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# Comparative Study of the Retrogradation of Intermediate Water Content Waxy Maize, Wheat, and Potato Starches

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The retrogradation of extruded starches from three different botanical sources was studied in concentrated conditions (34 ± 1% water) at 25 °C using differential scanning calorimetry (DSC) and isothermal calorimetry, Fourier transform infrared spectroscopy (FTIR), and wide-angle X-ray scattering. Potato starch showed the highest rate of retrogradation (~0.17 $h^{-1}$) followed by waxy maize (~0.12 $h^{-1}$), while the retrogradation of wheat starch was the slowest (~0.05 $h^{-1}$). In addition to the kinetics, the extent of molecular order in the retrograded samples was studied in detail in terms of "short-range" (helical) and "long-range" (crystalline) distance scales. The amylopectin crystallinity indices were essentially the same (~47−51% amylopectin basis) for the three starches. However, significant differences were found in the enthalpy of melting measured by DSC after "full" retrogradation (potato, 11.6 ± 0.7; waxy maize, 9.0 ± 0.5; and wheat, 6.1 ± 0.3 J/g of amylopectin). The degree of short-range molecular order in the retrograded state determined by FTIR was waxy maize > potato > wheat. The effect of amylopectin average chain length and the polymorphism of the crystalline phase were taken into account to explain the differences in the retrogradation enthalpies.

**KEYWORDS: Starch retrogradation; amylopectin; isothermal calorimetry; molecular order; staling**

## INTRODUCTION

While there have been several studies on the effect of starch botanical sources on its retrogradation kinetics, the majority of these were carried out in high water content conditions (i.e., starch concentrations ≤ 40%) (*1*−*3*); therefore, their findings may not be directly relevant to many starch-based foods such as baked goods, cooked cereals and pasta, and partially processed snacks and breakfast cereals. This view was recently supported by Zobel and Kulp (*4*). Although some work on more concentrated starch systems is available (*5*−*7*), these studies were concerned with starches from a single botanical source and thus did not address the relationship between the starch source and its retrogradation behavior.

Because the kinetics of starch retrogradation is strongly dependent on properties such as amylose/amylopectin content (*8*, *9*), lipid content (*8*), amylopectin fine structure (*2*), etc., which depend on the starch botanical source, it is therefore important to know how the combination of these different attributes affects the retrogradation kinetics of the individual starches. The aim of this study was to compare and understand the retrogradation behavior in concentrated conditions of three different starches, namely, waxy maize, potato, and wheat starches, at the same water content (~34% wwb) and storage temperature (25 °C).

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## MATERIALS AND METHODS

Waxy maize starch was obtained from National Starch and Chemical Ltd. (Manchester, United Kingdom). Potato starch was obtained from Sigma (Sigma-Aldrich Chemie GmbH, Germany). Wheat starch was obtained from Avebe (Avebe, The Netherlands).

**Sample Preparation.** Nonexpanded starch/water ribbons containing 34 ± 1% water (wwb) were prepared by extrusion through a 1 mm × 30 mm slit die using a Clextral BC-21 corotating, intermeshing twin-screw extruder. The extrusion conditions were as follows: screw speed, 300 rpm; solids feed rate, 5 kg/h; and applied temperature profile, 40, 90, 120, and 80 °C. Distilled water was pumped into the second zone at a flow rate of ~1.8 L/h. The extrusion specific mechanical energy values were 121, 169, and 133 W h $kg^{-1}$ for the waxy maize, potato, and wheat starches, respectively. The water contents of the extrudates were determined gravimetrically by drying in a 105 °C oven for 24 h.

To minimize the loss of water upon storage, the extrudates were wrapped in polyethylene film and placed inside heat-sealed aluminum foil-lined bags. The samples were stored at 25 °C (±0.5 °C) and monitored at regular time intervals. The only exception was the sample studied by microcalorimetry, which was sealed in the sampling calorimeter vessel and analyzed in situ.

**Wide-Angle X-ray Scattering (WAXS).** Diffractograms were acquired on the extruded ribbons over the $2\theta$ range 4−38° at 0.1° intervals with a scanning rate of 60 s/° using a Bruker D5005 wide-angle X-ray diffractometer equipped with a copper source operating at 40 kV and 30 mA producing a Cu Kα radiation with a wavelength of 1.54 Å. The diffractograms were holder subtracted and baseline corrected before calculating the crystallinity index.

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The crystallinity index was determined using the method described by Hermans and Weidinger (*10*), which relies on the numerical calculation of the area of the diffraction peaks resulting from the crystalline phase as a fraction of the overall diffractogram area. The amorphous reference diffractogram used for each starch was that of its freshly extruded sample. This approach for the choice of the amorphous standard is very important as the maximum $2\theta$ position of the amorphous diffractogram and its width are strongly water content dependent. The crystallinity index was calculated from triplicate diffractogram measurements performed on the "fully" retrograded sample and were reported as an average (±1 standard deviation).

**Differential Scanning Calorimetry (DSC).** The enthalpy associated with retrogradation was measured using DSC in the presence of excess water. Approximately 4 ± 1 mg of the extruded samples aged to different extents was placed in aluminum pans, and distilled water was added to obtain a water/starch ratio ≥3. The pans were then hermetically sealed and placed on mixing rollers overnight at ambient temperature in order to ensure homogeneous hydration. The thermograms were acquired between 5 and 95 °C at a heating rate of 10 °C/min using a DSC-7 (Perkin-Elmer, United Kingdom) calorimeter calibrated for temperature and heat flow using indium and cyclohexane. An empty aluminum pan was used as a reference. Baseline correction was performed before integrating the endotherm centered at ~53−58 °C (depending on the starch botanical source) corresponding to the melting of the retrograded amylopectin. For each storage time, duplicate analyses were carried out. The plateau melting enthalpy values for the samples were calculated as the average of at least four measured values determined on the fully retrograded sample. The same procedure was followed when studying the gelatinization of the native starches.

**Microcalorimetry.** Approximately 1 g of sample was sealed inside the stainless steel sample vessel of a Micro DSC III (Setaram, France). Distilled water was placed in the reference vessel. The weight of the water was such that its heat capacity was comparable to that of the starch/water sample. The vessels were introduced into the calorimeter and held at 25 °C (typically ±0.002 °C). The heat flux was measured continuously over a period of ~3 days in isothermal mode. The change of enthalpy during aging was calculated by numerical integration of the heat flux data. Triplicate experiments were carried out on each sample and were reported as an average (± 1 standard deviation).

