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Mobility of lipid in complexes of amylose–fatty acids by deuterium and ^{13}C solid state NMR

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Abstract

Palmitic and lauric acid complexes with amylose were studied by solid state methods: ^{13}C CP/MAS NMR, deuterium NMR, X-ray powder diffraction and differential scanning calorimetry (DSC). The crystalline amylose complexes were found to be in a V-type sixfold single chain helix. The melting points of the complexes were over 100°C, at least 40–50°C higher than the melting points of the free fatty acids. CP/MAS ^{13}C NMR spectra revealed two resonance peaks at 33.6 and 32.4 ppm for the palmitic acid, which were assigned as free and complexed fatty acid, respectively. A single resonance peak at 32.4 ppm was found for the lauric acid and assigned to the complex. The chemical shift of 32.4 ppm for the complexed fatty acids suggests a combined *trans* and *gauche* conformation for the fatty acid chain in the complex. T_1 relaxation measurements on the two palmitic acid resonances show different behavior: a very slow relaxation for the 33.6 ppm and a much faster relaxation (1.2 s) for the 32.4 ppm resonances. The latter was similar to the relaxation of the single resonance of the lauric acid (1.1 s). Temperature dependent deuterium spectra of the amylose–lauric acid and amylose–palmitic acid complexes suggest a complete complexation for the amylose–lauric acid, whereas the amylose–palmitic acid complex is partially disassociated by the thermal treatment. Based on the overall data, a partially disordered model is proposed: an imperfect helix with the fatty acid partly inside and partly out, depending on crystallization conditions and the necessity of placing the carboxyl head outside the V-helix.

Keywords: Amylose–fatty acids; Deuterium; Solid state NMR; Complex

1. Introduction

High amylose starch hybrids are known for their relatively greater content of fatty acid (Morrison, 1995) compared with the normal product. The exact state of the fatty acid as found in these starches, either nascent or after treatment such as gelatinization, has been the object of considerable study (Biliaderis, Page, Maurice & Juliano, 1986; Donovan 1979; Hoover & Vasanthan, 1994; Kulp & Lorenz, 1981; Russel, 1987). Solid state methods: ^{13}C CP/MAS NMR, X-ray powder diffraction and DSC (differential scanning calorimetry), suggest that the crystalline state of the amylose–fatty acid complex involves the V-amylose sixfold single chain left-handed helix, well-known among starch polymorphs, which are described in a recent review of starch structure (Buleon, Colonna, Planchot & Ball, 1998). Accordingly, it is often assumed that the fatty acid is a "stem" (planar zig-zag) inside the helix whose inner surface is hydrophobic because of the carbon–hydrogen matrix provided by the helically wound $\alpha(1-4)$ glucan (Godet, Tran, Delage & Buleon, 1993a). Given the pitch of the sixfold helix and the planar zig-zag geometry of the fatty acid, about 10% by weight of the latter would suffice to create 100% complexation. Since the head group of the fatty acid does not fit into the classical V-helix channel (Godet et al., 1993a), dislocations may be created, thus producing some disorder compared with an ideal crystal. Cereal starches contain from 0.54 to 1.3% lipid, which is present as free fatty acids or as lisophospholipids in a proportion characteristic of each plant species (Buleon et al., 1998). Their location and influence on starch crystalline texture, including the chainfold structure of lamellar amylose crystals, remains unknown.

A recent ^{13}C CP/MAS NMR study has identified a resonance at 31.7 ppm for samples of amylose–fatty acid complexes (Snape, Morrison, Maroto-Valer, Karkalas & Pethrick, 1998). This is characterized by a relaxation behavior for the fatty acid in the complex, compared with solid fatty acids in a physical mixture with amylose. The middle CH_2 in the physical mixture was found to relax five times

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more slowly than the CH_2 of the fatty acid in the complex. The 31.7 ppm resonance was taken as a signature of the amylose–fatty acid complex, which would suggest that the CH_2 sequences present a disordered arrangement (Snape et al., 1998). This conclusion is reasonable, given the polyethylene ^{13}C CP/MAS NMR result for semicrystalline material, which presents distinct peaks at 34.1 and 30.9 ppm, assigned to CH_2 groups in crystalline and noncrystalline domains (Earl & VanderHart, 1979), respectively. The postulate of a disordered structure for the complex prompted us to examine the deuterium NMR spectra of amylose–fatty acid complexes since such a spectrum usually reveals details of motional freedom (Fyfe, 1983).

