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ISOTHERMAL MICROCALORIMETRIC STUDIES ON STARCH RETROGRADATION

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Abstract

In the present study, isothermal microcalorimetry was introduced as a tool to investigate properties of starch retrogradation during the first 24 h. The study was made on purified amylose and amylopectin from corn, as well as on native starches, such as wheat, potato, maize, waxy maize and amylomaize, differing in their amylose content. The results were obtained in the form of *P–t* traces (thermal power *vs.* time), and integration of these traces gave a net exothermic enthalpy of reaction, caused by the crystallization of amylose and amylopectin. The *P–t* traces reflected the quantities of amylose and amylopectin in the starch studied. Depending on the amylose content and the botanical source of the starch, the rate of crystallization of amylose was high and predominated over that of amylopectin during the first 5–10 h. The contribution from amylose crystallization to the measured exothermic enthalpy was very substantial during this period. After ~10 h, amylose crystallized at a lower constant rate. During the first 24 h, amylopectin crystallized at a low steady rate. The exothermic enthalpies obtained by the isothermal microcalorimetric investigations during the first 24 h of retrogradation were generally low in relation to the endothermic melting enthalpies observed by differential scanning calorimetry (DSC) measurements after 24 h of storage. The discrepancies in enthalpy values between the two methods are discussed in relation to phase separation and the endothermic effects owing to the decrease in polymer–water interactions when polymer–rich regions in the starch gel separate. Besides the exothermic enthalpies obtained, the *P–t* traces also made it possible to study the initial gelation properties of amylose from different botanical sources. The present study further demonstrated that isothermal microcalorimetry can provide a possible way to investigate the anti-staling effect of certain polar lipids, such as sodium dodecylsulphate (SDS) and 1-monolauroyl-*rac*-glycerol (GML), when added to starches of different botanical origin. The net exothermic heat of reaction for starch retrogradation during the first 24 h was decreased when GML or SDS was added to the starch gels. The recorded *P–t* traces also showed how the effect of the added lipid influenced different periods during the first 24 h of starch retrogradation, and that the effect depended mainly on the amylose content, the botanical source of the starch, and the type of lipid used. When GML or SDS was added to waxy maize, the isothermal microcalorimetric studies clearly indicated some interaction between amylopectin and the polar lipids. These results concerning the action of anti-staling agents are further discussed in relation to the helical inclusion complexes formed between amylose-polar lipid and amylopectin-polar lipid.

Keywords: DSC, isothermal microcalorimetry, retrogradation, starch

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Introduction

Starch is mainly composed of the two α-polyglucans, amylose and amylopectin. Amylose is essentially linear, with α-(1 → 4)-*D*-linkages and a reported molecular weight around 10^5– 10^6 [1], whereas amylopectin is highly branched, with the same backbone of α-(1 → 4)-*D*-linkages and additional α-(1 → 6)-*D*-linkages at the branching points and molecular weights in the range 10^7– 10^9 [2]. Amylopectin has a bi- or polymodal distribution of chain lengths, with cereal starches generally having shorter chains than those found in tuber starches [3]. The chains of amylopectin can be classified as A-, B- or C-chains, owing to their molecular organisation [4]. It is the chain arrangement of amylopectin into double helices, joined in clusters, that gives native starch its semi-crystalline properties [5–8]. The granular composition differs, depending on the botanical source, from almost 100% amylopectin in waxy varieties to high-amylose varieties with about 70% amylose. The amylose content for normal varieties is usually in the range of 20–30%. Cereal starches also contain small amounts of lipids, mainly free fatty acids and lysophospholipids [9], whereas potato starch carries some phosphate groups [10]; these components also influence the physical properties of starch.

When starch is heated in aqueous solution, it is exposed to a number of non-equilibrium, irreversible changes collectively known as gelatinization [11]. It is well recognised that the gelatinization properties of starch are strongly influenced by the amount and distribution of water [12–14], and that a starch-water mixture can be looked upon as a polymer-diluent system, in which the water acts as a plasticiser of the amorphous regions of the polymer and controls the effective glass transition that precedes the melting of amylopectin [15]. Starch loses its crystalline properties during gelatinization, and its viscoelastic properties are changed owing to granular swelling and release of amylose. As a result of gelatinization, an opaque paste or gel of swollen granules in a continuous polysaccharide matrix is formed [16].

Starch gels are not in equilibrium, and the properties of the gels will change during storage [15, 17]. Retrogradation [11], mainly considered as a recrystallization process, leads to an undesirable firm and dry texture of many starch-based foods, with resulting quality losses of great expense to the food manufacturing industry. The development of crystalline order in amylopectin in the gel is a long-term effect compared to that of gelation of amylose. The increased crystallinity is usually studied by DSC, but can also be monitored by X-ray diffraction measurements characterizing the development of the B-pattern [18]. This pattern originates from hexagonal packing of double helices, and during retrogradation, it is possible for both amylose and amylopectin to contribute to the formation of double helices in clusters. The crystallization rates for amylose and amylopectin differ, and the latter dominates the recrystalliza-

tion process at long times. It is, however, possible to increase the shelf-lives of many starch-based foods by the addition of certain polar monoacyl lipids [19]. The added lipids affect the recrystallization properties of starch by forming helical inclusion complexes with the amylose fraction and with the outer branches of the amylopectin fraction [20, 21]. Several authors have extensively reviewed the behaviour of amylose-lipid complexes [22, 23]. The complex has further been characterized by Raman spectroscopy [24].

DSC measurements, in the temperature range 40 to 100°C, monitor the endothermic transitions of amylopectin during gelatinization and retrogradation [25, 26], whereas events in the range 120 to 170°C are associated with the melting of the amylose fraction [27]. A drawback of utilizing DSC for retrogradation studies is its limited sensitivity, owing to weak signals during the first period of recrystallization. However, by isothermal microcalorimetry, it is possible to investigate changes in thermal power in the range of microwatts [28]. The sensitivities of a typical isothermal microcalorimeter and a conventional power-compensated DSC are 0.1 and 10 μW, respectively. The amounts of sample able to be analyzed in the two instruments are 1000 and 10 mg for the microcalorimeter and the DSC, respectively. Therefore, the specific sensitivity of the microcalorimeter is 0.1 μW/g, compared to 1000 μW/g for the DSC. The use of microcalorimetry has recently gained wide interest for different food applications, mainly in the area of microbiological activity [29]. Microcalorimetry has also been used to characterize amylose complexes [30] and changes in enthalpy and heat capacity associated with gelatinization [31]. Thus, this highly sensitive method might also provide an opportunity to study the initial period of the recrystallization process in starch gels.