When analyzing the gelatinization of the native starches in the presence of excess water, ~0.2 g of starch was placed in the sample vessel, and distilled water was added to obtain a water/starch ratio ≥3. These vessels were then left on rollers overnight at room temperature to allow for a homogeneous water distribution throughout the sample. Thermograms were acquired by heating the samples at a heating rate of 1 °C/min between 25 and 120 °C. The samples were then cooled to 25 °C at the same rate. Duplicate measurements were carried out for each sample.

**Fourier Transform Infrared (FTIR) Spectroscopy.** Midinfrared spectra were acquired using an IFS48 (Bruker, United Kingdom) spectrometer equipped with a DTGS detector and a temperature-controlled single reflectance diamond attenuated total reflectance (ATR) accessory (Golden Gate, Graseby-Specac Ltd., United Kingdom) with a sealed sapphire anvil. For each spectrum, 32 scans acquired at a resolution of 4 $cm^{-1}$ were coadded. Spectra were acquired at 25 °C while the ATR accessory, excluding the sample compartment that was sealed, was constantly purged with dry air. The spectra were collected approximately every 2−3 h, and at least triplicate measurements were carried out for each sample at each storage time.

Data analysis was carried out using the OPUS 3.0 software (Bruker). The spectral region 1200−800 $cm^{-1}$, which has been shown to be sensitive to the degree of molecular order in starch (e.g., *11*, *12*), was used. The spectra were corrected for baseline offset relative to the absorbance at 1900 $cm^{-1}$ and then deconvoluted (*13*), using a deconvolution factor of 750, a noise reduction factor of 0.2, and a Lorentzian line shape. The ratio of the absorbencies 1045:1151 $cm^{-1}$ was used to monitor retrogradation.

**Statistical Analyses.** The significance of differences was assessed using the analysis of variance single factor test ($P < 5\%$).

**Normalization of Data.** To compare the retrogradation kinetics of the three starches, the measured results were normalized, presented as relative changes [$R(t)$], using the following equation:

$$R(t) = \frac{x(t) - x_0}{x_\infty - x_0} \quad (1)$$

where $x(t)$ = value of measured parameter (e.g., enthalpy or IR absorbance ratio) at time $t$, $x_0$ = value of $x$ for $t = 0$, and $x_\infty$ = value of $x$ for $t = \infty$.

The initial ($x_0$) and final ($x_\infty$) values were calculated by fitting the various experimental results to a stretched exponential equation derived from the Avrami kinetic equation, as described elsewhere (*14*):

$$x(t) = x_\infty - (x_\infty - x_0)\exp[(-Gt)^n] \quad (2)$$

where $G$ is the rate of retrogradation ($time^{-1}$) and $n$ is the Avrami exponent.

## RESULTS AND DISCUSSION

**Retrogradation Kinetics.** Three different techniques (DSC, isothermal calorimetry, and FTIR) were used to study the kinetics of retrogradation of the different starches (waxy maize, wheat, and potato). The results were normalized using eq 1 to demonstrate the scale of the difference in retrogradation kinetics. The differences in the extent of change after full retrogradation for each sample are discussed subsequently in the paper.

DSC was used to determine the melting enthalpy of retrograded starch. The DSC measurements were carried out in the presence of excess water (water/starch ≥ 3) in order to bring the temperature of amylopectin melting below 100 °C to facilitate its measurement using standard DSC aluminum pans (low pressure). Retrogradation was monitored through the enthalpy ($\Delta H$) of the endotherm centered at ~53−58 °C, which reports essentially on the melting of ordered amylopectin. Indeed, at these concentrations, the melting of retrograded amylose or that of amylose−lipid complexes would occur at much higher temperatures, typically ≥90 °C for the amylose−lipid complex (*15*, *16*) and even higher for retrograded amylose (*17*). For this reason, it is more accurate to report the $\Delta H$ values relative to amylopectin. These were calculated from the measured values using typical amylopectin contents reported for these starches: 98, 77, and 72% (dwb) for waxy maize, potato, and wheat starches, respectively (*18*).

For all three starches, the thermograms acquired shortly after extrusion showed no significant thermal events in the temperature range between 20 and 90 °C, suggesting that the extrusion process disrupted the native ordered structure of the starches. This was supported by the WAXS results described below (**Figure 4a**), which essentially showed an amorphous pattern, with the exception of the wheat starch sample, where some crystallinity was present. This, however, was not believed to be related to residual native starch crystallinity but to a crystalline amylose−lipid complex, as will be discussed in more detail below. As the samples were aged for increasing durations, the amylopectin retrogradation endotherm (peak temperature, ~53−58 °C) became more pronounced. For all three starches, the enthalpy of melting ($\Delta H$) of this endotherm showed a similar pattern of dependency on storage time, increasing rapidly before reaching a limiting value (**Figure 1a**) for the fully retrograded starch. The limiting enthalpy values $\Delta H_\infty$ (plateau) were calculated as described above. $\Delta H_\infty$ was the highest for potato starch (11.6 ± 0.7 J/g of amylopectin) followed by that for waxy maize starch (9.0 ± 0.5 J/g of amylopectin), while wheat starch yielded the smallest value (6.1 ± 0.3 J/g of amylopectin) (**Figure 1a**).

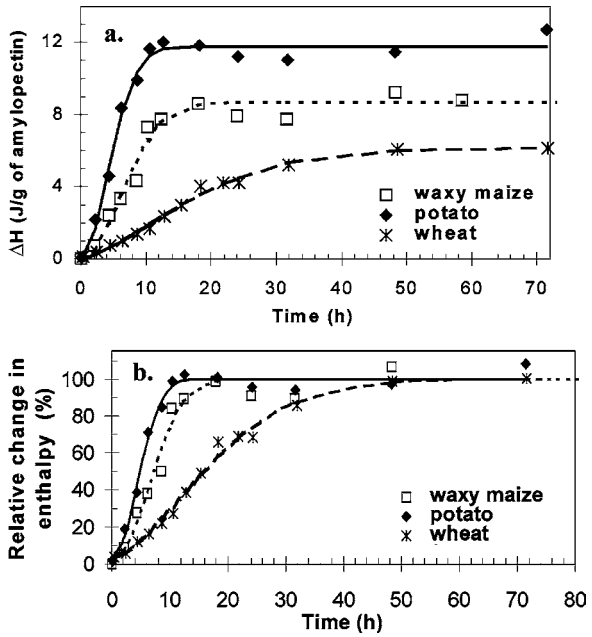


**Figure 1.** (**a**) Starch retrogradation monitored through the amylopectin melting enthalpy measured using DSC in the presence of excess water (lines were calculated using eq 2) and (**b**) the relative change in DSC melting enthalpy upon storage (from panel **a**).

