

Retrogradation of Maize Starch After Thermal Treatment Within and Above the Gelatinization Temperature Range

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ABSTRACT

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Studies of starch retrogradation have not considered the initial thermal treatment. In this article, we explore the effect of heating to temperatures within and above the gelatinization range on maize starch retrogradation. In the first experiment, 30% suspensions of waxy (*wx*) starch were initially heated to final temperatures ranging from 54 to 72°C and held for 20 min. On reheating in the differential scanning calorimeter immediately after cooling, the residual gelatinization endotherm peak temperature increased, the endotherm narrowed, and enthalpy decreased. Samples stored for seven days at 4°C showed additional amylopectin retrogradation endotherms. Retrogradation increased dramatically as initial holding temperature increased from 60 to 72°C. In a second experiment, *wx* starch was initially heated to final temperatures from 54 to 180°C and rapidly cooled, fol-

lowed by immediate reheating or storage at 4°C. Maximum amylopectin retrogradation enthalpy after storage was observed for initial heating to 82°C. Above 82°C, retrogradation enthalpy decreased as initial heating temperature increased. A similar effect for *ae wx* starch was observed, except that retrogradation occurred more rapidly than for *wx* starch. These experiments show that heating to various temperatures above the range of gelatinization may profoundly affect amylopectin retrogradation, perhaps due to varying extents of residual molecular order in starch materials that are commonly presumed to be fully gelatinized. This article shows that studies of starch retrogradation should take into account the thermal history of the samples even for temperatures above the gelatinization temperature range.

The many forms of starch in foods and processed foods are all in some metastable state with varying degrees of crystallinity or other forms of order. The noncrystalline material has been considered amorphous. Operationally, amorphous starch has been defined as material not exhibiting an X-ray diffraction pattern (Zobel et al 1988). Even a fully amorphous starch material may be assumed to reflect some combination of polyamorphous states, varying in free energy, as described by Angell (1995). Gidley (1988) has shown that amorphous starch gives a nuclear magnetic resonance (NMR) pattern consistent with a nonrandom relationship among glucose units, showing that some order exists in the amorphous starch. Several possible levels of noncrystalline polymer order over even longer than the short distances sensed by NMR have been presented by Sperling (1986). The distinction between crystalline and amorphous states is not absolute in terms of molecular order.

Starch chemists have long struggled to reconcile practical terms in common use with the precise nature of the changes in starch. These terms were in use before the structures responsible for the metastable nature of the polymers were understood. Pasting is a term that has been used to describe the cook-up behavior of native starch granules and, used broadly, it covers a wide range of changes at the molecular, supramolecular, granular, and extragranular levels. Set-back is a term that has been used primarily to describe the increase in viscosity that occurs on cooling a pasted starch (often as observed using the Brabender Viscoamylograph). As the events during cooking of starch granules in excess water have become better understood, the term gelatinization has been used to describe a portion of the changes involved in pasting starch, including the loss of crystallinity. Changes subsequent to pasting of starch may result in recovery of crystallinity under appropriate conditions. The original sense of the word retrogradation was related to the retrograde nature of the behavior with respect to crystallinity.

Atwell et al (1988) surveyed starch chemists and proposed more precise definitions for three terms in common use: gelatinization, pasting, and retrogradation. Gelatinization was proposed to include "the collapse of molecular orders within the starch granule," whereas pasting was proposed to include "the phenomena following gelatinization in the dissolution of starch." These authors struggled to distinguish clearly between these two terms: granule swelling and starch solubilization were considered to be involved in both processes. The most readily quantifiable aspects of the behavior of starch granules during heating involve loss of birefringence (quantified by polarized light microscopy), loss of double-helical structure (quantified by NMR), loss of crystallinity (quantified by X-ray diffraction), and an endothermic melting or dissociation event (quantified by differential scanning calorimetry [DSC]). All of these changes are related to the collapse of molecular orders and would be thought of as ways to monitor different aspects of gelatinization. Quantification of granule swelling, molecular solubilization, and loss of granule integrity is more problematic and is generally based on some empirical test.

Retrogradation has been used to describe changes in physical behavior following gelatinization (Cameron and Donald 1991, Wu and Eads 1993). Retrogradation has also been commonly used to describe increased order from initially dispersed starch molecules as well (Miles et al 1985a,b; Ring 1985; Ring et al 1987a; I'Anson et al 1988). Atwell et al (1988) proposed that retrogradation be considered "a process that occurs when the molecules comprising gelatinized starch begin to associate in an ordered structure." They recognized that changes in physical behavior resulted from a process of molecular ordering. However, the implicit presumption in this definition is that gelatinized starch retains no ordered structure. The possibility of residual amylopectin order at the completion of gelatinization has not been explored.

The various metastable semicrystalline forms of starch are due to the numerous ways that the nature and extent of the crystallinity may vary, the relationship of the crystalline regions to the remaining amorphous material, and the varied types of ordered (but noncrystalline) regions in the amorphous phase. Normal starch granules are understood to be semicrystalline polymeric materials. The crystallites in starch are composed of portions of the branched amylopectin molecules rather than involving the linear molecules. Polymer crystallinity and melting has been best

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studied for linear synthetic polymers of commercial importance (Sperling 1986, Wunderlich 1990). Although the crystallization and melting behavior of largely linear amylose might be understood based on the theoretical treatments developed for linear synthetic polymers, a rigorous theoretical basis for crystallization and melting of branched polymers has been more difficult to develop. Furthermore, although an analogy between amylopectin and branched copolymers (Wunderlich 1980) has been made (Slade and Levine 1991), the analogy is weak in that the ramified branching of amylopectin does not occur in a branched copolymer. Recently, the behavior of dendritic polymers has been described (Stutz 1995). Although an analogy between this class of polymers and amylopectin is in some ways better than for branched copolymers, branches of these dendritic molecules are regularly spaced rather than periodic.

The crystallinity of native starch granules is generated during granule synthesis, an example of a process that Wunderlich (1976) has termed crystallization during polymerization. Much evidence is consistent with the idea that tangential crystalline lamellae are added as the granule grows radially (Martin and Smith 1995). The lamellar nature of these crystallites is similar to that of the fringed micelle and the folded lamellar models for crystallinity of linear polymers. The amylopectin lamellae might be considered a special case of the fringed micelle model. The lamellae are formed from the branch chains, and one end of the branch chains has limited motion due to its attachment at a branch point. Given the periodicity in branch length (Hizukuri 1986), the crystallization behavior might be expected (Gidley and Bulpin 1987) to be analogous to oligomer crystallization, for which the lamellae are the width of the oligomer (Wunderlich 1976). The lamellar thickness of crystallites as observed by electron microscopy is consistent with the length of the amylopectin branch chains (Yamaguchi et al 1979). Ring et al (1987b) studied crystallization behavior of linear glucan chains of the length of amylopectin side chains and observed birefringent spherulites, as for crystallization of synthetic linear polymers. Crystallites in native starch are noteworthy relative to synthetic linear polymers in that the packing is not of individual glucan chains but of double helices of adjacent chains (Imberty et al 1991). Consequently, the crystallites include two levels of order: double-helical and crystalline. Cooke and Gidley (1992) have attempted to evaluate the nature of the losses of these two types of order during the process of gelatinization. They suggested that for DSC of native *wx* starch, loss of double-helical order may be a more important enthalpic event than the loss of crystalline order. Their work illustrates how ordered amylopectin structures (double helices) may be present in an amorphous (noncrystalline) state. (Others have shown that helical amylose-lipid complexes may be either amorphous or arranged in a crystalline array [Biliaderis et al 1986a, Karkalas et al 1995].)

