waxy starch (2.51 cal/g after 72 hr at 60°C versus 2.04 cal/g for waxy starch), indicating a greater loss of order in the amorphous and intercrystalline regions.

Annealing temperatures from 55 to 60°C caused only slight changes in primary or total enthalpy of normal or waxy maize
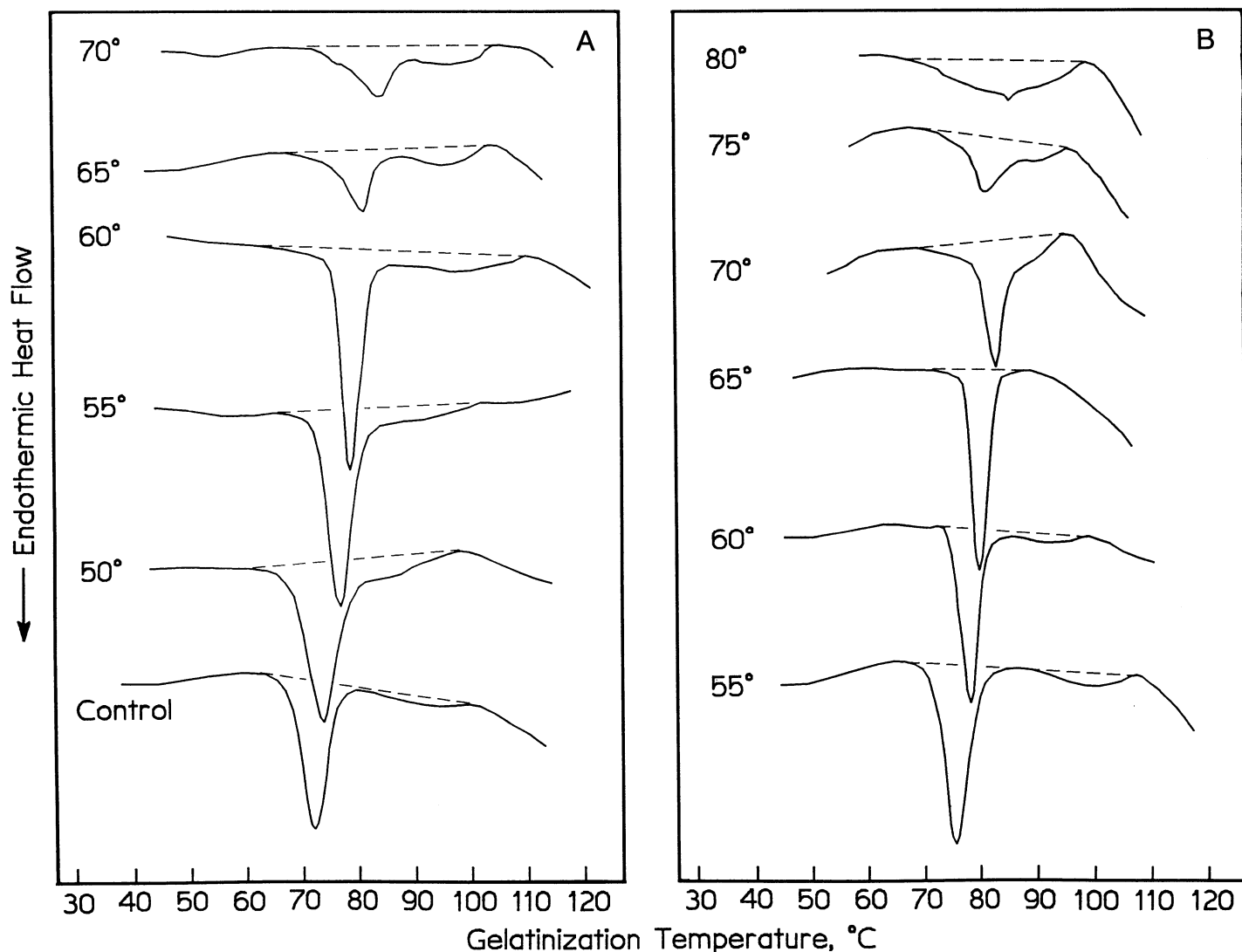


Fig. 2. Gelatinization endotherms of 24-hr annealed waxy maize starch: **A**, single-step annealing; **B**, multistep annealing.

starches, whereas the onset and peak temperature continued to increase and the temperature range ($T_P$–$T_O$) generally narrowed. The minimal change in this range indicates that some gelatinization of less "perfect" crystallites occurs, offsetting any increase of enthalpy that might be due to presence of more perfect crystallites. At temperatures above 60°C, enthalpy decreases further, indicating accelerated melting, so that only the most perfect crystallites, i.e., those which melt at the highest temperature, survive.