The difference in the retrogradation kinetics can clearly be seen from the normalized enthalpy plots (**Figure 1b**), where at the same storage temperature (25 °C) and water content (34 ± 1% wwb), the retrogradation of potato starch was slightly faster than that of waxy maize, while wheat starch showed the slowest kinetics. The retrogradation rates, $G$, were calculated using eq 2 and found to be 0.17 $h^{-1}$ for potato starch, 0.12 $h^{-1}$ for waxy maize starch, and 0.05 $h^{-1}$ for wheat starch. The exponent values $n$ were comparable at ∼1.5−2.

The difference between the retrogradation kinetics of potato and wheat agrees with the suggestion that the shorter outer chain lengths of cereal amylopectin result in slower retrogradation (*2*, *19*). This, however, would not explain the significant difference between wheat and waxy maize, both of which have comparable average chain lengths [∼18.5 and ∼18.6, respectively (*20*)], and suggests that the presence of amylose and lipids does hinder the retrogradation of amylopectin. Both amylose and lipids are present in much greater amounts in wheat starch than in waxy maize starch (*21*). This hindrance of amylopectin retrogradation is likely to occur through the entanglement of amylopectin with the amylose network. Such a network would be enhanced by amylose retrogradation and the existence of amylose−lipid complexes (in particular the crystallization/gelation of such a complex). Conde-Petit and Escher (*22*) demonstrated that the occurrence of amylose−ligand complexes accelerates the early stages of amylose gelation. Another possible reason for the slower retrogradation in wheat starch is the occurrence of amylopectin−lipid complexes, as described by Eliasson et al. (*8*, *23*), that might hinder the retrogradation of amylopectin. Conde-Petit and Escher (*22*), however, found no experimental evidence for a role played by amylopectin−lipid complexes in terms of retrogradation. Furthermore, in terms of steric constraints, because of the highly branched amylopectin structure, amylose−lipid complexes are likely to form preferentially, as suggested by Gudmundsson and Eliasson (*8*). It is interesting to note that the rates of retrogradation showed a positive correlation with the end temperature of the melting of the retrograded samples (**Table 1**), suggesting that the greater degree of supercooling in the rubbery state for potato leads to a larger "driving force" for retrogradation as compared to wheat, with waxy maize in an intermediate situation.

**Table 1.** Retrogradation Enthalpy as Measured Isothermally Using the Microcalorimeter as Compared to the Melting Enthalpy of Fully Retrograded Starch as Measured upon Heating in Excess Water Using DSC[a]

| | potato | waxy maize | wheat |
|---|---|---|---|
| isothermal microcalorimetry | | | |
| $\Delta H$ (J/g amylopectin) | 8.0 (±0.1) | 4.7 (±0.2) | 3.8 (±0.3) |
| scanning calorimetry (DSC) | | | |
| $\Delta H$ (J/g amylopectin) | 11.6 (±0.7) | 9.0 (±0.5) | 6.1 (±0.3) |
| $T_o$ (°C) | 46.8 (±0.9) | 45.6 (±0.8) | 45.4 (±0.5) |
| $T_p$ (°C) | 57.7 (±1.1) | 55.8 (±0.5) | 53.0 (±0.4) |
| $T_f$ (°C) | 70.2 (±1.6) | 69.3 (±2.1) | 61.2 (±0.8) |

[a] The onset ($T_o$), peak ($T_p$), and final ($T_f$) temperatures of the DSC transitions are also shown (average values are given ± standard deviation).

It may be argued that the characterization of the samples after hydration/suspension in excess water, which is widely used in studies of starch systems, and the fact that the analysis involved heating, may introduce artifacts. For this reason, the investigation was supplemented with isothermal calorimetry retrogradation studies. This approach addresses both concerns. Isothermal calorimetry requires a great degree of instrument stability over a relatively long time (∼50 h in this instance). This was best achieved using a microcalorimeter as standard DSC equipments are usually optimized for rapid heating/cooling rather than long-term baseline stability. Furthermore, because the heat flux at a given sampling time would be relatively small, as the DSC results suggested that the retrogradation occurs over several hours (**Figure 1a**,**b**), the microcalorimeter was the preferred choice as it samples ∼1 g of the starch extrudate as compared to the few milligrams for the DSC.

The isothermal calorimetry experiments were performed as described above, and for all three starch samples, the plot of heat flow vs aging time yielded relatively broad (∼15−30 h) exothermic patterns (**Figure 2a**) associated with retrogradation. The results suggested that potato starch retrograded the most rapidly, reaching a peak retrogradation time after ∼4 h, followed by waxy maize starch, reaching a peak after ∼7 h, and the wheat starch was the slowest to retrograde, reaching a peak after ∼12 h. To monitor the recovery of molecular order during aging, the retrogradation enthalpies were calculated by integrating the heat flow results (**Figure 2b**), which were subsequently normalized using eq 1 (**Figure 2c**). The retrogradation kinetics described by the normalized enthalpies obtained by isothermal calorimetry were very similar to those obtained using the scanning calorimetry in excess water (**Figure 1b**) with the same order of retrogradation rates: potato > waxy maize ≫ wheat. The retrogradation rates $G$ calculated by fitting eq 2 to the isothermal calorimetry $\Delta H$ vs time data were also very similar to those obtained for the DSC in excess water, with values of 0.17, 0.12, and 0.06 $h^{-1}$ for potato, waxy maize, and wheat, respectively. This agreement between the two approaches clearly demonstrates the validity of the protocol of DSC analysis in the presence of excess water in this context.

