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Effects of an added inclusion-amylose complex on the retrogradation of some starches and amylopectin

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The effects of added cetyltrimethylammonium bromide (CTAB)-amylose complex on retrogradation of some starches (waxy-maize, maize, and potato starch) and on amylopectin from potato have been studied by differential scanning calorimetry (DSC). The starches and amylopectin samples with added CTAB-amylose complex received four different heat treatments prior to storage and DSC measurements that either melted the complex or left the complex intact. The calorimetry measurements showed that intact CTAB-amylose complex had much less effect on decreasing the retrogradation of the starches and the amylopectin than samples with melted complex prior to measurements. This is discussed in relation to possible complex formation of amylopectin and lipids and the effects of adding uncomplexed lipids on the retrogradation of waxy starches and amylopectin.

INTRODUCTION

Products containing gelatinized starch undergo changes in texture when stored, owing to the physical changes in the starch. These textural changes are mainly attributed to the retrogradation of the starch, i.e. reordering of the starch into a crystalline state that makes the product less palatable.

It was first suggested by Shoch & French (1947) that the amylopectin component of the starch was responsible for the retrogradation of the starch in bread. This is further supported by findings in differential-scanning-calorimetry (DSC) studies (Eberstein *et al.*, 1980; Russell, 1983; Eliasson, 1985) and X-ray-diffraction studies (Miles *et al.*, 1985; Ring, 1985). The amylose component is considered to play a minor role in the retrogradation of bread, which concerns textural effects, since it recrystallizes very quickly, i.e. within a few hours after the gelatinization of the starch occurs.

It is well known that the addition of monoacyl lipids improves the shelf life of bread (Krog & Jensen, 1970; Lagendijk & Pennings, 1973; Knightly, 1977; Kulp & Ponte, 1981). These substances and other polar and non-polar organic compounds are known to form helical inclusion complexes with amylose (Mikus *et al.*, 1946; Takeo *et al.*, 1973). This complex formation has been used to explain the positive effects of emulsifiers either directly by preventing the amylose molecules from crystallizing (Zobel, 1973; Kulp & Ponte, 1981) or indirectly by changing the water distribution (Pisesook-bunterung & D'Appolonia, 1983). However, since it is the amylopectin and not the amylose that is responsible for the retrogradation of starch in connection with staling of bread, it is therefore not clear how lipids or emulsifiers delay or retard the retrogradation process.

It has been suggested recently that the amylopectin can form inclusion complexes with monoacyl substances and surfactants to some extent (Batres & White, 1986; Slade & Levine, 1987; Eliasson & Ljunger, 1988; Gudmundsson & Eliasson, 1990), and that could explain why, for example, waxy starches and amylopectin are affected by the addition of lipid compounds. However, since monoacyl lipids and surfactants mainly form inclusion complexes with amylose, it is of importance to know whether the intact amylose-lipid complex by itself affects the retrogradation of the amylopectin or if only lipids uncomplexed with amylose can have that effect.

This investigation was undertaken to make it clearer in what state lipids or other complexing agents affect

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the retrogradation of the amylopectin component, i.e. either through interaction with the amylose-lipid complex or directly by affecting the amylopectin. To accomplish this, a cetyltrimethylammonium bromide (CTAB)-amylose complex was added to three different starches (waxy maize, maize, and potato starch) and potato amylopectin. CTAB was used in this research since it easily complexes with amylose. It also does not give rise to any endothermic peak in the temperature interval used that can interfere with the crystallization peak obtained. The starch samples with a CTAB-amylose complex added received four different heat treatments before the DSC measurements, which either left the added complex intact or melted it. The results are compared with the retrogradation behaviour of pure starches and amylopectin under similar conditions.

MATERIALS AND METHODS

Materials

The starches used were commercial samples of maize, waxy maize, and potato (National Starch, Kristianstad Sweden). The amylopectin and amylose used were from potato starch (Sigma, St. Louis, MO, USA) and the cetyltrimethylammonium bromide (CTAB) was provided from BDH Biochemicals (Poole, UK).

Methods

Precipitation of the complex

The CTAB-amylose inclusion complex was precipitated as described by Eliasson (1988) except that the precipitated complex was washed three times with water and air-dried to about 80% dry weight before storage at +4°C. Before using the complex, it was rehydrated to contain 40% dry weight.