This study involves the preparation of amylose–fatty acid complexes and their characterization by ^{13}C CP/MAS NMR and T_1 measurements. Samples of the complexes of both high and low molecular weight amylose were prepared. Deuterium NMR studies on completely deuterated fatty acids in the complexes are included in the study.

2. Experimental

2.1. Materials

Amylose of DP900 from AVEBE, The Netherlands, was used. Its polydispersity was 1.8. Another sample of amylose was prepared by HCl hydrolysis (2.2 N) of wrinkled pea starch at 35°C for 35 days; amylose of DP30 was obtained by fractionation and crystallization.

Caprylic (C8), lauric (C12) and palmitic (C16) were purchased from Aldrich Chemie, Germany. Deuterated lauric acid (U-D23, 98%) was also purchased from Aldrich Chemie, Germany. Deuterated palmitic acid (U-D31 99%) was kindly provided by Dr Perly, France.

2.2. Sample preparations

Amylose–fatty acid complexes were prepared according to the method of Eliasson and Krog (1985). An amylose solution (60 mg/ml) was prepared using 95% DMSO while heating at 80°C for 30 min. At a temperature of 90°C, 60 mg of fatty acid which had been dissolved in 5 ml of DMSO was added to 10 ml of amylose solution and the heating was maintained for 4 h. Following this, 25 ml of water at 90°C was added to the mixture, which was then cooled to 20°C with downward ramping at 5°C/h. At the end of 24 h, the complex was centrifuged (Godet, Buleon, Tran & Colonna, 1993b), washed in a mixture of water/ethanol (50/50 v/v) and allowed to air dry, referred to as: "as-synthesized". This procedure was used to prepare complexes with amyloses with different degrees of polymerization for all three fatty acid samples. Also, with DP900 amylose, a set of complexes with decreasing amounts of fatty acid was prepared (10, 7, 5, 2 and 1%).

2.3. X-ray diffraction

X-ray diffraction data were collected using a diffractometer which operated in a transmission mode and was powered by an Inel (France) generator operated at 40 KV and 30 mA. A copper X-ray tube was used, along with a quartz monochrometer, which provided a CuKα1 beam, which was monochromatized using a bent crystal. The sample was surrounded by aluminum foil in order to maintain the moisture content. The full diffraction curves were recorded over a period of 2 h using 50 mg of sample conditioned at 75% relative humidity.

2.4. Differential scanning calorimeter (DSC)

DSC measurements were performed using a SETERAM DSC 121 instrument. The sample size was 20 mg to which 80 mg of water was added and the reference sample was 100 mg of water. Sample and reference were placed in specially designed capsules, which withstand high pressures thanks to aluminum seals. Temperature scans ranged from 20 to 200°C with a 3°C/min ramping.

2.5. Solid-state NMR

Solid-state NMR experiments were conducted on a Chemagnetics CMX-300 instrument operating at 75.4 MHz for the ^{13}C nucleus and 46.0 MHz for the ^{2}H nucleus (deuterium). The recycle delay for the ^{13}C CP/MAS experiment was 3 s, MAS spectra were accumulated with a spinning rate of approx. 3.5–4.5 KHz, a cross-polarization time of 3 ms and a line-broadening of 20 Hz was used. The T_1 experiment was done with the Torchia pulse sequence (Fyfe, 1983), a 4 s recycle delay was used, with T_1 delays (τ values) of 50 ms up to 1.6 s for the lauric acid complex and 100 ms up to 4 s for the palmitic acid complex. The deuterium experiments were done with quadrupolar echo sequences (Fyfe, 1983), with recycle delays varying from 500 ms up to 60 s. For the temperature dependent deuterium experiments, the recycle delays were 500 ms up to 2 s for the palmitic acid complex, and 4 s for the lauric acid complex. All spectra are processed with at least double the amount of data points by zero filling.

Spectra were recorded for "as-synthesized" samples and for the same samples "humidified" by a 72 h exposure in a desiccator at 100% relative humidity.