As found using the scanning calorimetry (**Figure 1a**), the final or limiting enthalpies ($\Delta H_\infty$) were significantly ($P < 5\%$) different for the three starches studied (**Figure 2b**) with values of 8.0 ± 0.1, 4.7 ± 0.2, and 3.8 ± 0.3 J/g of amylopectin for potato, waxy maize, and wheat starch, respectively. It was interesting to note that although the order of $\Delta H_\infty$ obtained from the scanning and isothermal calorimetry studies was the same

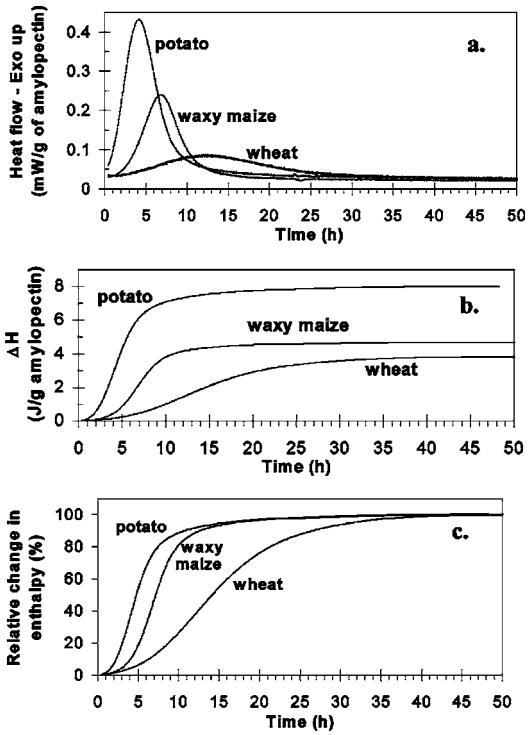


**Figure 2.** (**a**) Isothermal thermograms acquired using the microcalorimeter (heat flow was normalized to the amylopectin weight fraction of each sample), (**b**) extent of retrogradation with time as monitored through the integrated heat flow, and (**c**) relative change in enthalpy with time (from panel **b**).

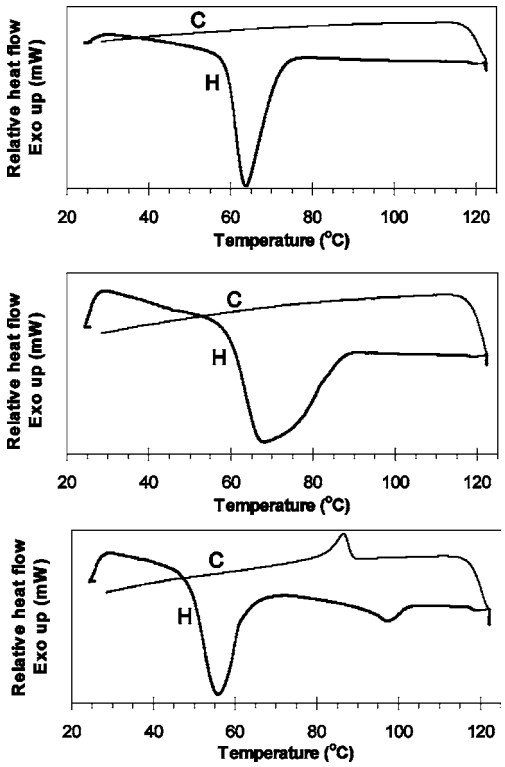


**Figure 3.** Thermograms acquired during heating (H) and cooling (C) in the microcalorimeter of the native potato (top), waxy maize (middle), and wheat starch (bottom) samples in excess water conditions.

for the three materials, the full retrogradation melting enthalpies measured by DSC were higher than those determined from the isothermal calorimetry results (**Table 1**).

To investigate the origin of the difference in the final retrogradation enthalpies ($\Delta H_\infty$) obtained between the scanning calorimetry study, performed in the presence of excess water on a starch sample of ~4 mg using DSC, and the isothermal calorimetry study, performed directly on ~1 g of sample containing ~34% water, it was important to assess the contribution of the use of different equipments with different technologies (power-compensated DSC vs heat flux calorimetry) and different sample sizes to these differences. For this, the native starches used in this study were adopted as "standards" and were analyzed in excess water conditions using both techniques (DSC and microcalorimetry) and the gelatinization behaviors obtained were compared.

The thermograms obtained using the microcalorimeter at the maximum reliably achievable heating/cooling rates of 1 °C (due to sample size) are shown in **Figure 3**. For both the waxy maize and the potato starches, the gelatinization endotherm was the only thermal event observed on heating to 120 °C and no significant events could be observed upon cooling. However, for wheat starch, there was another endotherm observed upon heating at a peak temperature of ~97 °C, which was attributed to the melting of the amylose−lipid complex. Upon cooling, an exotherm was observed at a peak temperature of ~86 °C and was attributed to the reforming and possible crystallization of this complex. The melting and reforming temperatures of the complex were comparable to those reported by Jovanovich et al. (*15*). It is believed that some of the amylose−lipid complex already existed within the native granule, as has been shown for other native starches (*24*, *25*), and that more was formed during the heating of the sample, as was shown for maize starch using synchrotron X-ray diffraction (*26*).

**Table 2.** Gelatinization Enthalpies and Onset ($T_o$), Peak ($T_p$), and Final ($T_f$) Melting Temperatures of the Native Starch Samples as Measured by Microcalorimetry and DSC in Excess Water Conditions (Values of Duplicate Runs Are Shown)

| | potato | waxy maize | wheat |
|---|---|---|---|
| | microcalorimetry | | |
| $\Delta H$ (J/g amylopectin) | 23.2, 23.3 | 16.4, 16.5 | 15.3, 15.6 |
| $T_o$ (°C) | 59.0, 59.0 | 59.8, 59.8 | 48.8, 49.2 |
| $T_p$ (°C) | 63.6, 63.7 | 67.7, 67.9 | 55.7, 55.8 |
| $T_f$ (°C) | 71.9, 72.0 | 85.5, 87.1 | 62.5, 62.7 |
| | DSC | | |
| $\Delta H$ (J/g amylopectin) | 22.7, 23.6 | 15.2, 16.4 | 15.6, 16.8 |
| $T_o$ (°C) | 61.1, 61.1 | 64.3, 64.8 | 55.0, 55.2 |
| $T_p$ (°C) | 65.7, 65.7 | 71.2, 71.3 | 59.0, 59.2 |
| $T_f$ (°C) | 70.8, 70.9 | 77.0, 77.0 | 64.1, 64.4 |