Our previous study (Krueger et al 1987b) indicated little change

### TABLE II
### Effect of Annealing Multistep Time on Gelatinization of Normal and Waxy Starch

| Annealing Conditions | | Primary Endotherm | | | | Secondary Enthalpy | Total |
|---|---|---|---|---|---|---|---|
| Temp (°C) | Time (hr) | Onset Temperature (°C) | Peak Temperature (°C) | $T_P$–$T_O$[a] | Enthalpy | | |
| Waxy | | | | | | | |
| Control | ... | 67.2 | 72.8 | 5.6 | 3.72 | 0.49 | 4.21 |
| 55 | 2 | 71.2 | 74.7 | 3.5 | 2.98 | 1.04 | 4.02 |
| 55 | 4 | 70.3 | 74.7 | 4.4 | 3.86 | 0.92 | 4.78 |
| 55 | 24 | 72.0 | 75.9 | 3.9 | 3.82 | 0.75 | 4.57 |
| 60 | 2 | 71.9 | 75.9 | 4.0 | 4.64 | 1.89 | 6.53 |
| 60 | 4 | 72.3 | 76.1 | 3.8 | 4.50 | 0.68 | 5.18 |
| 60 | 24 | 74.6 | 78.1 | 3.5 | 3.85 | 1.01 | 4.86 |
| 60 | 72 | 77.3 | 79.7 | 2.3 | 3.61 | 0.88 | 4.49 |
| 65 | 2 | 75.1 | 78.4 | 3.3 | 3.91 | 0.71 | 4.62 |
| 65 | 4 | 75.3 | 78.1 | 2.8 | 3.19 | 0.45 | 3.64 |
| 65 | 24 | 77.5 | 80.2 | 2.7 | 3.51 | 0.15 | 3.67 |
| 70 | 2 | 76.4 | 80.2 | 3.8 | 3.79 | 0.86 | 4.65 |
| 70 | 4 | 77.6 | 81.1 | 3.5 | 2.55 | 0.75 | 3.30 |
| 70 | 24 | 79.4 | 83.4 | 4.0 | 1.24 | 1.30 | 2.53 |
| Normal | | | | | | | |
| Control | ... | 69.2 | 73.2 | 3.9 | 3.28 | 0.53 | 3.80 |
| 55 | 2 | 70.1 | 74.6 | 4.5 | 3.67 | 0.39 | 4.06 |
| 55 | 4 | 71.2 | 74.4 | 3.2 | 3.42 | 0.62 | 4.04 |
| 55 | 24 | 72.6 | 75.7 | 3.1 | 3.36 | 1.17 | 4.54 |
| 60 | 2 | 72.5 | 75.7 | 3.2 | 3.56 | 2.16 | 5.72 |
| 60 | 4 | 73.0 | 76.0 | 3.0 | 3.29 | 0.15 | 3.44 |
| 60 | 24 | 75.5 | 78.2 | 2.8 | 3.34 | 0.71 | 4.05 |
| 60 | 72 | 77.6 | 79.6 | 2.0 | 2.36 | 0.85 | 3.21 |
| 65 | 2 | 74.8 | 77.9 | 3.0 | 3.00 | 0.84 | 3.84 |
| 65 | 4 | 74.7 | 77.8 | 3.1 | 3.22 | 2.10 | 5.32 |
| 65 | 24 | 78.4 | 80.4 | 2.0 | 2.18 | 0.47 | 2.64 |
| 70 | 2 | 75.4 | 79.1 | 3.7 | 1.98 | 0.27 | 2.25 |
| 70 | 4 | 73.0 | 79.7 | 6.7 | 1.35 | 0.00 | 1.35 |
| 70 | 24 | 77.9 | 82.9 | 4.9 | 0.95 | 0.31 | 1.26 |

[a] Peak minus onset temperature.