The present study was made in order to compare the retrogradation behaviour of various starches with different amylose contents, analyzed by microcalorimetry and DSC after the first 24 h and to investigate by microcalorimetry how monoacyl polar lipids influence retrogradation during the first 24 h.

Materials and methods

Materials

Amylose and amylopectin, both from corn, were obtained from Sigma Chemical Co., USA. Waxy maize, maize, amylomaize and potato starches were obtained from Lyckeby Stärkelsen, Sweden, and used without further purification. Wheat starch was prepared from a commercial flour (Kungsörnen, Sweden) at our laboratory. The dry-matter content of the starches was determined by drying in a heating cabinet at 135°C for 2 h.

For the retrogradation studies, the surfactant SDS (specially pure) was obtained from BDH Laboratory Supplies, UK. The concentration of SDS in the samples 2.0% (w/w) on starch basis. GML was obtained from Sigma, was also

used at a concentration of 2.0% (w/w) on starch basis, and was added in a lamellar phase. Double-distilled water was used for all experiments.

The apparent amylose content of the starches was determined according to the Blue Value method described by Morrison and Laignelet [32]. Urea, dimethyl sulphoxide and potassium iodine were all of analytical grade and obtained from Merck (Darmstadt, Germany), whereas the resublimed iodine was obtained from May & Baker (Dagenham, England). Absorbance was examined at 635 nm on a Beckman DU-50 spectrophotometer (Beckman Instruments, USA). The amylose content was calculated according to an equation given by Morrison and Laignelet [32], using two independent measurements with a deviation of 3.0% of the mean.

DSC

Samples were mixed to a starch:water solution ratio of 3:7 (w/w) in coated aluminium pans from TA Instruments. To achieve a uniform distribution of water, the samples were stored for 12 h at 25°C, before they were gelatinized in a heating cabinet at 105°C for 15 min. The DSC measurements of the retrograded starches were then performed after 24 h of storage at 22°C. DSC measurements were made with a Perkin-Elmer DSC-2C, using a heating rate of 10°C min^{-1} in the range 17–97°C. An empty DSC pan was used as a reference. The DSC results were evaluated, and the enthalpies of melting of recrystallised amylopectin (ΔH_{DSC}) and the temperatures at peak maximum (T_m) of the different starches were compared. After the measurements, the pans were punctured and dried at 105°C for 24 h to determine the exact water contents of the samples.

Isothermal microcalorimetry

For microcalorimetric studies, the samples were mixed to the same starch:water solution ratio (w/w) as for the DSC measurements, in 3 ml glass vials with PTFE/silicone septa and crimp seals of aluminium, and gelatinized after sealing in a heating cabinet at 105°C for 15 min. After heating, the septa were exchanged. The isothermal microcalorimeric experiments were performed at 25°C on a 4-channel Bio Activity Monitor, Thermometrics/LKB 2277 (Thermometrics AB, Järfälla, Sweden). Each twin calorimetric unit consists of two holders for insertion vessels. Each ampoule is surrounded by two Peltier-effect plates forming thermal bridges to small aluminium blocks. For each ampoule, the Peltier-effect plates are electrically connected in series, whereas the two thermopiles formed are connected in opposition, giving a differential voltage signal from the twin calorimetric unit [28]. The calibration constants of the different channels were determined using an insertion heater connected to a glass vial filled with quartz sand. Static glass vials filled with quartz sand were used as references, and heated glass vials (105°C, 15 min) filled with water were used as blanks to determine the baseline for each of the four channels of the microcalorimeter. Sample vials were transferred to the microcalorimetric

device and then held for a total lag time of 45 to 90 min before recording. The lag-time period included at least 15 min for the vials to cool to room temperature, and two times 15 min of temperature equilibration of the microcalorimeter during insertion of the sample holders. The signals from the microcalorimeter, i.e. thermal power, were measured by potentiometric recorders and a digital data-collection system, and the results were presented as P–t traces (power *vs.* time). The P–t traces shown in the figures below were all individually adjusted for their respective lag times.

Duplicate blank samples were analysed for accurate determination of the baselines of the four channels of the microcalorimeter. The reproducibility of the baselines of the P–t traces for the channels was, on average, within ±0.30 μW/(g glass vial). Each P–t trace was adjusted by multiplying by the appropriate calibration constant and then compensated for the blank, before normalization was done for a defined mass of sample. For a meaningful comparison, the recorded thermal power must be related to a defined mass (μW/g starch). The heat (ΔH_{MC}) produced or consumed by a sample was obtained by integration of the P–t trace during the first 24 h, except for amylomaize, for which only the first 19 h were registered.

Results and discussion

Amylose content

The apparent amylose contents (i.e. the amylose involved in complexation with native lipids of the starch was not included in this determination) of the different starches are given in Table 1. The amylose contents of wheat, maize and amylomaize starches were within the ranges reported in other studies [33, 34], whereas the amylose content of potato starch was somewhat high, since most mature potato starch granules normally contain 18–23% amylose [35, 36]. Because no native lipids are present in potato starch, the amylose content determined for potato starch is the true amylose content. For pure amylose, we obtained a low staining response around 80%. However, Morrison and Laignelet described a similar situation in their study and explained that the discrepancy was due to an amylose fraction with short chains [32].

DSC measurements

The main crystalline component in the native starch granule is the amylopectin fraction. On gelatinization, the crystalline order is lost, but on cooling and subsequent storage of the starch gel, the amylopectin slowly regains its crystallinity. The occurrence of amylopectin recrystallization can be observed by DSC as an endothermic transition in the temperature range of 40–100°C [25]. The extents of starch retrogradation, i.e. recrystallization of amylopectin,

Table 1 Amylose contents of the different starches and retrogradation-related enthalpy values from the DSC and isothermal microcalorimetric measurements