The gelatinization results are summarized in **Table 2**. The onset and peak gelatinization temperatures obtained using the microcalorimeter were consistently 2−6 °C lower than those observed using the DSC. There are two potential origins for this, both related to the differences in heating rates used (microcalorimeter, 1 °C/min; DSC, 10 °C/min): (i) heat transfer issues, although this contribution is expected to be small as thermal lags should be considered in relation to the heating rates and the sample mass and dimensions, and (ii) the nonequilibrium nature of gelatinization (*27*) even in excess water. The thermograms acquired on waxy maize starch using the microcalorimeter showed a bimodal gelatinization endotherm and a higher final gelatinization temperature (~9 °C higher than DSC). This behavior could be indicative of some annealing concurrent to the early stages of the gelatinization because of the relatively low heating rate. The gelatinization enthalpies measured in excess water were similar for the two techniques (**Table 2**). This is in contrast with the retrogradation enthalpy results described above (**Table 1**), clearly suggesting that the differ-

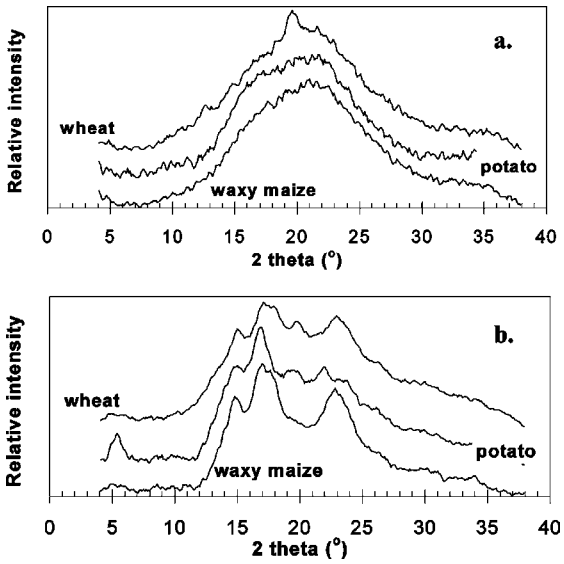


**Figure 4.** X-ray diffractograms of the (**a**) freshly extruded starch samples and (**b**) "fully" retrograded starch extrudates after 3 days of storage at 25 °C (data were smoothed using three point averaging).

ences observed are not due to differences in the operations of the two equipments/techniques or to differences in sample size. One possible explanation is the fact that the determination of the retrogradation enthalpies was carried out at different water contents, excess water for the DSC, and 34% for the isothermal microcalorimeter. Early work on native potato starch (*28*) demonstrated that the gelatinization endotherm depends strongly on water content (up to a water content of ~60% wwb) in terms of both temperature and enthalpy, whereby the higher the water content becomes, the lower is the gelatinization temperature and the greater is the melting enthalpy. However, when comparing the melting enthalpies measured by DSC in excess water to that measured at the original water content (~34% wwb) using the same technique, values of ~6.1 and ~11.3 J/g amylopectin were found for wheat starch, respectively. This is in contradiction with the previous findings on native potato starch, where the overall gelatinization enthalpy decreased from ~23 J/g amylopectin in excess water to ~18 J/g amylopectin at 33% water (*28*). The fact that the retrogradation $\Delta H$ measured in excess water is smaller than that at 34% water also excludes annealing in excess water as a reason for the difference in enthalpy between the DSC and the isothermal microcalorimetry retrogradation enthalpy results. The origin of this difference therefore remains unexplained.

**Starch Retrogradation: Polymorphism and Extent of Molecular Order.** WAXS was used to assess the amorphous/crystalline state of the fresh and retrograded samples. The diffractograms acquired shortly after extrusion for the waxy maize and potato starch samples showed essentially a diffuse pattern typical of amorphous materials, indicating that all native starch crystalline structures had been disrupted during the extrusion process (**Figure 4a**). The wheat starch extrudate showed essentially a similar pattern; however, its diffractogram also contained a small peak at a Bragg's angle value of $2\theta \approx 19.7°$ superimposed on the diffuse background. This peak is identifiable with the main diffraction peak of the $V_h$ polymorph of crystalline amylose−lipid complex (*29*). In this instance, the endogenous lipids associated with the native wheat starch granules [typically ~0.8% (*21*)] are responsible for the complex. This complex would not have been expected in the case of the two other starches due to the very low level of amylose in the waxy maize starch and the very low level of lipids normally associated with native potato starch granules. The lipid contents for these starches were found to be ~0.15, 0.05, and 0.8% (dwb) for the waxy maize, potato, and wheat starches, respectively (*21*).

After ~3 days of storage at 25 °C, the three extrudates were fully retrograded (as discussed below) and their WAXS diffractograms showed typical partially crystalline starch patterns (**Figure 4b**). The diffractograms suggested that waxy maize and wheat starch crystallized essentially to the A polymorph while potato starch crystallized to the B polymorph. The crystal structures of these two polymorphs have been reviewed by Imberty et al. (*30*). This suggests that under the conditions of this study (34% water and 25 °C), the starches reverted to their respective native starch crystal polymorphs. It is worth noting that the recrystallization polymorphic form is not necessarily the same as that of the native state. It is indeed well-documented that the temperature and water content at which the recrystallization proceeds largely affect the type of starch crystal polymorph formed upon retrogradation (*5*, *31*). The temperature and water content conditions used in this study lie on the boundary between forming the A and B type polymorphs, as was reported by Marsh (*5*) for wheat starch. Hence, it is interesting that although the three starch samples were aged under the same conditions, they retrograded to form different crystal polymorphs. This indicates that there is another underlying factor, besides those previously mentioned, that also influences the type of polymorph formed. This underlying factor could be the average chain length of the amylopectin, as suggested by Hizukuri et al. (*20*) and Gidley (*32*), who studied malto-oligosaccharides, and found that the A polymorph was favored by shorter chain lengths, whereas the B polymorph was favored by longer chain lengths. Waxy maize and wheat starches tend to have shorter average chain lengths than potato starches (*20*, *33*). Hizukuri et al. (*20*) found that the average chain lengths for the wheat and waxy maize starches were 18.5 and 18.6, respectively, and that of potato starch was 22.9, possibly explaining why the potato starch formed the B type polymorph and the waxy maize and wheat starches formed the A type polymorph.

Gidley (*34*) explained the effect of chain length on the polymorphic form by considering the negative change in entropy that occurs during crystallization. He stated that upon crystallization, the longer the chain length, the greater the negative entropy change would be and therefore the polymorph of highest entropy would be favored, which would be the B type polymorph, as it is less tightly packed than the A type polymorph.