DSC measurements

The DSC measurements were performed on a Perkin-Elmer DSC-2 instrument. The retrogradation of waxy maize, maize and potato starches and amylopectin (60% w/w water content) with or without added CTAB-amylose complex (60 mg of 60% w/w water content) was followed and measured as the melting enthalpy (ΔH_c) of the recrystallized starch or amylopectin. Samples (10–20 mg) of the test suspensions or gels were transferred to weighed sample pans, which were then sealed and reweighed. In this study, the sample pans were stored for 1, 2, 3, 7, or 14 days at the temperature of 23°C. All sample pans were heated from 17°C to 147°C at the heating rate of 10°C/min. An empty sample pan was used as reference. The water content of each sample pan was determined after each scan by puncturing the pans and drying them in an oven at 105°C overnight and reweighing. The melting enthalpy of recrystallized starch or amylopectin (ΔH_c) was calculated from the thermograms of the DSC measurements, and the melting enthalpy ($\Delta H_{c\times1}$, $\Delta H_{c\times2}$ and temperature ($T_{c\times1}$, $T_{c\times2}$) of the CTAB-amylose complex and the maize-starch-lipid-amylose complex were also determined. The values given are the means of two or three measurements and calculated on a dry-weight basis.

The starches and the amylopectin with a CTAB-amylose complex added were prepared in four different ways before they were stored in the sample pans and the DSC measurements conducted. These pretreatments were as follows.

(1) The CTAB-amylose complex was added to the dry starches and the amylopectin, and the right amount of water was added to make the water-to-starch ratio 60/40. The suspensions were transferred to sample pans and heated to 100°C in an oven for 15 min.
(2) This was the same as treatment (1) except that the sample pans were heated to 85°C.
(3) The starches and the amylopectin with the right amount of water added were heated to 100°C for 15 min and then cooled to room temperature before the CTAB-amylose complex was added.
(4) This was the same as treatment (3) except that the gel-like mixture was reheated to 100°C.

RESULTS

The CTAB-amylose complex used in this study had a single endotherm at the melting temperature of 96·6°C ± 1·0°C in excess water (80% w/w) and a melting enthalpy of 17·1 ± 0·5 J/g, which is in accordance with findings of Eliasson (1988). Retrogradation of the starches and the amylopectin with or without added CTAB-amylose complex was followed by DSC, by measuring the enthalpy of the endotherm assigned to the melting of crystallized amylopectin (ΔH_c).

In Figs 1 and 2, the retrogradation of waxy maize and maize starch is shown either without any addition or with CTAB-amylose complex added and prepared with the different heat treatments. Results for the potato starch and the amylopectin are not shown since they were very similar to that of maize starch. The waxy starch with the intact CTAB-amylose complex (treatments (2) and 3)) retrograded to a greater extent than untreated waxy starch, and the waxy starch with CTAB-amylose complex that had melted prior to DSC-measurements (treatments (1) and (4)) were the least retrograded. The retrogradation behaviour of maize, potato starch, and amylopectin was similar, since untreated starch and amylopectin retrograded to the greatest extent, and next in order were samples with intact CTAB-amylose complexes (treatments (2) and

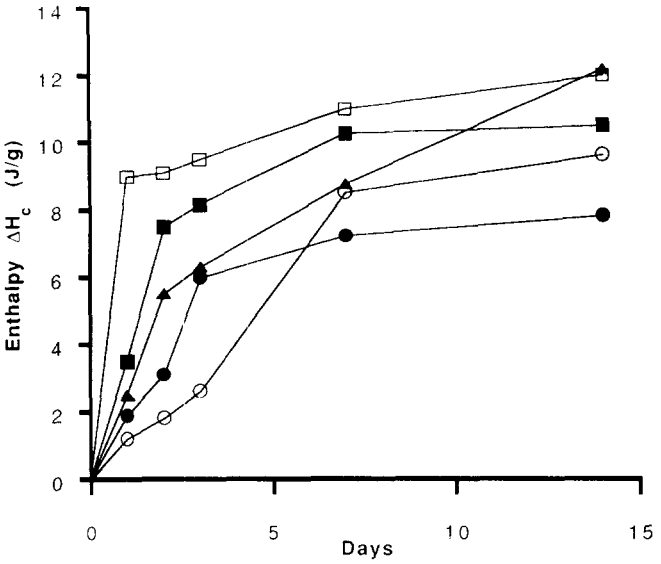

Fig. 1. Retrogradation of waxy-maize starch either untreated or with added CTAB-amylose complex and receiving four different heat treatments: (a) ■, untreated waxy starch; (b) ○, waxy starch with CTAB-amylose complex added (treatment (1)); (c) ▲, waxy starch (treatment (2)); (d) □, waxy starch (treatment (3)); (e) ●, waxy starch (treatment (4)).