The literature of starch granule cooking contains numerous references to the melting temperature (T_m) of the crystallites (Slade and Levine 1991). It is well known that homopolymers melt at a range of temperatures. For a homopolymer sample, the highest temperature in this range is commonly considered the melting temperature. In a strict sense, it is unlikely that, for a metastable system like starch, a true equilibrium melting temperature is ever closely approached, as Wunderlich (1980) argues is also the case for other high polymers. When the structural variation within and among starch molecules is considered, even the concept of a single equilibrium melting temperature becomes tenuous, as the chains involved in double helices and the resulting crystallites would be expected to vary in length.

The amorphous regions of the granule undergo glass transitions (T_g) at temperatures related to the moisture (plasticizer) content. Cameron and Donald (1992) have presented a model for native starch with two distinct types of amorphous regions, a suggestion consistent with a prior model based on DSC evidence (Biliaderis et al 1986b). This thinking would suggest at least two T_g

(Biliaderis et al 1986b, 1991). To the extent that the portions of some of the molecules of an amorphous region participate in neighboring crystallite structures, the mobility of the particular amorphous region will be more constrained, leading to an elevated T_g . Consequently, one might expect a range of T_g (Biliaderis 1991). If one considers the simple case of a polymer with a single T_g and melting temperature, one may understand one way that changes to a more stable state may occur. At temperatures above the T_g but below the melting temperature, the polymer would have sufficient thermal energy to change from one metastable semicrystalline state to a more stable (but still metastable) semicrystalline state that more closely approaches the theoretical equilibrium state. This process is termed annealing. The term has been used variously by starch chemists to apply to a variety of treatments, but it has not been precisely defined with respect to starch. On the basis of an increase in onset temperature (T_o) after treatment, Yost and Hoseney (1986) suggested that at 50% solids, annealing of wheat starch begins 3–8°C below the initiation of gelatinization. Based on their previous work, Jacobs et al (1995) defined annealing as a process in excess water (1:2, w/w) at a temperature 3–4% below the gelatinization peak temperature (in K). Common usage of annealing by starch chemists involves heating to just below the initiation of gelatinization. Others have shown that stepwise annealing of native starch granules can result in repeated increases in T_o (Knutson 1990, Jacobs et al 1994).

Wunderlich (1976) describes a common general usage of the term annealing as “imparting a certain property by heat treatment without large-scale melting.” In this sense, a variety of starch heat treatments at low moisture (in the starch literature, so-called heat-moisture treatments [Zobel 1992]) accomplish altered starch properties without melting and would be considered annealing. Annealing has been also applied in a more precise way in the study of polymers to mean “improvement of crystallization by heating to temperatures below the melting point,” leading to growth of crystal area, perfection of crystals, or change to a more stable crystal structure (Wunderlich 1976). In this definition, the melting point is the equilibrium melting point, a temperature difficult to conceptualize for molecules of variable structure. For starch, annealing in this sense will include recrystallization, in the sense that the latter term is used by Wunderlich (1976) to mean “the crystallization of a partially molten or dissolved material using the remaining crystalline parts as substrate.” In a molecularly complex starch system, recrystallization may be considered to be a mechanism for annealing within the gelatinization temperature range, as some crystallinity remains after melting of less well-ordered crystallite material. Biliaderis et al (1986b) have presented evidence that some annealing (likely involving recrystallization involving less stable crystallites) of starch occurs during heating at 10°C/min, a rate commonly used in DSC work. This observation is consistent with previous work (Wootton and Bamunuarachchi 1979), which showed an effect of heating rate on T_o , with more rapid heating leading to lower T_o . Whether a polymer undergoes recrystallization (in the sense above) or simply crystallizes from a disordered melt or solution through some nucleation process would be one basis for differentiating between annealing and crystallization. The problem of distinguishing these two processes is even more subtle when one considers the possibility of self-nucleation, a process in which small-scale residual order above the final heating temperature serves as a nucleation site for crystallization on cooling (Wunderlich 1976). For starch, self-nucleation might also be considered to include nucleation based on residual double helices which, although not necessarily crystalline, are prerequisite for subsequent crystallinity, and thus might be considered an immediate precursor to crystallinity. Particularly long double helices might only become disordered well above temperatures normally associated with gelatinization, and on cooling, these double helices could enhance crystallite formation. For starch, we propose that annealing be considered a proc-

ess of increased ordering based on residual order (including residual double helices), encompassing the effects both of treatment just below T_0 and the effects of treatments above T_0 that are associated with subsequent recrystallization or self-nucleation. We propose that crystallization be considered to result only from a disordered melt or solution. Although differentiating between annealing and crystallization is difficult with starch, failure to consider this distinction might confound an understanding of the changes in starch subsequent to a thermal treatment.

Retrogradation of gelatinized starch granules and retrogradation of starch molecules after molecular dispersion have been studied as if behavior of isolated molecular fractions were not different than their behavior in gelatinized starch. In some respects, the behaviors appear similar, as demonstrated by Eliasson (1985), who showed that stale bread had a DSC endotherm similar to that of retrograded amylopectin but not to that of retrograded amylose. For starch granules, it has been assumed that once the readily observed granule order (as observed by polarized light microscopy, NMR, X-ray diffraction, or DSC) has been lost, the order pertinent to the study of retrogradation has been lost. As a result, when retrogradation has been studied, it has not always been clear whether annealing (including recrystallization or self-nucleation) or crystallization behavior has been observed.

In the present work we chose to study native waxy-type maize starches to simplify the molecular composition and focus on the behavior of the metastable semicrystalline amylopectin in the

granules. We first describe the thermal behavior of waxy starch granules in excess water (30% starch, w/w) after gelatinization to varying extents (as determined by DSC); thus, the samples were partially gelatinized and partially annealed. Thermal behavior of the treated starches was observed either immediately after the initial heat treatment or after storage at 4°C for one week, allowing us to monitor the residual gelatinization enthalpy and the enthalpy associated with retrogradation. We then describe the thermal behavior of waxy-type starches heated to various temperatures above the temperature at which gelatinization enthalpy is lost in an attempt to study the effect of possible residual order unrelated to what may be observed by DSC on the subsequent increase in order. By this approach, we illustrate the complexity of the molecular processes that occur during what has been commonly considered retrogradation.