Starch	Amylose content/ %	DSC		Isothermal microcalorimetry		
		ΔH_{DSC}/ J(g starch)$^{-1}$	T_M/ °C	ΔH_{MC}/ J(g starch)$^{-1}$	$\Delta H_{MC,GML}$/ J(g starch)$^{-1}$	$\Delta H_{MC,SDS}$/ J(g starch)$^{-1}$
Wheat	26.2	0.70±0.08	54.3±0.5	0.68±0.10	0.24±0.04	0.28±0.01
Potato	33.4	4.35±0.40	63.3±0.5	0.75±0.05	0.31±0.02	0.47±0.01
Maize	26.2	1.43±0.13	53.1±1.0	1.53±0.04	0.82±0.03	0.38±0.03
Amylomaize	69.6	0.61±0.03	54.1±0.8	2.06±0.06*	1.62±0.03*	1.22±0.04*
Waxy maize	0	0.72±0.06	52.3±2.5	0.38±0.05	0.05±0.01	0.12±0.01
Amylopectin	0.2	0.71±0.12	–	0.18±0.02	0.07±0.03	0.07±0.01
Amylose	80.4	–	–	2.05±0.26	2.17±0.01	2.29±0.01

*All the enthalpy values from the isothermal microcalorimetric studies of amylomaize are based on 19 h of starch retrogradation.

after 24 h storage of the different gelatinized starch samples (30% (w/w) starch) are given in Table 1. The endothermic melting enthalpy of amylopectin recrystallization (ΔH_{DSC}) was found to be below 1 J g^{-1} starch, after aging gelatinized samples for 24 h, except for potato and maize starch. Potato starch showed a remarkably high enthalpy value, compared to those for the other starch samples. The retrogradation of wheat, potato and maize starch followed the order: potato > maize > wheat. A similar ranking was previously observed after storage for 7 days [35].

The role of moisture in the retrogradation of wheat starch gels was studied by Longton and LeGrys [37]. They found that the energy required to melt the retrograded amylopectin crystals in wheat starch gels, after 14 days of aging at 4°C, followed a bell-shaped curve as a function of starch content. The starch contents of all the samples in the present DSC study were 28.3–30.3% (w/w) starch. These would lie in the area of the leading inflection point of the bell-shaped curve. This could explan why most of the enthalpy values obtained in this study (Table 1) were lower than other data generally obtained at lower water contents [38, 39]. At the relatively low starch concentrations studied here, the effect on the melting enthalpy, caused by the dilution effect of water, would dominate the extent of crystallization; i.e. low enthalpy values were obtained for all the starch samples, including amylopectin and waxy maize, resulting in the amount of amylopectin in the samples appearing to be of minor significance. It is assumed that the relationship between melting enthalpy of recrystallized amylopectin and starch content exists not only for wheat starch gels but for gels of other starches of different botanical origin.

Isothermal microcalorimetry measurements

Due to the lag time, owing to the procedure of insertion of sample holders into the microcalorimeter, the earliest stages of starch retrogradation (i.e. the first 45–90 min) could not be recorded. To calculate the enthalpy of starch retrogradation (ΔH_{MC}) for the entire first 24 h of retrogradation, an extrapolation of each P–t trace back to $t=0$ was done. This was accomplished by curve-fitting the P–t traces to the second-order polynomial equation

$$P(t)=c_1+c_2t+\frac{c_3}{t}$$

where P is the thermal power at time t, and c_1, c_2 and c_3 are curve-fitting constants. The P–t curves are given as the average of at least two measurements, and all the curves shown were fitted to the above equation. The correlation factors for all the curve fittings were $R=0.96$–1.00, except for the P–t curves for waxy maize starch/2% SDS ($R=0.89$) and amylopectin/2% SDS ($R=0.77$). The reproducibility of all the P–t traces was, on average, within 8.0% of the mean value, based on the calculated enthalpy change (ΔH_{MC}).

Small positive power signals were recorded when blank samples were analysed in the microcalorimeter. The blank samples produced small amounts of exothermic heat. This modest heat production must be attributed to the glass vials used in the experiments. The heat treatment of the starch samples at 105°C for 15 min could introduce strains in the glass material. These strains would lead to exothermic relaxation processes that could be recorded because of the high sensitivity of the microcalorimeter. The relaxations of the glass material of the vials were reproducible to within ±0.30 μW/(g glass), as judged from duplicate measurements on the four channels of the microcalorimeter. All *P–t* traces were corrected for this effect by subtracting the blank runs.

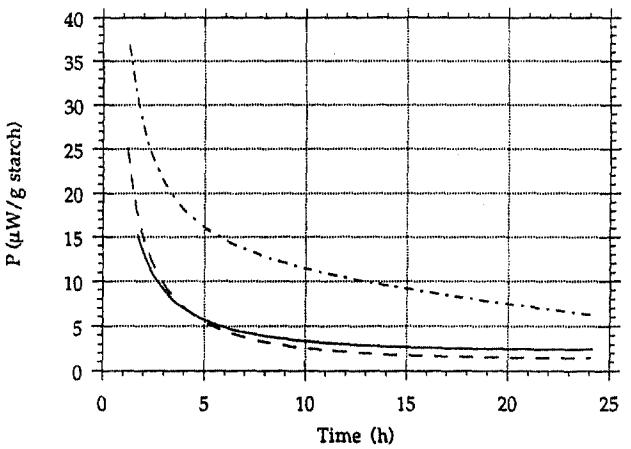

Fig. 1 *P–t* traces from the isothermal microcalorimetric analysis of wheat, potato and maize starches: —— wheat, – – potato, ·-· maize

The *P–t* traces for wheat, potato and maize starches are shown in Fig. 1. In general, all the *P–t* traces for the gelatinized starches started with a relatively high positive thermal power, indicating an exothermic process that decreased exponentially for approximately 5 h. After about 5 h, the traces levelled off to a steady state, in which the thermal power was nearly constant. The thermal-transition behavior of wheat and potato starches was very similar. The initial decay was almost identical; after 2 h, the thermal power was 13.2 or 15.0 μW/g starch for wheat or potato starch, respectively. The *P–t* traces levelled off to a steady state after 5–7 h, and the thermal power after 24 h was 2.4 μW/g wheat starch and 1.4 μW/g potato starch. The thermal-transition behaviour of maize starch was somewhat different from that of wheat and potato starch. For maize starch, the thermal power was higher during the entire first 24 h; it was 27.6 μW/g starch after 2 h and 6.3 μW/g starch after 24 h. The *P–t* trace for maize starch did not level off to a steady state during the first 24 h. The results shown in Fig. 1 suggest that maize starch retrograded to a greater extent during the first 24 h than did wheat or potato starch.