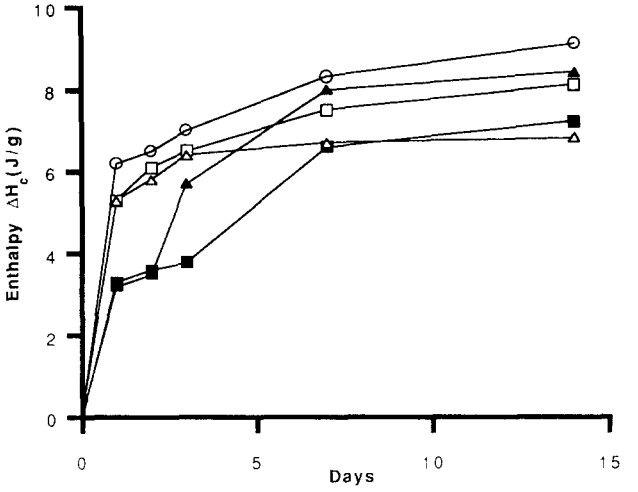

Fig. 2. Retrogradation of maize starch either untreated or with added CTAB-amylose complex and receiving four different heat treatments: (a) ○, maize starch untreated; (b) ■, maize starch (treatment (1)); (c) ▲, maize starch (treatment (2)); (d) □, maize starch (treatment (3)); (e) △, maize starch (treatment (4)).

(3)) and the least-retrograded samples were those that had the complex melted prior to DSC measurements.

In Table 1, the transition of the amylose-lipid complex in maize and CTAB-amylose complex in the different samples is shown. A single endotherm was obtained for all amylopectin, waxy-maize, and potato-starch samples. Two separate complex endotherms were seen for maize-starch samples with added CTAB-amylose complex. The maize starch was the only starch used in this study that had naturally occurring lipids, and that could be the explanation for the two separate complex endotherms obtained. The transition temperature of the complex endotherm of waxy-maize starch and the lower transition-temperature endotherm of maize starch were quite similar to that of the CTAB-amylose complex, but for the potato starch and amylopectin it was slightly higher. A rearrangement occurred in the maize-starch complexes as indicated in

Table 1. Melting enthalpies and temperatures of the lipid and CTAB-amylose complexes in waxy maize, maize, potato starch, and amylopectin

Sample	$\Delta H_{c\times1}$(J/g)	$T_{c\times1}$(°C)	$\Delta H_{c\times2}$(J/g)	$T_{c\times2}$(°C)
Waxy	—	—		
Waxy (treatment 1)	0·7 ± 0·15	96·8 ± 0·8		
Waxy (treatment 2)	0·7 ± 0·3	105·1 ± 2·0		
Waxy (treatment 3)	0·6 ± 0·2	96·3 ± 0·7		
Waxy (treatment 4)	0·6 ± 0·3	100·5 ± 1·5		
Maize	1·8 ± 0·3	102·6 ± 1·5		
Maize (treatment 1)	0·4 ± 0·1	93·3 ± 0·8	3·2 ± 0·3	119·8 ± 1·0
Maize (treatment 2)	3·3 ± 0·2	103·5 ± 1·5	0·6 ± 0·15	122·4 ± 1·5
Maize (treatment 3)	3·0 ± 0·3	96·6 ± 1·0	0·4 ± 0·1	113·6 ± 2·0
Maize (treatment 4)	0·4 ± 0·1	97·1 ± 0·9	2·6 ± 0·4	118·6 ± 1·0
Potato	—	—		
Potato (treatment 1)	0·4 ± 0·15	112·6 ± 2·5		
Potato (treatment 2)	0·5 ± 0·2	110·6 ± 1·5		
Potato (treatment 3)	0·6 ± 0·2	103·8 ± 2·0		
Potato (treatment 4)	0·5 ± 0·2	108·8 ± 2·2		
Amylopectin	—	—		
Amylopectin (1)	0·6 ± 0·2	111·3 ± 1·8		
Amylopectin (2)	0·8 ± 0·3	109·8 ± 2·2		
Amylopectin (3)	0·7 ± 0·3	106·8 ± 2·4		
Amylopectin (4)	1·1 ± 0·3	109·5 ± 1·7		