### TABLE III
### 24-hr Annealing: Effect of Annealing Temperature on Gelatinization Characteristics of High-Amylose Starches

| Annealing Temperature | Onset Temperature[a] | | Peak Temperature | | $T_P$ – $T_O$ | | Enthalpy | |
|---|---|---|---|---|---|---|---|---|
| | SS | MS | SS | MS | SS | MS | SS | MS |
| Amylomaize V | | | | | | | | |
| Control | 70.1 | ... | 75.6 | ... | 5.5 | ... | 2.06 | ... |
| 50 | 70.9 | ... | 77.6 | ... | 6.7 | ... | 3.20 | ... |
| 55 | 74.3 | 74.4 | 81.6 | 80.4 | 7.4 | 6.0 | 3.84 | 3.91 |
| 60 | 77.5 | 76.8 | 85.1 | 84.0 | 7.6 | 7.3 | 4.88 | 5.46 |
| 65 | 84.2 | 79.1 | 89.6 | 86.7 | 5.4 | 7.6 | 4.47 | 3.88 |
| 70 | 88.0 | 84.4 | 94.3 | 89.9 | 6.4 | 5.5 | 4.73 | 2.04 |
| 75 | 92.8 | 90.8 | 97.3 | 94.5 | 4.5 | 3.8 | 1.86 | 1.78 |
| 80 | 93.0 | 95.5 | 98.5 | 99.0 | 5.5 | 3.5 | 0.36 | 1.59 |
| Amylomaize VII | | | | | | | | |
| Control | 86.4 | ... | 94.0 | ... | 7.7 | ... | 2.52 | ... |
| 50 | 93.9 | ... | 101.1 | ... | 7.2 | ... | 3.79 | ... |
| 55 | 79.8 | 91.8 | 99.1 | 98.2 | 19.3 | 6.3 | 3.71 | 3.02 |
| 60 | 80.9 | 84.7 | 94.7 | 90.2 | 13.8 | 5.4 | 3.07 | 3.76 |
| 65 | 85.0 | 82.1 | 98.0 | 88.7 | 13.0 | 6.6 | 3.33 | 1.89 |
| 70 | 95.8 | 84.0 | 100.9 | 93.9 | 5.2 | 9.9 | 2.12 | 3.02 |

[a] SS = Single step; MS = multiple step.

in either the enthalpy or the temperature of gelatinization during annealing of waxy maize starch. However, the higher annealing temperatures used in the present study were found to cause more pronounced changes. Since waxy starch contains no amorphous amylose regions, annealing effects at higher temperatures occur in intercrystalline amylopectin regions or in crystallites; in either case, these regions are apparently more stable to disruption at lower annealing temperatures than are the amorphous amylose regions. With single-step annealing, the secondary endotherm becomes more evident (Fig. 2), suggesting increased formation of the structure responsible for this endotherm. In multistep annealing, a greater proportion of the primary endotherm survives to 70°C. At annealing temperatures above 70°C, both endotherms deteriorate.

The similarity in gelatinization behavior of the normal and waxy maize starches, both before and after annealing, suggests that 1) interaction between amylose and amylopectin in normal starch prior to annealing is relatively minor; and 2) little further interaction takes place as a result of annealing. In contrast, gelatinization behavior of class V amylomaize starch changed drastically as a result of annealing, suggesting substantial interaction between amylose and amylopectin. Specifically, amylomaize V starch prior to annealing displayed a characteristic endotherm with a rapid initial rise followed by a gradual decrease. Presumably, melting of crystallites occurs with the initial increase, comparable to the situation in normal and waxy starch. The gradual decrease is presumed to be a combination of the disruption of order in the amorphous amylose regions, as well as disruption of the amylose-lipid complex as described by Russell (1987a). With annealing, the endotherm becomes much sharper and more symmetrical. $T_O$ and $T_P$ increase greatly and occur at higher temperatures than are observed in normal or waxy starches. Furthermore, the structure represented by this new endotherm is capable of surviving to 80°C with multistep annealing. Enthalpy of gelatinization of the new endotherm is initially increased sub-

stantially. Higher temperatures and enthalpy values are both indications of increased order in the system, either due to amylose-amylopectin interactions, to increased association and ordering within the amylose amorphous regions, or to formation of the V-amylose complex interacting with the naturally occurring fatty acids.