MATERIALS AND METHODS

Materials

Maize genotypes homozygous for the waxy (*wx*) mutant and the amylose-extender waxy (*ae wx*) double mutant in the dent inbred W64A were grown at the Horticultural Research Farms of the Russell E. Larson Agricultural Research Center at Rock Springs, PA, during the summer of 1995. Both genotypes had previously been backcrossed at least nine times to W64A to produce isogenic lines. All plants were self-pollinated. Kernels were harvested at least 50 days after pollination and dried in a forced-air oven at 37°C for three days. Starch was isolated from 200 g (dry weight) of kernels by the procedure of Boyer et al (1976). Final starch samples were dried at 37°C and stored in a desiccator over CaCl_2 before analysis.

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). Starch gelatinization and retrogradation were monitored as described by Yuan et al (1993). Starch (~18.0 mg, dwb) was weighed into stainless steel sample pans (319-1605, Perkin-Elmer). Starches were assumed to contain 10% moisture. Deionized water was added to make 30% starch suspensions (w/w). Starch suspensions were sealed, and equilibrated for 15–30 min at room temperature. The reference cell contained a sealed, empty stainless steel pan. All samples were heated at a rate of 10°C/min. For initial thermal treatment, samples were heated from 15°C to the final treatment temperature. Analyses of endotherms allowed calculation of endotherm temperature of onset (T_0), peak temperature (T_p), and total enthalpy (J/g). Samples of indium were weighed into stainless steel pans and used as standards.

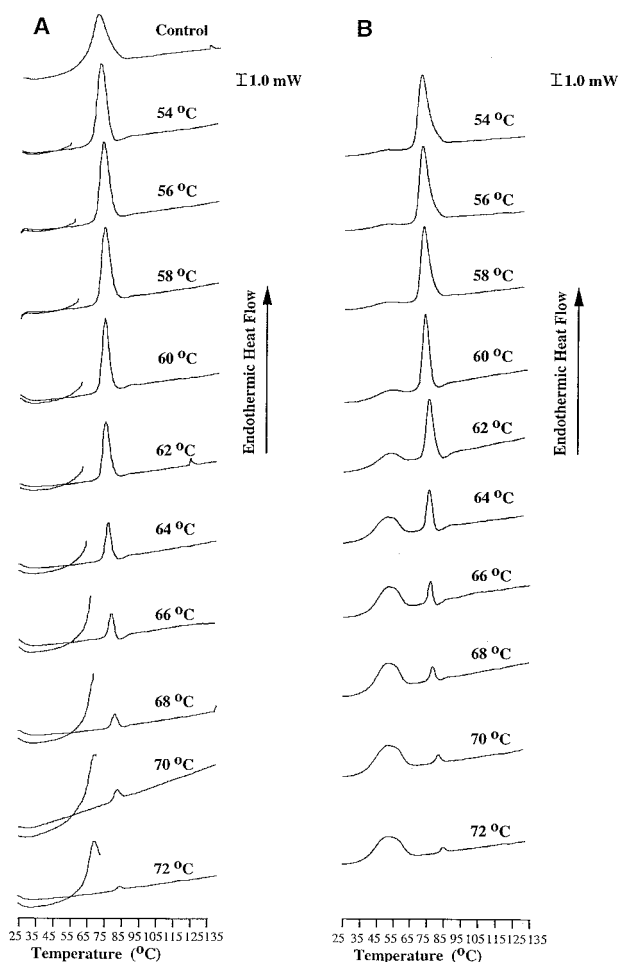


Fig. 1. Differential scanning calorimetry (DSC) thermograms for *wx* starch heated to temperatures within the range of gelatinization (Experiment I). **A)** Suspensions (30%, w/w) were heated to indicated final temperatures and held for 20 min, and then samples were cooled and immediately reheated. The control was not initially heated. **B)** Samples were cooled and stored for seven days at 4°C and then reheated.

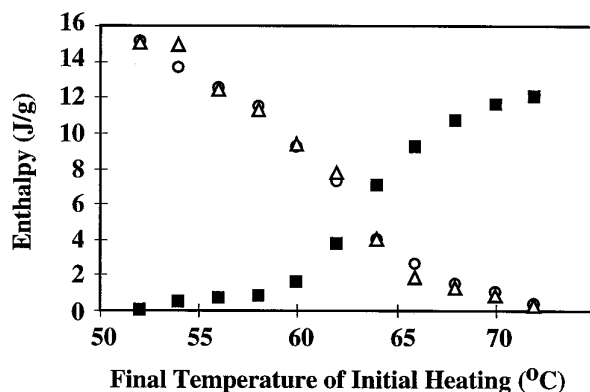


Fig. 2. Residual gelatinization enthalpy (O) after initial heating, and residual gelatinization enthalpy (Δ) and retrogradation enthalpy (■) after seven days at 4°C as a function of the final temperatures of initial heating (Experiment I).

Experiment I

All samples were heated as above and held at a final temperature for 20 min. Holding temperatures ranged from 54 to 72°C, just below the gelatinization onset ($T_o = 62^\circ\text{C}$) for the native starch and just above the endotherm maximum temperature ($T_{\text{max}} = 70^\circ\text{C}$), respectively. After heat treatment, samples were quench-cooled to 15°C, and held there for ~2 min (the time necessary for the sample to be cooled and for the DSC to be stabilized for the next heating cycle). Samples were then immediately reheated or removed for storage. Immediate reheating was from 5 to 140°C at 10°C/min. For evaluation of retrogradation, samples were held at room temperature for 15–30 min before storage at 4°C. After storage, samples were removed from the 4°C chamber and allowed to equilibrate to room temperature for 1 hr before DSC analysis. The second heating was from 5 to 140°C at 10°C/min.

Experiments II and III

Samples of *wx* and *ae wx* starch were heated to final temperatures ranging from 54 to 180°C. Samples were immediately quench-cooled to 15°C. Samples were held for 2 min at 15°C before removal or immediate reheating (as described above). Independent samples of each initial temperature treatment were prepared for each storage time. Storage was for one, three, seven, and ten days for *wx* starch samples and one day for *ae wx* starch samples. Immediate reheating for the *ae wx* samples was performed as above. The second heating for monitoring amylopectin retrogradation was performed from 5 to 140°C at 10°C/min.

RESULTS

Experiment I: Thermal Treatment of *wx* Starch Within the Range of Gelatinization

As holding temperatures were increased from 54 to 72°C, DSC thermograms obtained immediately after cooling showed an increase in residual gelatinization peak temperature and a decrease in residual gelatinization enthalpy (Fig. 1). In addition, all treatments produced a narrowing of the endotherm relative to that of the native starch gelatinization endotherm.