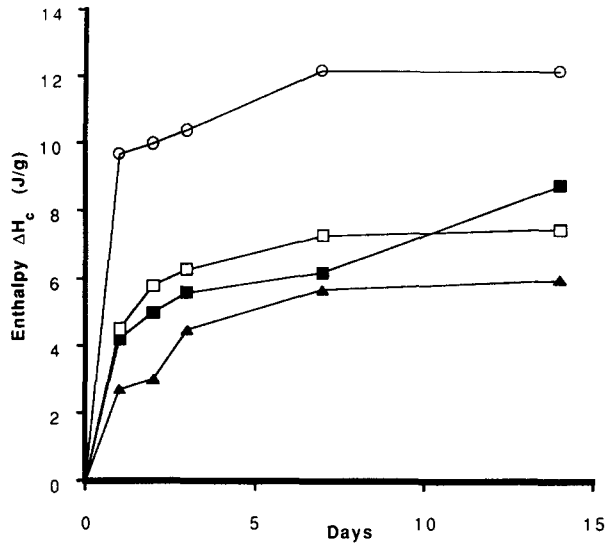

Fig. 3. Retrogradation of untreated amylopectin compared with amylopectin with uncomplexed CTAB added (2%, 3% w/w concentration) and amylopectin (treatment (4)): (a) ○, amylopectin, untreated; (b) □, amylopectin with 2% CTAB added; (c) ▲, amylopectin with 3% CTAB added; (d) ■, amylopectin (treatment (4)).

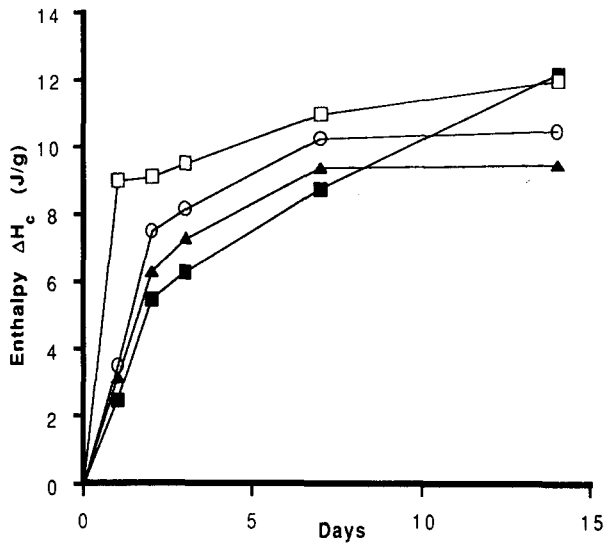

Fig. 4. Retrogradation of waxy-maize starch with intact CTAB-amylose complex (treatments (2) and (3)) compared with the expected dilution effects of the CTAB-amylose complex with untreated waxy-maize starch as reference: (a) ○, waxy-maize starch untreated; (b) ■, waxy-maize starch (treatment (2)); (c) □, waxy-maize starch (treatment (3)); (d) ▲, waxy-maize starch, expected dilution effects.

Table 1. The low-transition-temperature endotherm had a much higher enthalpy value than the high-transition-temperature endotherm for samples with intact CTAB-amylose complex but the order is reversed for samples with the CTAB-amylose complex melted before DSC measurements.

In Fig. 3, the retrogradation of untreated amylopectin is compared with amylopectin that has received heat treatment (4) (melted complex) and amylopectin with uncomplexed CTAB added (in 2 and 3% w/w concentration). The amylopectin with uncomplexed CTAB added retrograded the least, but untreated amylopectin retrograded to the greatest extent, whereas amylopectin with added CTAB-amylose complex was between the two. The amount of CTAB in the complex is equal to 2·4% w/w concentration.

In Figs 4 and 5, the expected dilution effect of the added CTAB-amylose complex on the retrogradation of waxy-maize and maize starches is compared with that for the same starches with added CTAB-amylose complex in intact form (treatments (2) and (3)), with the untreated starches as a reference. The results for the potato starch and amylopectin are not shown, but they were very similar to that of maize. Dilution effects are expected if the intact complex does not interact with the starches or the amylopectin. The added complex was about 12% of the total dry weight, so one can expect that the dilution would decrease the enthalpy values of the starches and amylopectin to about 88% of the values of untreated samples. There is a small difference between the expected dilution effects of the CTAB-amylose complex and the measured effects of the intact CTAB-amylose complex except for waxy-maize starch.