Annealing amylomaize VII starch produces similar but smaller changes in the DSC endotherms. If the annealed endotherms of high-amylose starches are indeed due to amylose-amylopectin interaction, these smaller changes are what would be expected with amylomaize VII; the maximum opportunity for interaction would occur when concentrations of amylose and amylopectin are most nearly equal, as is the case with amylomaize V starch. However, the possibility exists that less reaction occurs because the amylose molecules in the amorphous regions of amylomaize VII starch are more strongly associated and therefore are harder to disrupt and reorient. Also, higher amylose content enhances the possibility of the presence of amylose V crystalline structures, which would be less likely to reorient.

In addition to amylose-amylopectin interaction, other possibilities for the increased gelatinization enthalpy and the change in shape of the endotherm in annealed high-amylose starches are enhanced formation of amylose-lipid complexes and enhanced order within the amorphous, glassy, amylose-containing regions, as described by Zobel et al (1988). These explanations, however, are less consistent with our data and our observations than is amylose-amylopectin interaction. The increases in onset and peak temperature of the endotherms make amylose-lipid complex formation unlikely, since the temperature range of the amylose-lipid complex is constant (Kugimiya et al 1980). Furthermore, amylose-lipid complex formation would be expected to be as great, and possibly greater, in starch with higher amylose content. This argument also applies to annealing of amorphous regions. There may, of course, be some contribution to the increased enthalpy by either or both of these actions.
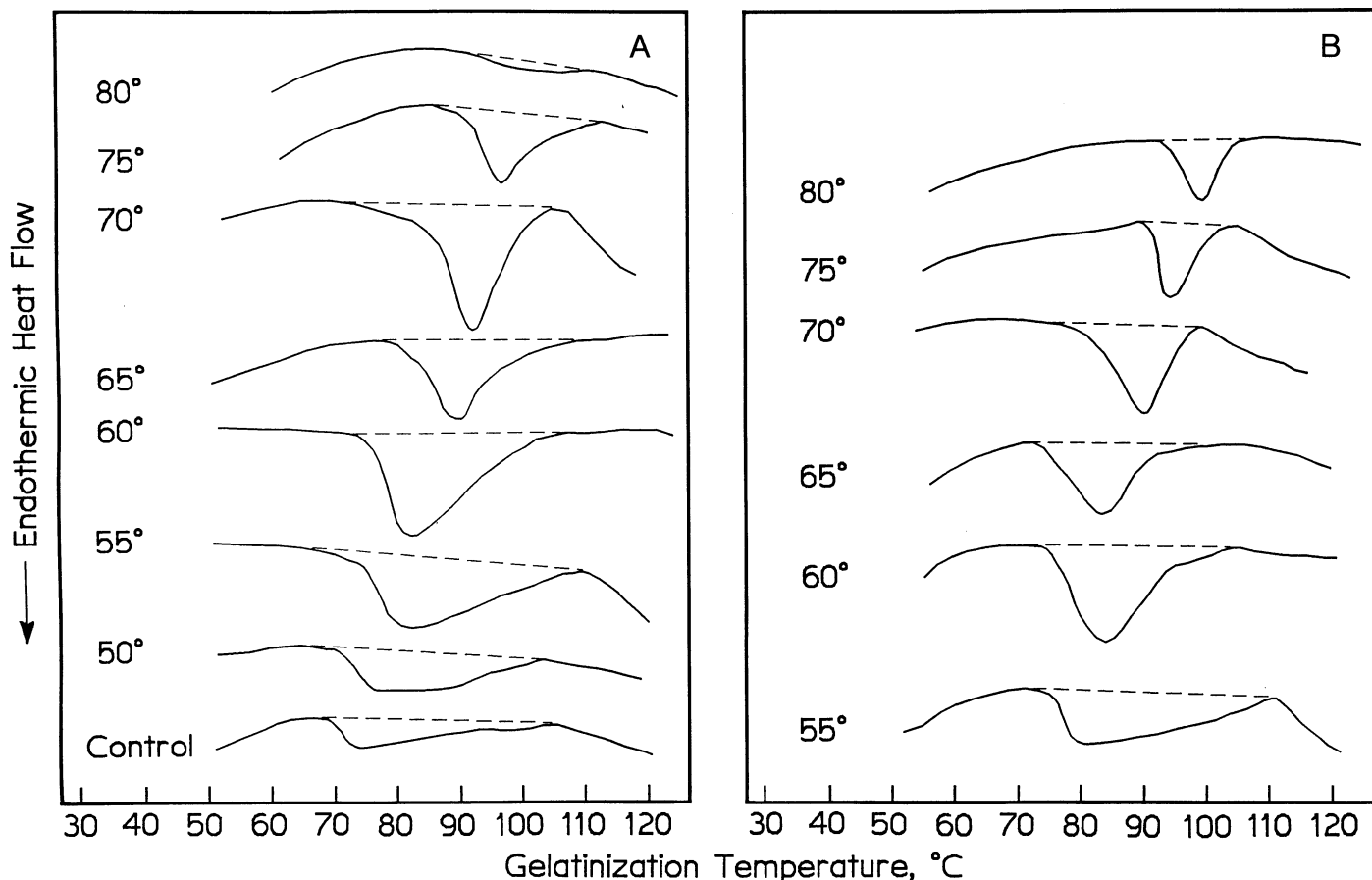