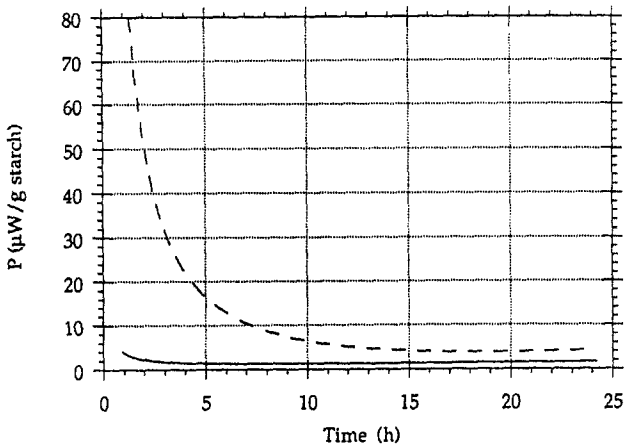

Fig. 2 *P–t* traces from the isothermal microcalorimetric analysis of amylose and amylopectin: – – amylose, —— amylopectin

In Fig. 2, *P–t* traces for amylose and amylopectin are shown. The *P–t* trace for amylose started with a high positive thermal power, i.e. 51.3 μW/g starch after 2 h. This was followed by a relatively large exponential decrease in thermal power over the first 10 h of retrogradation, before the *P–t* trace levelled off to a steady state. The thermal power for amylose was 4.3 μW/g starch after 24 h. The amylopectin trace in Fig. 2 had a very different appearance from the one for amylose or the ones for the starches in Fig. 1. The large initial exponential decay was absent, and very low positive thermal power was recorded; after 2 h, the thermal power was only 2.6 μW/g starch. It should be observed that there was also a relatively rapid exothermic relaxation process during the first 2–4 h for amylopectin, but its magnitude was much smaller than that for amylose. An almost constant thermal power was developed after only about 3 h, and after 24 h, the thermal power was 1.5 μW/g starch. The *P–t* traces in Fig. 2 indicate that retrogradation of starch is dominated by amylose during the first 5 to 10 h and dependent on amylose content. From the steady-state values of the amylose and amylopectin traces in Fig. 2, it is evident that the steady state for a whole starch would be influenced by both starch fractions.

In Fig. 3, the *P–t* traces for waxy maize, maize and amylomaize starches are presented. The *P–t* trace for amylomaize (69.6% amylose) showed a strong resemblance to that for pure amylose, and the *P–t* trace for waxy maize (0% amylose) was similar to that for amylopectin. The initial exponential decay of the *P–t* trace for maize starch (26.2% amylose) during the first few hours was intermediate to those of amylomaize and waxy maize. It appears that there was also a relatively rapid exothermic relaxation process during the first 5 h for waxy maize, but the magnitude was, as for amylopectin above, much smaller than that for amylomaize. The initial microcalorimetric response of thermal

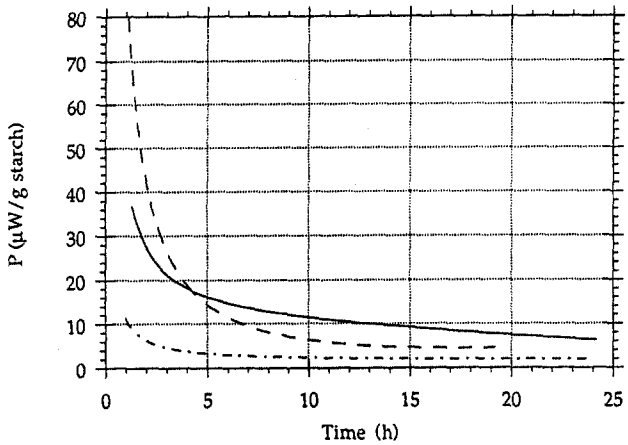

Fig. 3 *P–t* traces from the isothermal microcalorimetric analysis of maize, amylomaize and waxy maize starches: —— maize, – – amylomaize, -·- waxy maize

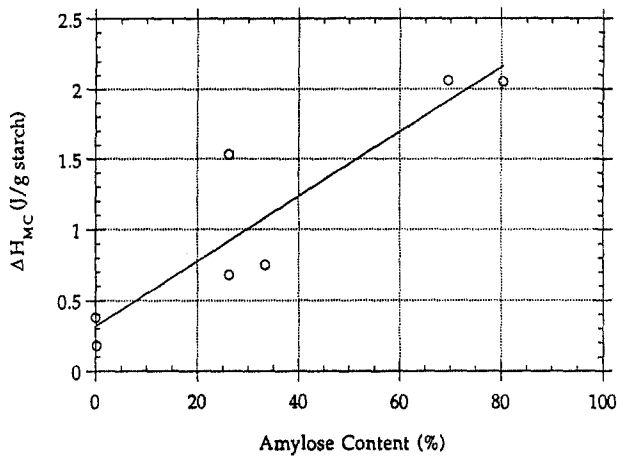

Fig. 4 Exothermic enthalpies from the isothermal microcalorimetry measurements, ΔH_{MC}, as a function of amylose content of the starch samples; water content: ~30% (w/w) (R=0.92)

power and the decrease in thermal power during the first 5 h of retrogradation seem to be directly related to the amylose content of the starch samples.

When the *P–t* traces were integrated, the enthalpy change, ΔH_{MC}, for the first 24 h of starch retrogradation was obtained. Integration of all the *P–t* traces gave negative enthalpies; i.e. the net enthalpy of reaction for starch retrogradation during the first 24 h, measured by microcalorimetry, was, as expected, exothermic. The derived enthalpy values (ΔH_{MC}) for the different starches are given in Table 1; they were between 0.2–2.1 J (g starch)$^{-1}$. The lowest exother-

mic enthalpy values were obtained for amylopectin and waxy maize, while amylose and amylomaize gave the highest values. Complex formation between lipids (native and added) and amylose, and the phase transition of added lipids not involved in complexation, were not taken into account in the estimation of ΔH_{MC}. Figure 4 shows the enthalpy change, ΔH_{MC}, as a function of amylose content for the different samples (water content ~30% (w/w)). It can be seen that there is a clear linear correlation ($R=0.92$) between ΔH_{MC} and amylose content of the samples. This underlines the importance of the amylose fraction during the early stages of retrogradation. The influence of amylose on ΔH_{MC} depends on the amount and botanical variation.