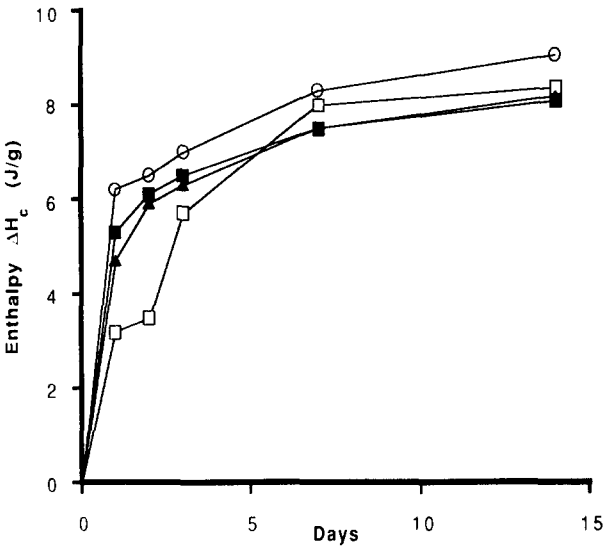

Fig. 5. Retrogradation of maize starch with intact CTAB-amylose complex (treatments (2) and (3)) compared with the expected dilution effects of the CTAB-amylose complex with untreated maize starch as reference: (a) ○, maize starch untreated; (b) □, maize starch (treatment (2)); (c) ■, maize starch (treatment (3)); (d) ▲, maize starch, expected dilution effects.

DISCUSSION

It is well known that the retrogradation of the amylopectin component is responsible for the long-term textural changes of bread and that many kinds of monoacyl and other substances can influence that process. It is therefore important to know the mechanism

behind these effects, i.e. whether the lipids influence the amylopectin directly or indirectly through the amylose-lipid inclusion complex.

As can be seen in Figs 1 and 2, starches with an intact CTAB-amylose complex added (treatments (2) and (3)) retrograded to a greater extent than the same starches which had the CTAB-amylose complex melted prior to storage (treatments (1) and (4)). This shows within reasonable doubt that monoacyl compounds and surfactants have much greater effect on decreasing the retrogradation of the amylopectin component than inclusion complexes. In an effort to explain the effects of lipids on amylopectin, the possibility has been discussed that amylopectin could form a complex, at least to some extent, with lipid compounds (Kugimiya *et al.*, 1980; Batres & White, 1986; Evans, 1986; Slade & Levine 1987; Eliasson & Ljunger, 1988; Gudmundsson & Eliasson, 1990). Such a complex formation would explain why the retrogradation of the amylopectin component is affected by lipid compounds. This has, however, been difficult to confirm for ordinary waxy starches, but, for pure amylopectin in non-granular form with added surfactants, an endotherm measured with DSC has been obtained as well as an X-ray-diffraction pattern of the V type that could be assigned to an amylopectin complex (Gudmundsson & Eliasson, 1990). The amylopectin has much less affinity for lipids and surfactants than amylose. Hahn & Hood (1987) have estimated that a normal maize starch binds about seven times as many lipids as waxy-maize starch (g lipids/g starch). In most cases, the complexing ability of the amylopectin is therefore masked by that of amylose. This could be due to the crystalline state in which the amylopectin is found within starch granules that render it unsusceptible to interaction with lipid compounds. It has been shown by Steeneken (1984) that amylose has much higher etherification reactivity than amylopectin in granule starch, whereas in solution both starch fractions show almost equal degree of substitution. It has also been shown by Sarko and Wu (1978) that crystalline amylose does not form a complex with iodine. Thus, since the amylopectin is in a crystalline state in granular starch and the amylose is in an amorphous state, this could explain to some extent why monoacyl substances and surfactants react more intensively with amylose. However, this cannot be the only explanation, since waxy-maize starch with added surfactants, even after reheating and therefore in an amorphous state, did not show any sign of transition that could be related to a complex between amylopectin and surfactant (Evans, 1986; Eliasson *et al.*, 1988). The explanation that Evans (1986) puts forward could be relevant here. Since linear regions in amylopectin are 20–30 glucose units long according to Harbitz (1983) or 16–20 glucose units long according to Hizukuri (1985), there will probably be room for one or perhaps two alkane chains. French & Murphy (1977) have estimated that the hydrocarbon chain of a monoacyl lipid forms an inclusion complex with three turns of amylose with six glucose residues in each turn. Owing to the short outer branches of amylopectin, the co-operativity of the complex formation is greatly reduced. If a transition endotherm is to be seen in DSC measurements, the enthalpy change must occur over a fairly narrow temperature range, within 20–30°C, and, if the transition is non-co-operative, this is hardly obtained.