After storage for seven days at 4°C, very small endotherms associated with amylopectin retrogradation (enthalpy <0.5 J/g) were detected for samples initially heated to 54, 56, and 58°C. For samples initially heated to >58°C, retrogradation enthalpy increased more dramatically with increases in initial holding temperature (Figs. 1 and 2). After retrogradation of each sample, residual gelatinization endotherms similar to those observed immediately

after the initial holding treatment could still be observed (Fig. 1). This work indicates that the extent of residual gelatinization in general is inversely related to the extent of partial retrogradation (Fig. 2).

The relationship of lost gelatinization enthalpy to retrogradation enthalpy is shown in Fig. 3. Losses in gelatinization enthalpy up to ~6 J/g do not result in a similar increase in retrogradation enthalpy after seven days of storage at 4°C. However, losses of gelatinization enthalpy due to initial temperature treatment do lead to a similar increase in retrogradation enthalpy over the range of gelatinization enthalpy losses between 6 and 14 J/g. In this region the slope is ~1.

Experiment II: Thermal Treatment of *wx* Starch Above the Range of Gelatinization

Residual gelatinization enthalpy and retrogradation enthalpy values for the *wx* starch samples (for all storage times at 4°C) are plotted versus initial treatment temperature in Fig. 4. DSC thermograms for four selected initial temperature treatments are shown in Fig. 5.

Retrogradation was observed after one, three, seven, and ten days for initial temperature treatments from 66 to 78°C, temperatures within the range of gelatinization (Fig. 4). Over this same range of initial temperatures, some residual gelatinization enthalpy could also be observed. The residual gelatinization enthalpy decreased with increasing temperature treatment. As initial temperature treatments increased, retrogradation enthalpy after one day increased to a maximum and then decreased. Other storage times showed a similar increase and then decrease in retrogradation enthalpy as initial temperature treatment increased. Maximum retrogradation for each storage period occurred for the 82°C initial temperature treatment, just above the native gelatinization endotherm completion temperature. For one day of storage of *wx* samples at 4°C, increases in initial temperature treatments from 82 to 140°C resulted in the most dramatic decreases in retrogradation enthalpy. No retrogradation was observed at >140°C after one day (Figs. 4 and 5). After three days, retrogradation enthalpy was higher than after one day for all initial temperature treatments, gradually decreasing to near zero enthalpy for the 160°C treatment (Fig. 4). After seven days, retrogradation enthalpies were still higher for all treatments. For both seven and ten days of storage, samples initially heated to temperatures from 82 to 112°C showed the highest enthalpy observed for all samples at ~11 J/g. Even at seven and ten days, the retrogradation enthalpy was less for the 160 and 180°C initial treatments. Storage for ten days resulted in slightly higher enthalpies than storage for seven days for the samples previously heated to 160 and 180°C.

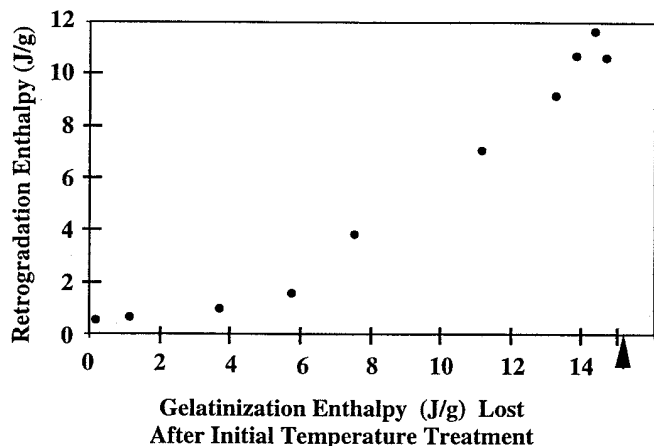


Fig. 3. Relationship between gelatinization enthalpy lost (native gelatinization enthalpy – residual gelatinization enthalpy) and retrogradation enthalpy, both determined after seven days at 4°C (Experiment I). Native gelatinization enthalpy is indicated by the arrow.

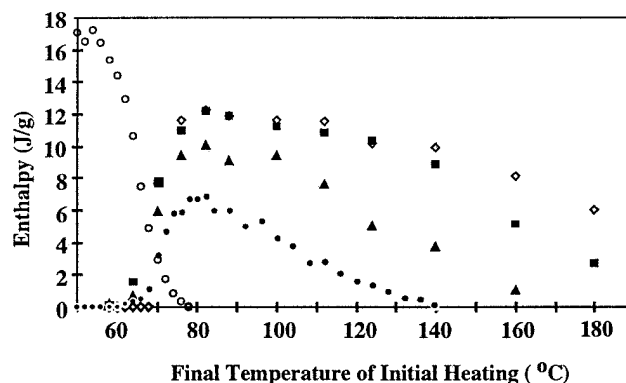


Fig. 4. Heating of *wx* starch to various temperatures within and above the gelatinization temperature range (Experiment II). Residual gelatinization enthalpy immediately after initial heating (○). No retrogradation was observed on immediate reheating. Retrogradation enthalpy was observed after 1 (●), 3 (▲), 7 (■), and 10 (◇) days.

Experiment III: Thermal Treatment of *ae wx* Starch Above the Range of Gelatinization

The native gelatinization endotherm for *ae wx* starch is broader and occurs over a higher temperature range than that of *wx* starch (Figs. 1 and 6). Residual gelatinization enthalpy could be detected in initial treatments from 54 to 92°C, immediately and after one day (Fig. 7), with residual enthalpy decreasing with increasing treatment temperature over that range. When samples initially heated to from 88 to 140°C were cooled and immediately reheated, appreciable enthalpy was observed on reheating. This immediately observed enthalpy increased to a maximum (~4 J/g) for the 100°C treatment. As initial temperature treatments increased from 100 to 140°C, immediately observed enthalpy decreased. At 140°C, there was no enthalpy observed on immediate reheating. Enthalpy after one day increased as initial treatments increased from 76 to 100°C, where it reached a maximum. Maximum enthalpy for immediate reheating, as well as the initial plateau for one-day retrogradation, were observed for the 100°C initial treatment. This treatment temperature coincides with the completion of the *ae wx* native gelatinization endotherm.

DISCUSSION

Experiment I: Thermal Treatment of *wx* Starch Within the Range of Gelatinization

Initial temperature treatments from 54 to 60°C led to a narrowing of the gelatinization endotherm relative to the control, an

effect interpreted to be due to annealing (Fig. 1). Annealing of starch after holding just $<T_0$ is well documented in the literature (Krueger et al 1987a,b; Knutson 1990; Seow and Teo 1993; Hoover and Vasanthan 1994). The narrowing of the gelatinization endotherm and the peak temperature shift to higher temperatures are thought to be related to increased proportion of crystallinity or increased crystallite perfection of crystalline regions of amylopectin molecules. The interpretation of the present results is more complex because initial holding temperatures are within the initial gelatinization range, and the residual gelatinization enthalpy decreases as a result (Knutson 1990). At these temperatures, it is likely that a portion of the starch sample is melted (Gidley and Cooke 1991) and only a portion is annealed. Because some melting occurred, the annealing may well involve some recrystallization.