**Fig. 3.** Gelatinization endotherms of 24-hr annealed amylomaize V starch: **A,** single-step annealing; **B,** multistep annealing.
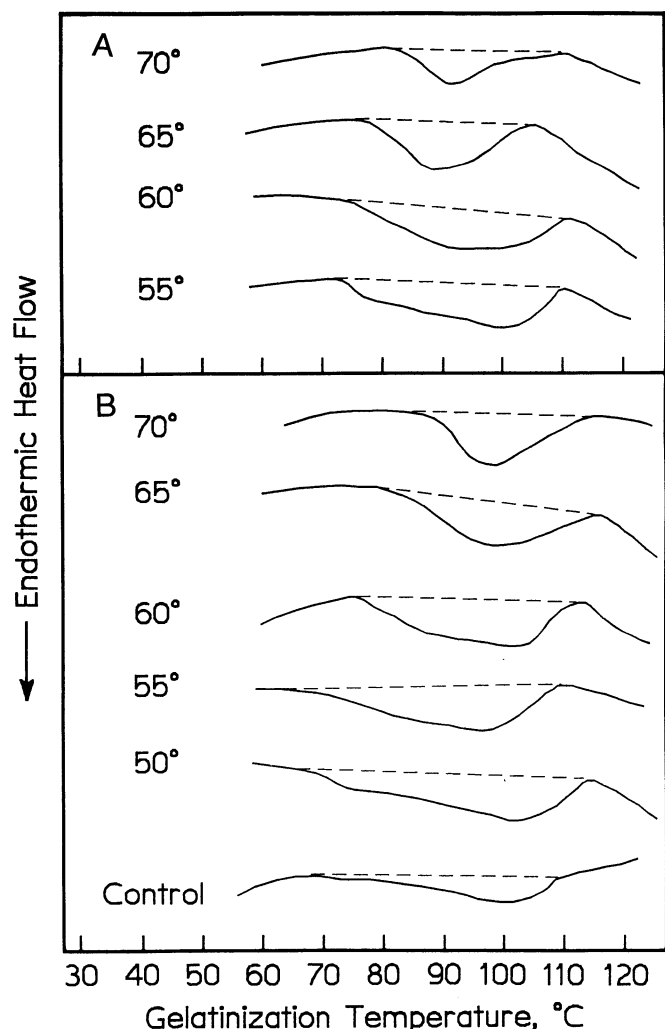
**Fig. 4.** Gelatinization endotherms of 24-hr annealed amylomaize VII starch: **A**, single-step annealing; **B**, multistep annealing.

The extent of amylose-amylopectin interaction can be estimated if the enthalpic contributions that involve only amylopectin (either melting of amylopectin crystallites or disruption of order in the intercrystalline amylopectin regions) are eliminated. This can be achieved by subtracting from the total enthalpy of an amylose-containing starch a value equal to the enthalpy of waxy maize starch multiplied by the proportion of amylopectin in the starch. The resulting value is the enthalpy required to disrupt any other interactions: amylose-amylopectin interactions, amylose double helix interactions, and amylose-lipid complexes. It is recognized that this value does not measure the interaction enthalpy exactly because of the possibility of variations in enthalpy required to melt amylopectin crystallites from different starches, and amylopectins from high-amylose starches are known to have longer side chains than those from normal maize starch (Hizukuri 1985). However, this calculation provides a first approximation of the enthalpy of interaction, and a way for evaluating the events that occur during gelatinization.