These results from isothermal microcalorimetry pointed to the plausible possibility of different events taking place simultaneously in the starch gel during the early stages of retrogradation, when the influence of amylose content of the starch is of great importance. The main factor contributing to the features of the *P–t* traces in Figs 1, 2 and 3 could be expected to be crystallization of amylose and amylopectin. This is consistent with the findings of Miles *et al.* [40] and Ring *et al.* [41]. Amylose crystallization, i.e. association of double helices, dominated during the first 10 h (Figs 1 and 2). The amount of amylose double helices aggregating into more polymer-rich domains was high during this initial period. This was followed by an increasing structural order between the double helices, resulting in crystalline domains. This might explain the constant crystallization rate after 10 h, seen as constant, steady-state thermal powers of the *P–t* traces (Fig. 2).

Judging from the *P–t* trace for amylopectin in Fig. 2, the crystallization rate was almost constant from the very beginning. This could be explained by the difference in molecular order of the two starch components. Domains rich in double helices already existed in amylopectin molecules, owing to the branching points and due to the fact that double helices of the side chains were still connected to the main polymer chain. The constant rate of amylopectin crystallization, seen as the constant thermal power of the *P–t* traces in Fig. 2, was a result of a diffusion-controlled process, in which the chains regained their structural order [15, 42]. In gelatinized starch, crystallization of amylopectin is secondary to amylose crystallization in the first 10 h, during which the amylose must first be organised into local polymer-rich regions of double helices. Once a steady state is reached, amylose crystallization is no longer a dominating factor.

It should be noted that the events measured in the microcalorimeter and the endothermic melting transition studied in the DSC were examined under two different temperature conditions. The investigations in the isothermal microcalorimeter were carried out at 25°C, i.e. a normal storage temperature for starch-based products. The temperature range for the endothermic peak examined in the DSC was located at higher temperatures (45–65°C). DSC measurements in the range 20–100°C monitor only endothermic transitions representing the dissociation of double helices of amylopectin, while microcalorimetric measurements cover a broader range of both exo- and endothermic events. When the

exothermic enthalpies from the microcalorimeter (ΔH_{MC}) were compared to the endothermic enthalpies from the DSC (ΔH_{DSC}), we found that $|\Delta H_{MC}| \leq |\Delta H_{DSC}|$ for all the starches except amylomaize. When amylose was analyzed in the DSC, no enthalpy change was observed, due to the absence of endothermic transitions in the temperature range studied. It is reasonable to expect the opposite relationship, $|\Delta H_{MC}| > |\Delta H_{DSC}|$. If we assume, hypothetically, that microcalorimetry only monitors the crystallization of amylopectin then $|\Delta H_{MC}| = |\Delta H_{DSC}|$, provided that the difference in measurement temperatures can be neglected. Because the microcalorimeter monitors the exothermic crystallization of both amylopectin and amylose, and because the contribution of exothermic heat from amylose crystallization is quite substantial, judging from the features of the *P–t* traces in Fig. 2, this exothermic heat of reaction should, of course, be superimposed on that for amylopectin to give $|\Delta H_{MC}| > |\Delta H_{DSC}|$. As this was not the case, the observed differences between the two methods might be explained by additional endothermic processes during the beginning of gelation of the amylose fraction. A simultaneous endothermic process would result in a reduction of the sum of the exothermic enthalpies of amylose and amylopectin crystallization, and if the endothermic process were of sufficient magnitude, the observed relationship, $|\Delta H_{MC}| \leq |\Delta H_{DSC}|$, would result.

The behaviour of amylose and amylopectin is, of course, profoundly influenced by differences in molecular structure. The gelation process, i.e. aggregation of amylose resulting in gelation and network formation, starts immediately after starch dispersions of sufficient concentration are cooled to room temperature after gelatinization. Amylose gelation has been studied by several groups and with a number of different techniques; e.g. Miles *et al.* used several physical methods to study it [40]. Their viscometry measurements showed that gels are formed on cooling entangled amylose solutions. Their results from rheological measurements, which reflect the development of network structure, and from dilatometry, which indicates changes in interactions between molecules, showed that gelation and volume changes took place in the early stages of starch retrogradation. These changes in shear modulus and gel volume reached a limiting value after approximately 5 h. The development of opacity, manifested by an increase in turbidity, was a much faster process and was completed after 1 h, suggesting that changes in density distribution occurred within the sample. The conclusion was that entanglement of polymer chains precedes the aggregation and gelation of amylose. The randomly coiled amylose separated into a polymer-rich network phase, leaving polymer-deficient, i.e. more water-rich, regions within the gel. This phase separation should be completed after approximately 5 h. However, an X-ray diffraction study, monitoring the development of crystallinity in the amylose gel, showed changes up to 25 h, before a limiting value was approached. Miles *et al.* [40] concluded that crystallinity subsequently developed within the separated polymer-rich phase at a slower rate than that of phase separation. Several other workers have confirmed that amylose gelation proceeds through a phase separation [43–45]. It is impor-

tant to remember that results of all such experiments are very dependent on polymer concentration. It has been shown that the rate of gelation increases with increasing concentration. Contrary to Miles *et al.* [40], Gidley proposed that molecular entanglement does not determine the critical concentration for gelation [46]. Gidley's model for gelation of amylose involved the formation of double helices and aggregates of helices via crosslinking during phase separation. A recent computer study [47] of structural features of amylose solutions and gels has favoured the gelation model proposed by Gidley.

The gelation and crystallization of waxy maize amylopectin have been extensively studied by Ring *et al.* [41]. Amylopectin gelation is much slower than the gelation of amylose. Polymer aggregation, seen as an increase in turbidity, became more evident with decreasing temperature; therefore, their experiments were conducted at 1 °C. Rheological experiments showed that changes in shear modulus, G', with time for a 20% amylopectin solution exhibited a sigmoidal dependence, and the modulus approached a limiting value after 30–40 days. Turbidity measurements showed that a limiting value was approached after 4–5 days. Dilatometry indicated slow positive volume changes, before reaching a constant value after 30–40 days. In contrast to the gelation and subsequent crystallization of amylose, the crystallization of amylopectin could be reversed by heating to 100 °C. Amylopectin gels were thermoreversible. Gel melting occurred in the range of 45–65 °C, as monitored by DSC measurements. The enthalpy of gel melting increased slowly with time, approaching a limiting value after 30–40 days. Thus, the observed phase separation of amylopectin was a much slower process than the relatively rapid phase separation exhibited by amylose.