Initial heating to final temperatures from just above 60 to 72°C led to further loss of residual gelatinization enthalpy for both the immediately reheated samples and for the samples stored for seven days at 4°C (Fig. 1). Residual gelatinization enthalpy observed on immediate reheating and after seven days of storage could be the result of: 1) some or all granules not yet being fully gelatinized; 2) some granules not being gelatinized at all; 3) annealing of all or some of the granules; or 4) a combination of these factors. After a particular initial temperature treatment, it is likely that some granules are annealed while other granules may be partially or fully gelatinized. Variation in thermal behavior within a population of granules has been observed (Liu et al 1991, Liu and Lelièvre 1992). It is interesting to note also that the shape and location of the residual gelatinization endotherm generally did not change after seven days of storage at 4°C (Fig. 1), except that the peak height diminished slightly and some tailing was evident for the 54, 56, and 58°C treatments on storage. It would appear that subsequent to initial heating, a limited capacity for reordering of residual native starch structures might exist. The generally similar shape and location of the residual gelatinization endotherm for all treatments indicates that the residual ordered structure after initial heating is essentially independent from subsequent reordering events. This observation suggests further that, for starch initially heated to final temperatures from just above 60 to 72°C, the gelatinization enthalpy lost by a subpopulation of granules is related to the retrogradation enthalpy observed for that same subpopulation.

Amylopectin retrogradation is assumed to be responsible for the endothermic event occurring from ~35 to ~65°C for heated samples that were stored at 4°C for seven days. Amylopectin retro-

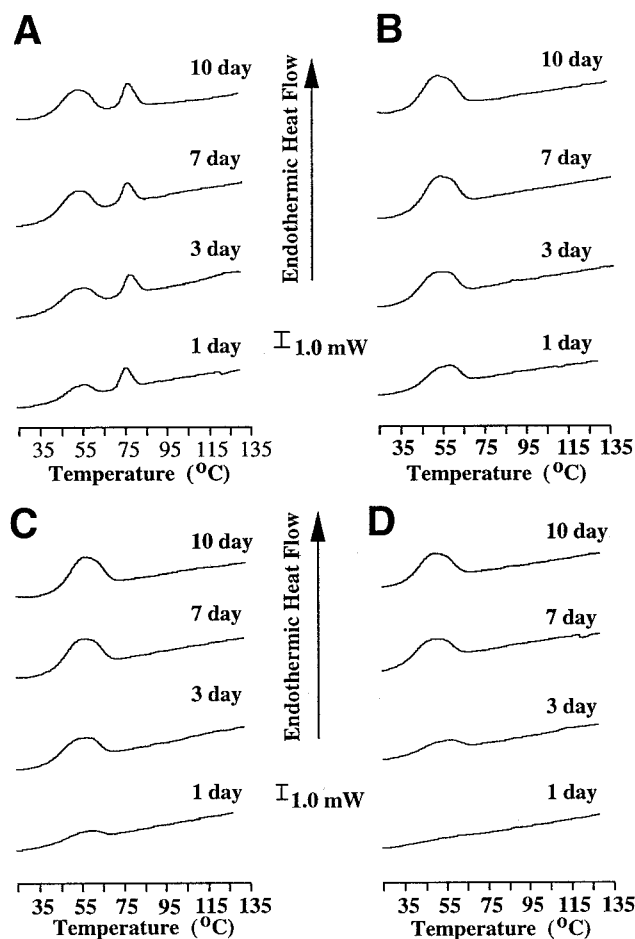


Fig. 5. Differential scanning calorimetry (DSC) thermograms for *wx* starch heated to final temperature, immediately cooled, and stored for 1, 3, 7, or 10 days at 4°C for selected final temperatures of initial heating. A–D: 70, 82, 112, and 140°C, respectively (Experiment II).

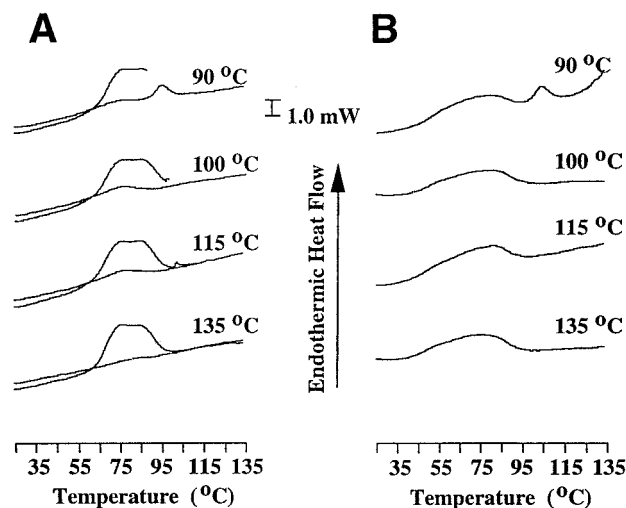


Fig. 6. Differential scanning calorimetry (DSC) thermograms for *ae wx* starch heated to the indicated final temperatures. A) on initial heating and immediate reheating; B) after one day of storage at 4°C (Experiment III).

gradation could be observed even for samples that contained substantial residual gelatinization enthalpy (those initially heated to final temperatures from 54 to 72°C). This experiment demonstrates that, for amylopectin retrogradation to occur, complete gelatinization of a starch preparation is not necessary.

Numerous researchers have observed enthalpy of amylopectin retrogradation at similar temperatures well below the temperature range for gelatinization (Ring 1985, Ring et al 1987a, Russell 1987, Kalichevsky et al 1990, Ward et al 1994). Reorientation or reassociation of the amylopectin branch chains in a less ordered manner than what existed for the native starch is an explanation for the observed amylopectin retrogradation endotherm at a temperature range below that for gelatinization (Ward et al 1994). This temperature range for retrogradation enthalpy may be modified. Jouppila and Roos (1996) showed that when gelatinized starch is stored at 60°C, retrogradation enthalpy can occur above that temperature, showing that storage temperature can influence the degree and perfection of molecular reordering and suggesting that retrogradation at lower storage temperatures generally leads to crystallites that melt far below the equilibrium melting temperatures.