The crystallinity indices were calculated as described above from the WAXS diffractograms acquired after full retrogradation, i.e., after ~3 days of aging. A value of ~32% (±1.1%) (wwb), i.e., ~50% (dwb), was found for waxy maize starch, while the values for wheat starch and potato starch were of the same order, ~24% (±1.7%) (wwb), i.e., ~36% (dwb). The crystallinity indices therefore did not depend on the polymorphic form of the starch and seemed to be directly correlated with the amylopectin content. It is therefore more appropriate to recalculate and compare these results on an amylopectin basis. Using the typical amylose content values for these starches listed above, the calculated crystallinity indices (amylopectin basis) were not significantly different ($P < 5\%$) with values of ~51% (±1.7%) for waxy maize, ~50% (±3.2%) for wheat, and ~47% (±3.3%) for potato. The contribution from the amylose−lipid complex crystallinity in the case of wheat would be excluded

from this value as the amorphous reference used was the freshly extruded wheat starch, which exhibited the complex (**Figure 4a**,**b**).

While both the scanning and the isothermal calorimetry techniques showed a significant difference ($P < 5\%$) in the degree of order in the fully retrograded starch extrudates (**Figures 1a** and **2b**) with the $\Delta H_\infty$ of potato approximately twice that of wheat starch, the crystallinity measured by WAXS was not significantly different between the three starches. This lack of direct correlation between calorimetry and WAXS is consistent with previous findings (*35*, *36*) where it was suggested that different molecular structures, in terms of distance scales, are probed by DSC and WAXS during starch gelatinization and retrogradation. While WAXS reports on so-called "long-range" order, i.e., in this instance the packing of the A chain double helices into crystalline lamellae, DSC is believed to report on the overall enthalpy changes involved in the melting of the crystallites (long-range) but also the dissociation of the double helices (short-range order), which may or may not be involved in crystals, as defined by the WAXS criteria of crystal size and perfection. Using this argument, it is postulated that the potato starch may have a higher degree of noncrystalline short-range molecular order. This suggestion is partly supported by the possibility of cocrystallization (*8*) or more likely ordered zones composed of both amylopectin and amylose. In the case of wheat starch, a significant fraction of the amylose is involved in amylose−lipid complexes and is therefore not available for interaction with amylopectin. The results also, somehow surprisingly, suggest that waxy maize starch exhibits, although to a lesser extent, a higher degree of order as measured by DSC than by WAXS.

It is important to note that in this discussion, $\Delta H$ was assumed to be directly proportional to the amount of ordered phase and that the latent heat of melting/dissociation was independent of the starch botanical source or the crystalline polymorph formed. This is somehow simplistic as in many instances the melting enthalpy is strongly affected, in addition to the mass fraction of the ordered/crystalline phase, by its physical characteristics. For example, for the same triglyceride (e.g., tri-myristin), $\Delta H$ depends strongly on polymorphism, with a variation from 85 kJ/mol for the $\alpha$ polymorph to as high as 137 kJ/mol for the $\beta$ polymorph (*37*).

FTIR measurements were carried out in an attempt to determine the degree of short-range molecular order (helical order) developed in these starch samples during storage and obtain a molecular insight into the discrepancies between the degree of order determined by calorimetry and WAXS. The absorbance at ~1045 $cm^{-1}$ was assigned to the ordered phase of starch (*38*). It is often necessary to perform an internal normalization to account for variation in sample thickness in transmittance measurements or the quality of the contact between the sample and the ATR crystal in reflectance measurements, as was the case in this study. For this, the band at ~1151 $cm^{-1}$ is often used as an "internal standard" (*39*) and the ratio of the absorbencies 1045:1151 $cm^{-1}$ can be used to probe the degree of short-range molecular order in starch, e.g., during gelatinization (*40*) and retrogradation (*6*).

While the FTIR results strongly supported the calorimetry findings in terms of the retrogradation kinetics (**Figure 5a**,**b**), i.e., potato starch retrograding the fastest, followed by waxy maize and then wheat, the extent of molecular order after full retrogradation showed only limited agreement with the calorimetry results and the interpretation put forward above. Both techniques suggested that potato and waxy maize starches

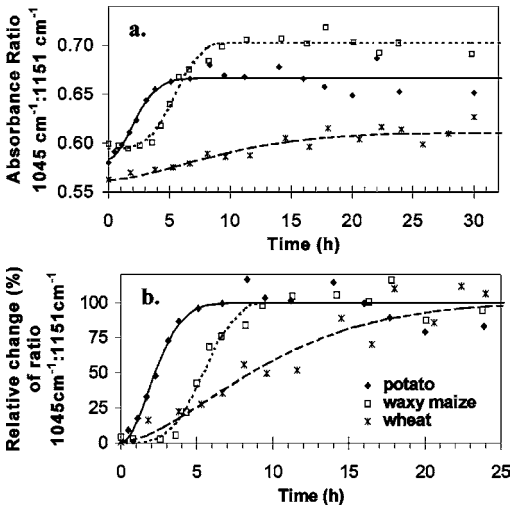


**Figure 5.** (**a**) Retrogradation monitored using the FTIR absorbance ratio 1045:1151 $cm^{-1}$ (each point is the average of four measurements; lines were calculated using eq 2) and (**b**) relative change of the absorbance ratio 1045:1151 $cm^{-1}$ (from panel **a**).

contain a higher degree of molecular order than that reflected by their WAXS determined crystallinity. However, while calorimetry suggested that potato starch contained the highest degree of amylopectin order, the FTIR data implied, despite a high degree of scatter (linked to sampling artifacts), that retrograded waxy maize starch is more ordered at the molecular level than both potato and wheat starches. The implication of this is that (i) either the ratio of the absorbencies 1045:1151 $cm^{-1}$, which is taken in this context as a measure of short-range molecular order, is also sensitive to other molecular characteristics (e.g., chain length) or (ii) that the difference in the retrogradation enthalpy between the three starches is due, in addition to variations in the degree of molecular order, to other properties such as the chain length and the polymorphism of the retrograded amylopectin, the latter being the more likely interpretation. Indeed, Wursch and Gumy (*19*) reported a linear correlation to exist between the DSC measured retrograded amylopectin enthalpy and the average external chain length. This is also indirectly supported by the gelatinization enthalpies obtained on the native starches (**Table 2**) where potato starch yielded a higher $\Delta H$ (~23 J/g amylopectin) than both waxy maize and wheat, which gave similar values of ~16 J/g amylopectin (values were calculated as the average of both calorimetric techniques). On this basis, it would be expected that the enthalpy of retrogradation of potato would be the highest while waxy maize and wheat should yield comparable values.