Yamamoto *et al.* (1984) have shown that iodine and SDS bind co-operatively to amylose, and the co-operativity increases with an increase in the degree of polymerization of amylose. The most stable complexes of linear amylose with iodine are formed by chains with more than 60 glucose residues. Since some of the outer brances of amylopectin can be as short as 12 glucose residues (Hizukuri, 1985), it is not only non-co-operativity that makes an endotherm due to the amylopectin-lipid complex hard to obtain but also the fact that many linear regions of the amylopectin are unable to form complexes of any form. However, if the amylopectin forms an inclusion complex with monoacyl substances, then it is plausible that amylopectin in the amorphous state is more susceptible to interaction with lipids than amylopectin in the crystalline state.

Since the intact CTAB-amylose complex has much less effect on decreasing the retrogradation than a melted complex or uncomplexed CTAB, it is probable that the complex does not interact with the starches in the intact form but mainly dilutes the starch. The intact complex can therefore be said to have a dilution effect on the starch in most cases without affecting the amylopectin component (except for the waxy-maize starch as shown in Fig. 4), since little difference is observed between the expected dilution effects and the actual effects of the intact complex.

For the waxy-maize starch, the intact CTAB-amylose complex increases the retrogradation as compared with untreated waxy-maize starch. Why this is so is not known. The amylopectin and maize and potato starches with an intact complex added reached nearly the same final retrogradation level as the untreated samples, but the crystallization rate was slower for the first three days. The added complex in these samples must have interfered with the crystallization process of the amylopectin component in the first days of storage, either by steric hindrance or by entanglement between loose amylose segments from the complex and the amylopectin. This kind of interaction is only seen for waxy-maize starch that received treatment (2) but not treatment (3).

The melted complex (treatment (4)) has less effect on decreasing the retrogradation of amylopectin than added CTAB (Fig. 3). Some of the added CTAB-amylose complex, which was melted in the heat treatment had probably reformed during storage so

the CTAB had less opportunity to affect the amylopectin, than when it is added uncomplexed.

The difference between heat treatments that have similar effect, i.e. melt or leave the CTAB-amylose complex intact, can be considerable. Treatments (1) and (4), which both melt the CTAB-amylose complex, give different results, since samples that received treatment (4) retrograde less than samples receiving treatment (1). In treatment (4), the starch and the amylopectin are treated with heat before the complex is added and then reheated, so the starch is already in an amorphous-gel state when the complex melts, and thus the starch is more susceptible to interaction with the CTAB. In treatment (1), on the other hand, the starch and the complex are heated together simultaneously. Treatments (2) and (3), which left the complex intact, give quite similar results, though treatment (2) usually results in slightly lower enthalpy values, maybe because some of the least stable complex has melted during the heat treatment.

As may be seen in Table 1, the complex has only one form, i.e. one endothermic transition is seen, except for maize starch, which has two separate transitions for all heat treatments. However, these two separate complexes and their rearrangements do not seem to affect the retrogradation results because a similar effect of heat treatments on the retrogradation was found for potato starch.

It has been speculated that amylose can co-crystallize with amylopectin to some extent (Russell, 1987), and the effects of added lipids or surfactants should be to form a complex with the amylose, so that it cannot take part in the crystallization process. Schierbaum *et al.* (1986) have also observed that rapidly aggregating amylose can co-operatively interact with linear segments of amylopectin. From our previous studies, it is known that the amylose component can affect the retrogradation of the amylopectin if the amylose is over 50% w/w (Gudmundsson & Eliasson, 1990). In this study, the additional amylose in the form of a CTAB-amylose complex did not exceed 10% of the total weight, so the influence of the additional amylose is judged to be minimal.

CONCLUSION

Lipids and surfactants have their greatest effect on the retrogradation of starches when added in uncomplexed form but are most ineffective when they are added in the form of an intact amylose-inclusion complex. These findings give further support for the opinion that lipids, surfactants, or other monoacyl substances can directly interact with the amylopectin component and in that way decrease the retrogradation.

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