In the present work, initial losses in gelatinization enthalpy from 0 to ~6 J/g did not result in similar increases in retrogradation enthalpy after seven days (Figs. 2 and 3). A plot of gelatinization enthalpy lost versus retrogradation enthalpy gained (Fig. 3) suggests that there may be two distinct linear regimes, above and below ~6 J/g. The minimal increase in retrogradation enthalpy in initial temperature treatments up to 60°C might be explained in several ways. If a small subset of granules in the population gelatinize at lower temperatures, then gelatinization enthalpy is lost only from these granules. They may swell somewhat under the excess water conditions. If more granules gelatinized, the water uptake by these later gelatinizing granules would be more limited (Liu and Lelièvre 1992) leading to a higher concentration of the disordered branch chains for the amylopectin within these granules. A minimum concentration of disordered amylopectin branch chains may be required for retrogradation to occur under the conditions (% starch, storage time, storage temperature) of this experiment. The close correspondence between gelatinization enthalpy lost and retrogradation enthalpy observed for samples heated to >60°C could be due to the later gelatinizing granules not swelling to the same extent as the earlier gelatinizing granules. Alternatively, the structural differences which cause them to gelatinize at slightly higher temperatures might also allow them to retrograde more efficiently. As others have observed, the retrogradation enthalpy observed is generally less than the initial gelatinization enthalpy (Ring et al 1987a). This observation might be related to the lower temperature range commonly observed for retrogradation. Crystallization during polymerization in synthesis may produce a more stable metastable semicrystalline state than can be achieved by retrogradation at or below room temperature by crystallization or annealing from a more fully disordered state.

Experiment II: Thermal Treatment of wx Starch Above the Range of Gelatinization

Our results obtained with the wx starch samples suggest that the degree of retrogradation of a starch preparation after one to ten days at 4°C may be profoundly influenced by initial temperature treatment above the range of native gelatinization.

Significant remaining molecular order may be responsible for the observation that the highest retrogradation enthalpies were observed at all storage times for initial heating to a temperature just above the range of gelatinization, 82°C. It can be envisioned that temperature treatments just above gelatinization may allow residual ordered structures, leading to more rapid retrogradation (Wunderlich 1976). At 82°C, this phenomenon would likely involve recrystallization in the sense of the term used by Wunderlich (1976). Slade and Levine (1991) have pointed out that amy-

lopectin crystallization is nucleation-limited. Consequently, the effect of residual order might be very important in the retrogradation of amylopectin.

Over the range of initial heating to temperatures from 82 to 140°C, retrogradation enthalpy observed after one day decreased dramatically from ~7 J/g to 0. This behavior suggests that one-day retrogradation may be due to residual order that decreases as the final temperature of initial heating increases. For preparation of starch products from wx starches, this observation may mean that large differences in retrogradation in the short term may result from relatively small differences in treatments to accomplish gelatinization. All initial temperature treatments resulted in increased retrogradation after three days of storage compared to one day of storage. Retrogradation enthalpies after seven and ten days were very similar over the range of initial heating temperatures from 80–90°C, indicating that maximum amounts of retrogradation for a 30% starch suspension may have been approached by seven days in these treatments. The decreasing slope from 90–140°C for all storage times shows that observed retrogradation enthalpy is a function of initial heating temperature. It is possible that the maximum enthalpy of retrogradation at a given storage temperature may remain the same, but longer storage times will be required for higher temperatures of initial heating to reach maximum enthalpy. It is noteworthy that lower retrogradation enthalpies are observed even for an initial heating temperature of 180°C as compared to 160°C. It is possible that residual order is decreased even in this range well above temperatures normally associated with gelatinization. Although residual crystallinity is unlikely, some double-helical order could remain up to 160°C, allowing self-nucleation on cooling. It is difficult to say whether further losses of order might occur for initial heating temperatures at >180°C. Even if retrogradation enthalpy further decreases, it would be difficult to rule out chemical changes in the starch at such temperatures.

Previous research in this laboratory has shown that W64A wx starch granules are easily disrupted, and the constituent molecules are thoroughly dispersed on heating in excess water at 90°C for 8 min, whereas Ia5125 wx starch granules retained their integrity under these conditions (R. C. Yuan, unpublished data). Thus, the observed loss of retrogradation enthalpy for W64A wx starch after heating to >82°C might be related to a loss of residual granule structure, and the effect on retrogradation might be peculiar to a particularly delicate type of wx starch granule. However, DSC studies of Ia5125 wx starch also led to a similar effect of initial heating temperature on retrogradation (*data not shown*), with no retrogradation observed for the 140°C treatment after one day.

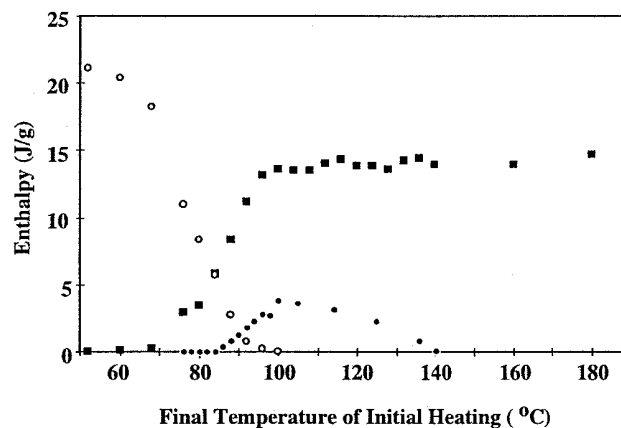


Fig. 7. Heating of *ae* wx starch to various temperatures within and above the gelatinization temperature range (Experiment III). Residual gelatinization enthalpy observed immediately after heating (○). Retrogradation enthalpy observed on immediate reheating (●) and after one day of storage at 4°C (■).

Consequently, a loss of granule integrity does not explain the decreased extent of retrogradation on heating *wx* starch to $>82^{\circ}\text{C}$. Nevertheless, retrogradation within intact granules may be an important factor in rheology of gelatinized starch, as gelatinized granules have been shown to shrink on storage (Ellis et al 1988).

Keetels et al (1996) recently reported an effect of heating temperature on the rheological properties of potato and wheat starch gels heated to 70, 90, or 120°C . These authors suggested that less amylopectin entanglement might result from the higher temperature treatment and hypothesized that less entanglement would allow more rapid retrogradation. They proposed that DSC be employed to monitor retrogradation of starch after heating to various temperatures. Contrary to the hypothesis of Keetels et al (1996), our DSC results show that, for this *wx*-type starch, retrogradation rate diminishes for starch initially heated to a higher temperature.

Experiment III: Thermal Treatment of *ae wx* Starch Above the Range of Gelatinization

Retrogradation of *ae wx* starch preparations occurs over a much shorter time scale than for *wx* starch. The general pattern seen for *wx* starch, in which retrogradation enthalpy increases to a maximum and then decreases as the final temperature of initial heating increases, is observed for *ae wx* starch only on immediate reheating. When the *ae wx* starch is stored for one day, the effect of initial heating temperature above the range of native gelatinization is lost, and the maximum retrogradation enthalpy remains constant.