It is interesting to note that for all three starches, the gelatinization enthalpies were significantly higher (approximately a factor of 2) than the retrogradation enthalpies ($\Delta H_\infty$) measured under the same conditions of excess water (**Tables 1** and **2**) suggesting that on retrogradation, amylopectin recovers only a fraction of its native extent of molecular order. This is not surprising when considering the intricate way native amylopectin crystals are formed during biosynthesis (*41*), which is very likely to occur under more ideal conditions and result in more perfected structures than those formed by retrogradation in more limited time scales. Waigh et al. (*42*) suggested that after starch gelatinization, i.e., when the amylopectin is in the random coil conformation, the free ends of the outer amylopectin chains entangle resulting in topological constraints, which lead to a degree of randomness in A chain pairing during the reformation of double helices. Furthermore, the temperature range over which the melting of molecular order formed by

retrogradation was much broader than the native one (**Tables 1** and **2**). It is tempting to suggest that the fact that the gelatinization temperatures for each starch were also higher than those of the melting of the corresponding retrograded system (**Tables 1** and **2**) supports the hypothesis of more perfect crystalline structures in the native materials. This, however, would be inaccurate as it is now well-established that gelatinization is nonequilibrium in nature and depends strongly on the plasticization of the amorphous phase of the granule in which the crystallites are embedded (*27*). Such plasticization depends very strongly on the extent and kinetics of hydration of this amorphous phase, which would be much more limited in the granular starch form than in a material, which was first "destructured" by extrusion and then allowed to recrystallize. Furthermore, the WAXS crystalline values obtained in this study on the retrograded samples are very comparable to those reported for the native starches (*35*) once the effect of moisture content on crystallinity has been accounted for (*43*).

In summary, the retrogradation behavior in concentrated starch systems (water content ~34% wwb) at 25 °C depended on the botanical source. The kinetics of retrogradation determined using scanning and isothermal calorimetry and FTIR were consistent. The retrogradation rate was the highest for potato starch, followed by waxy maize and then wheat. The extent of molecular order in the fully retrograded materials was studied. While the amylopectin crystallinity indices determined from WAXS were not significantly different between the three retrograded starch systems, FTIR and calorimetry suggested variations in the degree of short-range molecular order, with wheat starch consistently the least ordered material. This was attributed to the possible interference of the networks of amylose and amylose−lipid complexes with amylopectin retrogradation. In addition to the proximate composition of the three starches, the amylopectin fine structure (average chain length, outer chain length) was used to interpret the findings. It is suggested that implying differences in the degree of molecular order between different starches by comparing their retrogradation enthalpies is not accurate as the enthalpy may depend on several other parameters, such as average chain length and crystal polymorphism.

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## LITERATURE CITED

(1) Orford, P. D.; Ring, S. G.; Carroll, V.; Miles, M. J.; Morris, V. J. The effect of concentration and botanical source on the gelation and retrogradation of starch. *J. Sci. Food Agric.* **1987**, *39*, 169−177.

(2) Kalichevsky, M. T.; Orford, P. D.; Ring, S. G. The retrogradation and gelation of amylopectin from various botanical sources. *Carbohydr. Res.* **1990**, *198*, 49−55.

(3) Roulet, P.; MacInnes, W. M.; Gumy, D.; Wursch, P. Retrogradation kinetics of eight starches. *Starch−Starke* **1990**, *42*, 99−101.

(4) Zobel, H. F.; Kulp, K. The staling mechanism. In *Baked Goods Freshness. Technology, Evaluation and Inhibition of Staling*; Hebeda, R. E., Zobel, H. F., Eds.; Marcel Dekker: New York, 1996; pp 1−64.

(5) Marsh, R. D. L. A study of the retrogradation of wheat starch systems using X-ray diffraction. Doctoral dissertation, University of Nottingham, 1986.

(6) Wilson, R. H.; Goodfellow, B. J.; Belton, P. S.; Osborne, B. G.; Oliver, G.; Russell, P. L. Comparison of Fourier transform mid infrared-spectroscopy and near-infrared reflectance spectroscopy with differential scanning calorimetry for the study of the staling of bread. *J. Sci. Food Agric.* **1991**, *54*, 471−483.

(7) Farhat, I. A.; Blanshard, J. M. V.; Mitchell, J. R. The retrogradation of waxy maize starch extrudates: Effects of storage temperature and water content. *Biopolymers* **2000**, *53*, 411−422.

(8) Gudmundsson, M.; Eliasson, A.-C. Retrogradation of amylopectin and the effects of amylose and added surfactants/emulsifiers. *Carbohydr. Polym.* **1990**, *13*, 295−315.

(9) Klucinec, J. D.; Thompson, D. B. Amylopectin nature and amylose-to-amylopectin ratio as influences on the behavior of gels of dispersed starch. *Cereal Chem.* **2002**, *79*, 24−35.

(10) Hermans, P. H.; Weidinger, A. Quantitative X-ray investigations on the crystallinity of cellulose fibers. A background analysis. *J. Appl. Phys.* **1948**, *19*, 491−506.

(11) Wilson, R. H.; Kalichevsky, M. T.; Ring, S. G.; Belton, P. S. A Fourier transform infrared study of the gelation and retrogradation of waxy-maize starch. *Carbohydr. Res.* **1987**, *166*, 162−165.

(12) Sevenou, O.; Hill, S. E.; Farhat, I. A.; Mitchell, J. R. Organisation of the external region of the starch granule as determined by infrared spectroscopy. *Int. J. Biol. Macromol.* **2002**, *31*, 79−85.

(13) Kauppinen, J. K.; Moffatt, D. J.; Mantsch, H. H.; Cameron, D. G. Fourier self-deconvolution: A method for resolving intrinsically overlapped bands. *Appl. Spectrosc.* **1981**, *35*, 271−276.

(14) Farhat, I. A.; Blanshard, J. M. V. Modeling the kinetics of starch retrogradation. In *Bread Staling*; Chinachoti, P., Vodovotz, Y., Eds.; CRC Press LLC: Boca Raton, 2001; pp 163−172.

(15) Jovanovich, G.; Zamponi, R. A.; Lupano, C. E.; Anon, M. C. Effect of water-content on the formation and dissociation of the amylose lipid complex in wheat-flour. *J. Agric. Food Chem.* **1992**, *40*, 1789−1793.