As discussed above, it is known that the formation of an amylose network is accompanied by changes in volume of the gel, that the volume change is related to the amount of polymer separated from the gel, and that these changes are completed within 5 h [40]. This might relate indirectly to the present results, in that the influence of the amylose fraction was of minor importance after 5 h of retrogradation, as shown in Fig. 3. Interactions between solute and solvent are important for polymer separation from solution, and the magnitude of the interaction depends on solute-solvent hydrogen bonds [40]. In terms of polymer-water interactions, there are three well-known types of forces that bind water to macromolecules: hydrogen bonds, hydrophobic interactions and ionic bonds [48]. Since starch has a relatively unsubstituted structure, compared to that of other polysaccharides such as agar and carrageenan, a high proportion of intra- and intermolecular hydrogen bonds exists. But at least one of the four free hydroxyls on the glucose repeat unit of starch is available to interact with water. The other three hydroxyls are either intra- or intermolecularly hydrogen-bonded [49, 50]. During phase separation, a decrease in polymer-water interactions, an increase in polymer-polymer interactions, and an increase in water-water interactions take place. One possible way to estimate these interactions would be by

studying the enthalpies of dehydration of starch gels. Collison and Dickson have performed this kind of experiment by simultaneous differential thermal analysis and thermogravimetric analysis [51]. Dehydration involves the evolution of water vapour, and this is, of course, not the case in the starch gelation process described above. However, similarities in the changes in interactions between the molecules in a starch gel at 25°C can be assumed. Collison and Dickson [51] stated that the process of rupturing bonds (mainly hydrogen bonds) between starch and water is endothermic. An increase in hydrogen-bonding interactions between starch molecules is exothermic. Another study by the same authors showed that water-water interactions are weaker than starch-water interactions [52]. Given that the increase in polymer-polymer interactions, manifested by increasing crystallinity of amylose, is a process that takes place during the first 24 h [40], the predominant heat effect from changes in the interactions between molecules in the gel during the first 5–6 h is endothermic, resulting from the disruption of hydrogen bonds when the starch and water phases separate. This endothermic heat lowers the observed exothermic heat from the microcalorimetric measurements, resulting in the relationship, $|\Delta H_{MC}| \leq |\Delta H_{DSC}|$ The endothermic contribution from phase separation depends on the amylose content and botanical source of the different starches [35, 53].

Isothermal microcalorimetric studies on starch-lipid complexation

Recrystallization events in starch-based products are usually not desirable, and the associated aging process can be delayed or sometimes eliminated by addition of certain polar lipids that act as anti-staling agents [54]. To obtain a maximum anti-staling effect, it is important to control the phase behaviour of the polar lipids used [55]. Monoglycerides are widely used in the baking industry. Monoglycerides such as GML, added in a lamellar liquid-crystalline phase, favour formation of more highly ordered complexes with starch, in comparison to the addition of cubic or mixed phases. Positive anti-staling effects are also achieved by the addition of the ionic surfactant SDS, which is commonly used in model systems [56].

The effects of GML and SDS on the retrogradation of maize starch are illustrated in Fig. 5. The thermal power was reduced for the entire first 24 h, and the addition of the polar lipids had a large influence on the initial exponential decay of the *P–t* traces. This indicated that the added lipids had a large influence on the amylose fraction. A constant thermal power was reached within 5 h for the lipid-containing samples, in contrast to the maize starch alone, for which no steady state was achieved during the first 24 h. Our interpretation is that the contribution of amylose crystallization to the measured exothermic enthalpies ceased to predominate after 5 h, in the presence of lipid. This could be attributed to the amount of amylose complexed to the added lipid. The thermal power

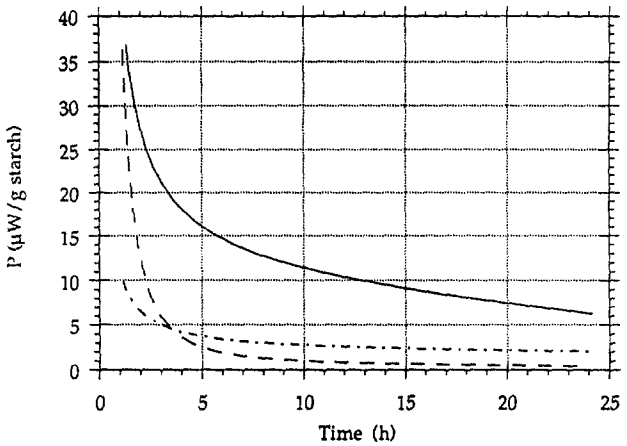

Fig. 5 *P–t* traces from the isothermal microcalorimetric analysis of maize starch with added SDS or GML (2.0% (w/w) on starch basis): —— maize, no added lipids, – – maize, 2.0% GML, ·-· maize, 2.0% SDS

after 24 h was 6.3 μW/g starch without added lipids, 2.1 μW/g starch with SDS, and 0.4 μW/g starch with GML; i.e. in the steady state, GML had a greater effect on the reduction of starch crystallization. The effect of the lipids on the crystallization of both amylose and amylopectin is also seen in the derived enthalpy values given in Table 1. Lower exothermic enthalpies were obtained when SDS or GML was added. In the initial stages of retrogradation, i.e. the first 3–4 h, SDS had a greater reducing effect on the thermal power, compared to that of GML, due to the greater influence of SDS on the amylose

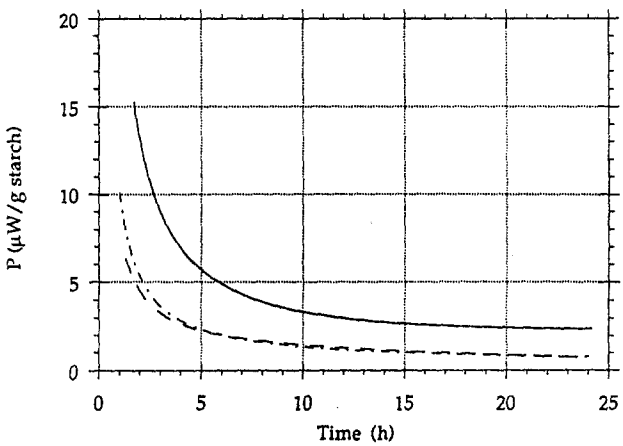

Fig. 6 *P–t* traces from the isothermal microcalorimetric analysis of wheat starch with added SDS or GML (2.0% (w/w) on starch basis): —— wheat, no added lipids, – – wheat, 2.0% GML, ·-· wheat, 2.0% SDS

fraction. This was revealed by the higher exothermic enthalpy obtained for the entire 24 h for the sample with GML.