Several workers have shown that the chain length distribution of *ae wx* starch differs from that of *wx* starch with a higher proportion of B2 chains and longer B2 chain fractions, as well as longer A and B1 fractions (Yuan et al 1993, Shi and Seib 1995). These longer chains would more readily form double helices, thus promoting nucleation and subsequent crystallization. Alternatively, double helices in the native starch could be harder to dissociate, leading to more significant recrystallization or self-nucleation. After one day of storage of the *ae wx* starch (Fig. 7), there is no indication of an effect of initial heating temperature on subsequent retrogradation, in contrast to the effect observed on immediate reheating. The lack of an effect after one day may be due to the rapid retrogradation subsequent to all initial heating treatments, allowing the same maximum enthalpy to be achieved by the end of that time. Previous research has shown that retrogradation of *ae wx* starch occurs rapidly within one day and does not increase further thereafter (Thompson and Blanshard 1995). We hypothesize that the retrogradation enthalpy for *wx* starch would ultimately reach a plateau at ~ 12 J/g, even for the sample initially heated to 180°C . We suggest that the structure of the *ae wx* starch effectively overcomes the nucleation-limited nature of the process for *wx* starch. This observation implies that relatively small differences in amylopectin structure may lead to major differences in retrogradation kinetics when retrogradation is evaluated at variable temperatures above the gelatinization temperature range.

One might suggest that the reason that *wx* starch retrogradation decreases as the final temperature of initial heating increases is that chemical degradation due to the higher temperatures inhibits retrogradation. The same argument might be used to explain the retrogradation behavior of *ae wx* starch on immediate reheating. However, the observation that one-day retrogradation enthalpy of *ae wx* starch remains constant for initial heating temperatures from 100 to 180°C does not lend support to the idea that chemical degradation caused the reduced retrogradation.

CONCLUSION

This article describes for the first time an influence of the final temperature of initial heating of native starches on what has been considered retrogradation behavior of these starches, when the final temperature of initial heating varies within and above the temperature range for native starch gelatinization (as observed by

DSC). This work calls into question the common use of the terms retrogradation and annealing, as applied to the increase in order subsequent to heating of starch granules. Retrogradation has been commonly used to describe a return to an ordered crystalline structure from a state presumed to be fully disordered. Annealing has been most commonly used to describe formation of increased crystallinity as a result of heating just below the melting temperature. Neither of these usages is entirely appropriate. For a population of amylopectin molecules varying in structure both within and among the molecules, variable extents of residual order may be retained after gelatinization appears complete by DSC, leading to behavior on storage that might be best described as annealing, in the sense that it involves recrystallization or self-nucleation. If the possibility of residual order is not considered, conclusions regarding the increase in order on storage of variably gelatinized starch materials may only apply to the particular final temperature employed. The present work may lay the groundwork for an improved understanding of starch retrogradation as a process that may commonly involve certain types of annealing. The extent to which annealing-type behavior is involved in retrogradation will vary according to the initial thermal treatment. Understanding of residual order in heated starch will be important because the rate of self-association of starch molecules is a function of the extent to which pertinent elements of the double-helical and crystalline order generated during polymerization are lost on heating prior to storage.

Retrogradation would be considered synonymous with crystallization only if the state after initial heating were one in which all pertinent residual order has been lost. Since this state will be extremely difficult to achieve or discern, researchers studying retrogradation behavior should be cautious about comparing the retrogradation kinetics of different starches with different thermal histories, even when the starches are cooked to temperatures typically assumed to cause complete gelatinization.

LITERATURE CITED

- Angell, C. A. 1995. Formation of glasses from liquids and biopolymers. *Science* 267:1924-1935.
- Atwell, W. A., Hood, L. F., Lineback, D. R., Varriano-Marston, E., and Zobel, H. F. 1988. The terminology and methodology associated with basic starch phenomena. *Cereal Foods World* 33:306-311.
- Biliaderis, C. G. 1991. The structure and interactions of starch with food constituents. *Can. J. Physiol. Pharmacol.* 69:60-78.
- Biliaderis, C. G., Page, C. M., and Maurice, T. J. 1986a. On the multiple melting transitions of starch/monoglyceride systems. *Food Chem.* 22:279-295.
- Biliaderis, C. G., Page, C. M., Maurice, T. J., and Juliano, B. O. 1986b. Thermal characterization of rice starches: a polymeric approach to phase transitions of granular starch. *J. Agric. Food Chem.* 34:6-14.
- Boyer, C. D., Shannon, J. C., Garwood, D. L., and Creech, R. G. 1976. Changes in starch granule size and amylose percentage during kernel development in several *Zea mays* L. genotypes. *Cereal Chem.* 53:327-337.
- Cameron, R. E., and Donald, A. M. 1991. Small-angle X-ray scattering and differential scanning calorimetry from starch and retrograded starch. Pages 301-309 in: *Food Polymers, Gels and Colloids*. E. Dickinson, ed. R. Soc. Chem: Cambridge, UK.
- Cameron, R. E., and Donald, A. M. 1992. A small-angle X-ray scattering study of the annealing and gelatinization of starch. *Polymer* 33:2628-2635.
- Cooke, D., and Gidley, M. J. 1992. Loss of crystalline and molecular order during starch gelatinisation: Origin of the enthalpic transition. *Carbohydr. Res.* 227:103-112.
- Eliasson, A.-C. 1985. Retrogradation of starch as measured by differential scanning calorimetry. Pages 93-98 in: *New Approaches to Research on Cereal Carbohydrates*. R. D. Hill and L. Munck, eds. Elsevier Sci.: Amsterdam.
- Ellis, H. S., Ring, S. G., and Whittam, M. A. 1988. Time-dependent changes in the size and volume of gelatinized starch granules on storage. *Food Hydrocolloids* 2:321-328.