Interaction enthalpy was calculated in this manner for normal and high-amylose starches from the enthalpy values given in Tables I and III. The results are shown in Table IV. A relatively small amount of interaction was found for normal maize starch under any annealing condition. The small amount of interaction enthalpy observed in the normal starch samples is presumably due to amylose-amylose interaction, or to amylose-lipid complexes, because of the lack of intermingling of amylose and amylopectin areas in A-type starches, as proposed by Zobel (1988a). On the other hand, interaction enthalpy for high-amylose starches was substantial.

The difference in behavior between the normal and waxy

**TABLE IV**
**Interaction Enthalpy of Amylose-Containing Starches**

| Annealing Temperature | Amaizo[a] | | Amylo V | | Amylo VII | |
|---|---|---|---|---|---|---|
| | SS | MS | SS | MS | SS | MS |
| Control | 0.61 | ... | 0.01 | ... | 1.09 | ... |
| 50 | 0.18 | ... | 0.68 | ... | 2.03 | ... |
| 55 | 0.00 | 1.08 | 1.36 | 1.69 | 1.98 | 1.47 |
| 60 | 0.31 | 0.37 | 2.65 | 3.10 | 1.51 | 2.20 |
| 65 | 0.00 | 0.00 | 3.31 | 2.10 | 2.52 | 0.86 |
| 70 | 0.39 | 0.00 | 4.04 | 0.48 | 1.64 | 1.93 |
| 75 | ... | ... | 1.86 | 1.10 | ... | ... |
| 80 | ... | ... | 0.36 | 0.75 | ... | ... |

[a] SS = Single step; MS = multiple step.

starches and those with high amylose content must be considered in view of differences in the crystalline nature of the raw starches. Zobel (1988a,b) in his recent reviews of starch crystallinity and granule structure, points out that X-ray diffraction analysis of normal and waxy starches gives typical A patterns, whereas starches with amylose content of 49% or higher display the B pattern. In A-type starches, amylose and amylopectin appear to exist in separate domains, whereas in B-type starches, using potato starch as an example, Zobel summarized evidence for intermingling of amylose and amylopectin. The increased intermingling provides greater opportunity for interaction between these molecules. It should be noted that the same situation may not necessarily exist in high-amylose maize starches, because the B crystalline pattern has been ascribed at least in part to the greater length of the amylopectin side chains in these starches (Zobel 1988a). In addition, because the A structure is more densely packed and contains less water, conversion of B to A conformation is energetically favored over the reverse. Our data are consistent with this concept of starch crystallization. For example, the degree of interaction of amylose and amylopectin in normal starch shows no significant change with annealing, consistent with Zobel's contention that there is little mixing of amylose and amylopectin in typical A-type starches (1988b). If the molecules are not naturally intermingled in starches with high amounts of amylopectin, there is not likely to be enough flexibility and mobility to allow greater mixing during annealing. However, when high-amylose starches are annealed, total enthalpy and interaction enthalpy increase significantly, consistent with the presence of less densely packed crystallites and with greater intermingling of amylose and amylopectin, to provide opportunity for amylose-amylopectin interactions of the type proposed by Russell (1987a) for short-range ordering.

Additional study of the annealing effect, beyond the scope of this report, remains to be done. For example, apparent changes in crystallinity indicated by these studies need to be confirmed by X-ray crystallography, especially whether the high-amylose starches show transformation from one type of crystal structure to another. Also, Zobel et al (1988) recently confirmed that enthalpy values in starch gelatinization are affected by the formation of amylose-lipid complexes. The starches used in this study are certainly affected in a similar manner; X-ray studies should provide insight into the contribution that amylose-lipid complexes make to the enthalpy of the annealed high-amylose starches. Annealing studies on defatted starches would further aid understanding the effect of such complexes on starch properties. Finally, annealing studies on starch under limited water conditions also should contribute to our overall understanding of starch crystallinity and gelatinization.

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