The reduction in retrogradation when SDS or GML was added to wheat starch is illustrated in Fig. 6. As for maize starch, the effect of lipids on wheat starch was evident during the entire first 24 h, but the time taken for the *P–t* traces to reach constant thermal power was approximately the same, with or without lipid. The thermal power after 24 h was 2.4 μW/g starch without added lipid, 0.8 μW/g starch with SDS, and 0.7 μW/g starch with GML (Fig. 6). It was observed that the differences between SDS and GML, with respect to the

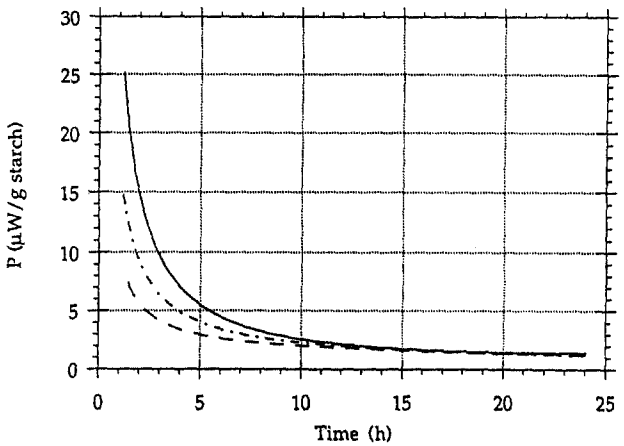

Fig. 7 *P–t* traces from the isothermal microcalorimetric analysis of potato starch with added SDS or GML (2.0% (w/w) on starch basis): —— potato, no added lipids, – – potato, 2.0% GML, -·- potato, 2.0% SDS

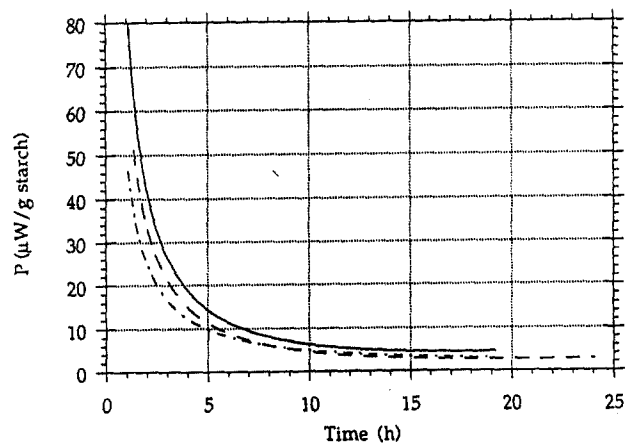

Fig. 8 *P–t* traces from the isothermal microcalorimetric analysis of amylomaize starch with added SDS or GML (2.0% (w/w) on starch basis): —— amylomaize, no added lipids, – – amylomaize, 2.0% GML, -·- amylomaize, 2.0% SDS

effect on crystallization, were small. This is also shown by the calculated exothermic enthalpies in Table 1.

The decay of the *P–t* traces for potato starch with GML or SDS is shown in Fig. 7; the traces differed only in the initial period, i.e. the first 10–12 h. Potato starch without added lipid had the highest value, followed by potato-SDS and then potato-GML. After approximately 12 h, a steady state was reached for the three *P–t* traces, and the thermal power after 24 h was similar, with 1.4 μW/g starch without added lipid, 1.2 μW/g starch with SDS, and 1.2 μW/g starch with GML (Fig. 7). It was remarkable that the lipids had no effect on the crystallization of amylose and amylopectin after 12 h. The retrogradation enthalpies (ΔH_{MC}) for the first 24 h followed the order: no added lipid > SDS > GML (Table 1).

The effects of SDS and GML on the retrogradation of amylomaize are illustrated in Fig. 8. As for the previous starches, the lipids had a reducing effect. Because of the high amount of amylose, the reductions in both thermal power and retrogradation enthalpy were not so dominant. The thermal powers after 19 h were 4.5 μW/g starch with no lipid, 3.0 μW/g starch with SDS, and 2.6 μW/g starch with GML.

As demonstrated above, isothermal microcalorimetry can be a useful complement to other, established methods. The reduction in the exothermic nature of the events when SDS or GML was added, is illustrated in Fig. 9, which shows a linear relationship between the enthalpies, ΔH_{MC}, for GML and SDS and the amylose content, similar to that found for samples without any added lipid. These results also indicated that it was mainly the amylose fraction that was involved in complexation with lipid. The mechanism of formation of amy-

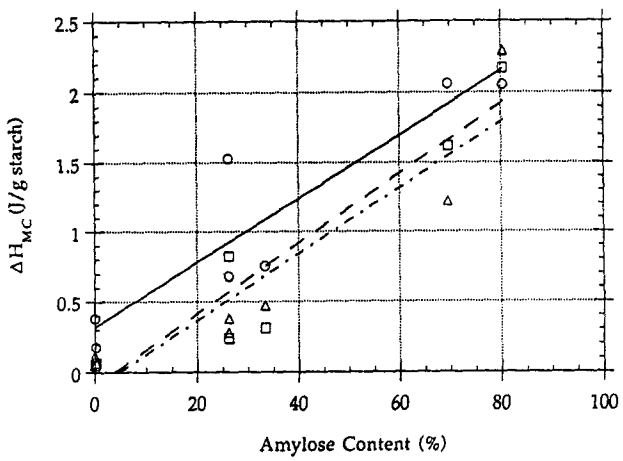

Fig. 9 Exothermic enthalpies from the isothermal microcalorimetry measurements, ΔH_{MC}, as a function of amylose content of the starch samples, when 2.0% (w/w) on starch basis SDS or GML was added; R (no lipids) = 0.91, R (GML) = 0.94, and R (SDS) = 0.93; — no added lipids (circles), – – 2.0% GML (squares), -·- 2.0% SDS (triangles)

lose-lipid complex is related to the amphiphilic character of amylose, owing to its α-(1 → 4)-*D*-glucosidic linkages; the hydrocarbon chain of the monoacyl polar lipid is protected inside the hydrophobic cavity of the amylose coil [20]. The inclusion complexes between starch and lipid are formed when a gelatinized starch dispersion is cooled to room temperature. Pure amylose-GML complexes exhibited an exothermic DSC transition at 68°C on cooling [57]. In a native starch gel, this complexation would result in reduced amylose crystallization. X-ray measurements have shown that a V-pattern, characteristic of single-stranded amylose-lipid complex, is superimposed on the B-pattern originating from the association of double helices during retrogradation [58].