- Gidley, M. J., and Bociek, S. M. 1988. ^{13}C CP/MAS NMR studies of amylose inclusion complexes, cyclodextrins, and the amorphous phase of starch granules. Relationships between glycosidic linkage conformation and solid-state ^{13}C chemical shifts. *J. Am. Chem. Soc.* 110:3820-3829.
- Gidley, M. J., and Bulpin, P. V. 1987. Crystallisation of malto-oligosaccharides as models of the crystalline forms of starch: Minimum chain-length requirement for the formation of double helices. *Carbohydr. Res.* 161:291-300.
- Gidley, M. J., and Cooke, D. 1991. Aspects of molecular organization and ultrastructure in starch granules. *Biochem. Soc. Trans.* 19:551-555.
- Hizukuri, S. 1986. Polymodal distribution of the chain lengths of amylopectins, and its significance. *Carbohydr. Res.* 147:342-347.
- Hoover, R., and Vasanthan, T. 1994. The effect of annealing on the physicochemical properties of wheat, oat, potato and lentil starches. *J. Food Biochem.* 17:303-325.
- I'Anson, K. J., Miles, M. J., Morris, V. J., and Ring, S. G. 1988. A study of amylose gelation using a synchrotron X-ray source. *Carbohydr. Polym.* 8:45-53.
- Imberty, A., Buléon, A., Tran, V., and Pérez, S. 1991. Recent advances in knowledge of starch structure. *Starch/Staerke* 43:375-384.
- Jacobs, H., Eerlingen, R. C., Clauwaert, W., and Delcour, J. A. 1995. Influence of annealing on the pasting properties of starches from varying botanical sources. *Cereal Chem.* 72:480-487.
- Jacobs, H., Eerlingen, R. C., Hauwaert, D. V., and Delcour, J. A. 1994. Multistep annealing: Impact on the properties of pea starch. (Abstr.) *Cereal Foods World* 39:558.
- Jouppila, K., and Roos, Y. H. 1996. The effect of physical state and glass transition on crystallization in starch. Pages 196-199 in: *Crystal Growth of Organic Materials*. A. S. Myerson, D. A. Green, and P. Meenan, eds. Am. Chem. Soc.: Washington, DC.
- Kalichevsky, M. T., Orford, P. D., and Ring, S. G. 1990. The retrogradation and gelation of amylopectins from various botanical sources. *Carbohydr. Res.* 198:49-55.
- Karkalas, J., Ma, S., Morrison, W. R., and Pethrick, R. A. 1995. Some factors determining the thermal properties of amylose inclusion complexes with fatty acids. *Carbohydr. Res.* 268:233-247.
- Keetels, C. J. A. M., Van Vliet, T., and Walstra, P. 1996. Gelation and retrogradation of concentrated starch systems: 3. Effect of concentration and heating temperature. *Food Hydrocolloids* 10:363-368.
- Knutson, C. A. 1990. Annealing of maize starches at elevated temperatures. *Cereal Chem.* 67:376-384.
- Krueger, B. R., Knutson, C. A., Inglett, G. E., and Walker, C. E. 1987a. A differential scanning calorimetry study on the effect of annealing on gelatinization behavior of corn starch. *J. Food Sci.* 52:715-718.
- Krueger, B. R., Walker, C. E., Knutson, C. A., and Inglett, E. G. 1987b. Differential scanning calorimetry of raw and annealed starch isolated from normal and mutant maize genotypes. *Cereal Chem.* 64:187-190.
- Liu, H., and Lelièvre, J. 1992. A differential scanning calorimetry study of melting transitions in aqueous suspensions containing blends of wheat and rice starch. *Carbohydr. Polym.* 17:145-149.
- Liu, H., Lelièvre, J., and Ayoung-Chee, W. 1991. A study of starch gelatinization using differential scanning calorimetry, X-ray, and birefringence measurements. *Carbohydr. Res.* 210:79-87.
- Martin, C., and Smith, A. M. 1995. Starch biosynthesis. *Plant Cell* 7:971-985.
- Miles, M. J., Morris, V. J., Orford, P. D., and Ring, S. G. 1985a. The roles of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydr. Res.* 135:271-281.
- Miles, M. J., Morris, V. J., and Ring, S. G. 1985b. Gelation of amylose. *Carbohydr. Res.* 135:257-269.
- Ring, S. G. 1985. Observations on the crystallization of amylopectin from aqueous solution. *Int. J. Biol. Macromol.* 7:253-254.
- Ring, S. G., Colonna, P., I'Anson, K. J., Kalichevsky, M. T., Miles, M. J., Morris, V. J., and Orford, P. D. 1987a. The gelation and crystallisation of amylopectin. *Carbohydr. Res.* 162:277-293.
- Ring, S. G., Miles, M. J., Morris, V. J., Turner, R., and Colonna, P. 1987b. Spherulitic crystallization of short chain amylose. *Int. J. Biol. Macromol.* 9:158-160.
- Russell, P. L. 1987. The ageing of gels from starches of different amylose/amylopectin content studied by differential scanning calorimetry. *J. Cereal Sci.* 6:147-158.
- Seow, C. C., and Teo, C. H. 1993. Annealing of granular rice starches—Interpretation of the effect on phase transitions associated with gelatinization. *Starch/Staerke* 45:345-351.
- Shi, Y.-C., and Seib, P. A. 1995. Fine structure of maize starches from four wx-containing genotypes of the W64A inbred line in relation to gelatinization and retrogradation. *Carbohydr. Polym.* 26:141-147.
- Slade, L., and Levine, H. 1991. Beyond water activity: Recent advances based on an alternative approach to the assessment of food quality and safety. *Crit. Rev. Food Sci. Nutr.* 30:115-360.
- Sperling, L. H. 1986. *Introduction to Physical Polymer Science*. John Wiley and Sons: New York.
- Stutz, H. 1995. The glass transition temperature of dendritic polymers. *J. Polymer Sci.: Pt. B: Polymer Phys.* 33:333-340.
- Thompson, D. B., and Blanshard, J. M. V. 1995. Retrogradation of selected wx-containing maize starches. (Abstr.) *Cereal Foods World* 40:670.
- Ward, K. E. J., Hosenev, R. C., and Seib, P. A. 1994. Retrogradation of amylopectin from maize and wheat starches. *Cereal Chem.* 71:150-155.
- Wootton, M., and Bamunuarachchi, A. 1979. Application of differential scanning calorimetry to starch gelatinization. II. Effect of heating rate and moisture level. *Starch/Staerke* 31:262-264.
- Wu, J. Y., and Eads, T. M. 1993. Evolution of polymer mobility during ageing of gelatinized waxy maize starch: A magnetization transfer ^1H NMR study. *Carbohydr. Polym.* 20:51-60.
- Wunderlich, B. 1976. *Macromolecular Physics*. Vol. 2. Crystal Nucleation, Growth, Annealing. Academic Press: New York.
- Wunderlich, B. 1980. *Macromolecular Physics*. Vol. 3. Crystal Melting. Academic Press: New York.
- Wunderlich, B. 1990. *Thermal Analysis*. Academic Press: New York.
- Yamaguchi, M., Kainuma, K., and French, D. 1979. Electron microscopic observations of waxy maize starch. *J. Ultrastr. Res.* 69:249-261.
- Yost, D. A., and Hosenev, R. C. 1986. Annealing and glass transition of starch. *Starch/Staerke* 38:289-292.
- Yuan, R. C., Thompson, D. B., and Boyer, C. D. 1993. Fine structure of amylopectin in relation to gelatinization and retrogradation behavior of maize starches from three wx-containing genotypes in two inbred lines. *Cereal Chem.* 70:81-89.
- Zobel, H. F., Young, S. N., and Rocca, L. A. 1988. Starch gelatinization: An X-ray diffraction study. *Cereal Chem.* 65:443-446.
- Zobel, H. F. 1992. Starch granule structure. Pages 1-36 in: *Developments in Carbohydrate Chemistry*. R. Alexander and H. Zobel, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.

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