(16) Eliasson, A.-C. Effect of water content on the gelatinization of wheat starch. *Starch−Starke* **1980**, *32*, 270−272.

(17) Miles, M. J.; Morris, V. J.; Orford, P. D.; Ring, S. G. The roles of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydr. Res.* **1985**, *135*, 271−281.

(18) Fredriksson, H.; Silverio, J.; Andersson, R.; Eliasson, A.-C.; Aman, P. The influence of amylose and amylopectin characteristics on gelatinization and retrogradation properties of different starches. *Carbohydr. Polym.* **1998**, *35*, 119−134.

(19) Wursch, P.; Gumy, D. Inhibition of amylopectin retrogradation by partial beta-amylolysis. *Carbohydr. Res.* **1994**, *256*, 129−137.

(20) Hizukuri, S.; Kaneko, T.; Takeda, Y. Measurement of chain length of amylopectin and its relevance to the origin of crystalline polymorphism of starch granules. *Biochim. Biophys. Acta* **1983**, *760*, 188−191.

(21) Swinkels, J. J. M. Composition and properties of commercial native starches. *Starch−Starke* **1985**, *37*, 1−5.

(22) Conde-Petit, B.; Escher, F. Influence of starch-lipid complexation on the aging behavior of high-concentration starch gels. *Starch−Starke* **1994**, *46*, 172−177.

(23) Eliasson, A. C.; Ljunger, G. Interactions between amylopectin and lipid additives during retrogradation in a model system. *J. Sci. Food Agric.* **1988**, *44*, 353−361.

(24) Morrison, W. R.; Law, R. V.; Snape, C. E. Evidence for inclusion complexes of lipids with v-amylose in maize, rice and oat starches. *J. Cereal Sci.* **1993**, *18*, 107−109.

(25) Morrison, W. R.; Tester, R. F.; Snape, C. E.; Law, R.; Gidley, M. J. Swelling and gelatinisation of cereal starches. 4. Some effects of lipid-complexed amylose and free amylose in waxy and normal barley starches. *Cereal Chem.* **1993**, *70*, 385−391.

(26) Le Bail, P.; Bizot, H.; Ollivon, M.; Keller, G.; Bourgaux, C.; Buleon, A. Monitoring the crystallization of amylose-lipid complexes during maize starch melting by synchrotron X-ray diffraction. *Biopolymers* **1999**, *50*, 99−110.

(27) Slade, L.; Levine, H. Nonequilibrium melting of native granular starch: Part I. Temperature location of the glass transition associated with gelatinization of A-type cereal starches. *Carbohydr. Polym.* **1988**, *8*, 183−208.
(28) Donovan, J. W. Phase transitions of the starch-water system. *Biopolymers* **1979**, *18*, 263−275.
(29) Mercier, C.; Charbonniere, R.; Grebaut, J.; De La Guerivière, J. F. Formation of amylose-lipid complexes by twin-screw extrusion cooking of manioc starch. *Cereal Chem.* **1980**, *57*, 4−9.
(30) Imberty, A.; Buleon, A.; Tran, V.; Perez, S. Recent advances in knowledge of starch structure. *Starch—Starke* **1991**, *43*, 375−384.
(31) Hizukuri, S. X-ray diffractometric studies on starches Part VI. Crystalline types of amylodextrin and effect of temperature and concentration of mother liquor on crystalline type. *Agric. Biol. Chem.* **1961**, *25*, 45−49.
(32) Gidley, M. J.; Bulpin, P. V. Crystallisation of malto-oligosaccharides as models of the crystalline forms of starch: Minimum chain-length requirement for the formation of double helices. *Carbohydr. Res.* **1987**, *161*, 291−300.
(33) Ong, M. H.; Jumel, K.; Tokarczuk, P. F.; Blanshard, J. M. V.; Harding, S. E. Simultaneous determinations of the molecular weight distributions of amyloses and the fine structures of amylopectins of native starches. *Carbohydr. Res.* **1994**, *260*, 99−117.
(34) Gidley, M. J. Factors affecting the crystalline type (A−C) of native starches and model compounds: A rationalisation of observed effects in terms of polymorphic structures. *Carbohydr. Res.* **1987**, *161*, 301−304.
(35) Cooke, D.; Gidley, M. J. Loss of crystalline and molecular order during starch gelatinization—Origin of the enthalpic transition. *Carbohydr. Res.* **1992**, *227*, 103−112.
(36) Ottenhof, M.-A. A multi-technique study of the retrogradation of concentrated starch systems. Doctoral dissertation, University of Nottingham, 2003.
(37) Hagemann, J. W.; Rothfus, J. A. Polymorphism and transformation energetics of saturated monoacid triglycerides from differential scanning calorimetry and theoretical modeling. *J. Am. Oil Chem. Soc.* **1983**, *60*, 1123−1131.
(38) Smits, A. L. M.; Ruhnau, F. C.; Vliegenthart, J. F. G.; van Soest, J. J. G. Aging of starch based systems as observed with FT-IR and solid-state NMR spectroscopy. *Starch—Starke* **1998**, *50*, 478−483.
(39) van Soest, J. J. G.; de Wit, D.; Tournois, H.; Vliegenthart, J. F. G. Retrogradation of potato starch as studied by Fourier transform infrared-spectroscopy. *Starch—Starke* **1994**, *46*, 453−457.
(40) Sevenou, O.; Hill, S. E.; Farhat, I. A.; Mitchell, J. R. In-situ study of the changes in starch and gluten during heating of dough using attenuated-total-reflectance Fourier transform-infrared (ATR-FTIR). In *Plant Biopolymer Science: Food and Non-Food Applications*; Renard, D., Della Valle, G., Popineau, Y., Eds.; The Royal Society of Chemistry: Cambridge, 2002; pp 275−283.
(41) Buleon, A.; Colonna, P.; Planchot, V.; Ball, S. Starch granules: Structure and biosynthesis. *Int. J. Biol. Macromol.* **1998**, *23*, 85−112.
(42) Waigh, T. A.; Gidley, M. J.; Komanshek, B. U.; Donald, A. M. The phase transformations in starch during gelatinisation: A liquid crystalline approach. *Carbohydr. Res.* **2000**, *328*, 165−176.
(43) Hartley, L.; Chevance, F.; Hill, S. E.; Mitchell, J. R.; Blanshard, J. M. V. Partitioning of water in binary biopolymer mixtures at low water content. *Carbohydr. Polym.* **1995**, *28*, 83−89.

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