The *P–t* traces for waxy maize-SDS and waxy maize-GML (Fig. 10) indicated reduced thermal power, compared to that for waxy maize with no added polar lipid. As indicated in Fig. 10, a relatively rapid and minor exothermic relaxation process took place during the first 5 h, for waxy maize with or without lipid. The magnitude was, however, much lower than that for amylose. Due to the absence of amylose in waxy maize, this small initial decay cannot be explained by amylose crystallization. The thermal powers after 24 h were 1.9 μW/g starch for waxy maize without added lipid, 0.5 μW/g starch with SDS, and ~0 μW/g starch with GML. A comparison of the enthalpies for waxy maize with or without SDS or GML indicated, from the reduced enthalpy values, that the polar lipids interacted with amylopectin. One possible kind of interaction described in the literature is complex formation, but it has previously been difficult to obtain direct evidence of such interactions [59–61]. It has generally been presumed that the branches of amylopectin are too short to interact with lipids, or that the α-(1 → 6)-*D*-linkages would act as a barrier [62]. However, since the recrystallization of waxy maize is reduced by addition

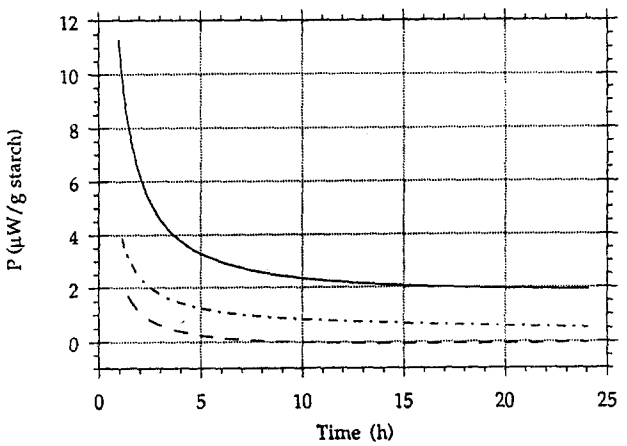

Fig. 10 *P–t* traces from the isothermal microcalorimetric analysis of waxy maize starch with added SDS or GML (2.0% (w/w) on starch basis): —waxy maize, no added lipids, – – waxy maize, 2.0% GML, -·- waxy maize, 2.0% SDS

of certain polar lipids, it is plausible to implicate amylopectin-lipid complexation. If this were the case, one could assume a mechanism similar to that for amylose-lipid complexation. The hydrocarbon chains would be protected in a helical inclusion complex by the chains of amylopectin, and thus it seems likely that at least the outer branches would be capable of this kind of starch-lipid interaction. An amylopectin-SDS complex has recently been reported from microcalorimetric measurements showing very small exothermic enthalpies [30]. The interaction between SDS and purified amylopectin has also been quantified by surface tension measurements, and observed as a distinct shift in the ordinary and apparent critical micelle concentration [63]. Amylopectin-lipid complex has not been detected by X-ray measurements, and does not seem to be joined in any crystalline junction zones. Still, the existence of even a small amount of amylopectin-lipid complex could cause large dislocations in amylopectin clusters and thus disturb the reorganization of the double-helical order of amylopectin; this is believed to be the major, long-term mechanism behind the anti-staling effects of lipids [61, 64, 65].

Conclusions

Isothermal microcalorimetry was found to be a convenient method to study the properties of starch during the first 24 h of retrogradation. The high specific sensitivity of the method made it possible to identify different, simultaneous events taking place in a starch gel during the initial stages of retrogradation. Because of the continuous recording of the thermal power evolved by various starch gels, different profiles, in the form of P–t traces, were obtained for different starches. The appearance of the profiles reflected the amounts of amylose and amylopectin in the starch studied, and integration of these P–t traces gave the negative enthalpy change for the different, ongoing processes collectively known as starch retrogradation. The net enthalpy of reaction for retrogradation during the first 24 h was, as expected, exothermic, and the main contributing factor was crystallization of amylose and amylopectin. Depending on amylose content and botanical variation, the crystallization of amylose in a starch gel was found to predominate over amylopectin crystallization during the first 5–10 h of retrogradation. In this first period, amylopectin crystallized at a much lower, constant rate than did amylose. The extents of association and aggregation of double helices of amylose into more polymer-rich domains were considered to be high in this first period, and after ~10 h, amylose was found to reach a steady state and crystallize at a more constant rate.

When results from the isothermal microcalorimetric measurements were compared to results from corresponding DSC studies, it was found that $|\Delta H_{MC}| \leq |\Delta H_{DSC}|$. The phase separation in an amylose gel, which took place during the first 5–6 h of retrogradation, caused a decrease in polymer-water in-

teractions, i.e. a disruption of the binding forces, mainly hydrogen bonding, between starch and water. It is concluded that this phase separation produced an endothermic heat of reaction and lowered the net exothermic heat observed from the isothermal microcalorimetric studies. The endothermic contribution from the phase separation again depended on the amylose content and botanical source of the starch.

The anti-staling effect of polar lipids and ionic surfactants on starch was demonstrated using isothermal microcalorimetry. The net exothermic heat of reaction for starch retrogradation during the first 24 h was decreased, when GML or SDS was added to the starch gels. The recorded P–t traces also showed how the effect of added lipids influenced different periods during the first 24 h of starch retrogradation, and that the effect depended mainly on amylose content, botanical source of the starch, and type of lipid used. In the initial period, i.e. the first 5–10 h, the crystallization of amylose was reduced to various extents for all the starches studied, and a steady state, in which crystallization was constant, was achieved in a shorter time. This was probably a result of the helical inclusion complex formed between amylose and the added lipid. During the following period, i.e. from 10–24 h, when the crystallization of amylose and amylopectin was constant, maize and wheat starches, but not potato starch, were affected by the addition of lipid. The constant rate of crystallization of the two starch fractions during this later period was decreased for maize and wheat starches. When GML or SDS was added to waxy maize starch, a clear reduction was seen in the exothermic enthalpy measured by isothermal microcalorimetry during the first 24 h of retrogradation. This indicated that amylopectin interacted with the polar lipid. The exact nature of this interaction could not be determined from these microcalorimetric studies, but the possibility of complex formation between amylopectin and polar lipid must be considered as a plausible explanation.

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