3. Results and discussions

3.1. X-ray analysis

X-ray diffractometer scans (Fig. 1) on all of the complexes show three main reflections at $2\theta = 7.45°$, 12.9° and 19.84°, all of which are characteristic of the V_h polymorph of amylose (Buleon et al., 1998). A number of weaker reflections were found and correspond to

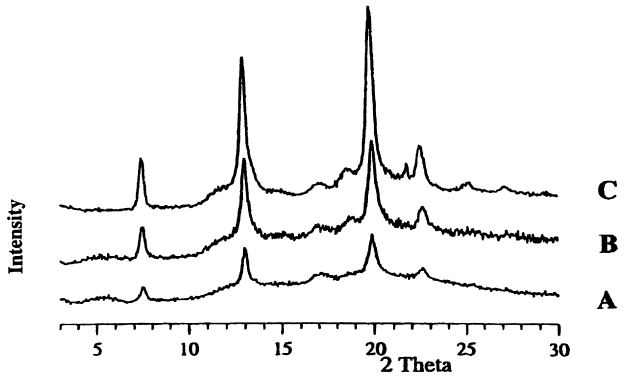

Fig. 1. X-ray powder diffraction patterns obtained from: (A) amylose–caprylic acid complex, (B) amylose–lauric acid complex and (C) amylose–palmitic acid complex.

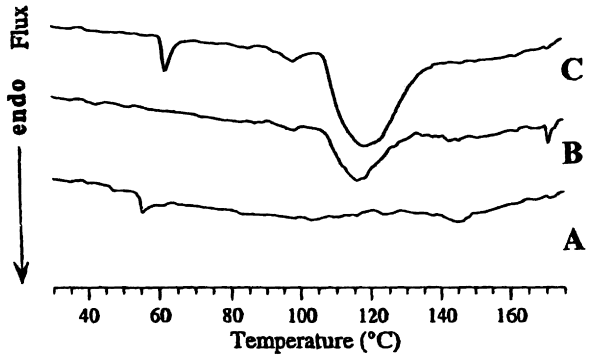

Fig. 2. Differential scanning calorimetry (DSC) thermograms of high molecular weight amylose complexes: (A) amylose–caprylic acid complex, (B) amylose–lauric acid complex and (C) amylose–palmitic acid complex.

$2\theta = 9.88°$, 17°, 18.62° and 22.55°. These diffractograms show very little background scatter and narrow peaks, which demonstrates that the samples themselves are of high crystallinity with rather large crystallites. Line broadening varies from one sample to the other but is greatest for the C-16 samples (Godet et al., 1993a). Nevertheless, it can be said that all three fatty acids generate the same V_h amylose polymorph. However, the palmitic acid complexes display a sharp peak at 22°, which corresponds to pure crystalline fatty acid, as also demonstrated by the endotherm in the DSC spectrum as 62°C, corresponding to the melting of palmitic acid itself.

3.2. DSC analysis

Fig. 2 shows that the melting endotherm for the complexes are found between 100 and 140°C and they are broad, suggesting a poor degree of order. The C8 sample (in the higher degree of polymerization) failed to display the presence of a complex. The higher molecular weight amylose complexes show a slightly greater endotherm than the DP30 complexes (not shown). The enthalpy of fusion of the complexes made with the high molecular weight amylose increases with the length of the fatty acid chain. Finally, the thermogram of DP 900-C8 did not display an observable endotherm and no further study was performed on this material.

3.3. C CP/MAS nmr

The ^{13}C CP/MAS spectra of complexes with palmitic and lauric acid are shown in Fig. 3. Traces of ethanol (58.4 and 19.0 ppm) from the preparation washing procedure and the 31–32 ppm signature resonance of the fatty acid are present, along with the usual resonances for V-amylose (Veregin, Fyfe & Marchessault, 1987). The V-polymorph of starch is the one always found for the complexes (Buleon et al., 1998). An analytical method for fatty acid determination based on methylation of the fatty acid was used (Morisson & Smith, 1964; Wolff & Fabien, 1989) to demonstrate that the complexes studied had about 10% fatty acid content.

In Fig. 3, several of the resonances are caused by the fatty acid. The band assignments for these materials are well known and are included in Table 1. The resonance in the 31–32 ppm range is a characteristic of the lipid complex (Snape et al., 1998), but this resonance was not observed in the spectrum of the amylose–caprylic complex, which confirms our conclusion from the DSC measurements that the complex did not form under our synthesis conditions for the 900-C8 sample. Godet et al. (1993a) confirmed the

Table 1
T_1 results (in s) and chemical shifts for fatty acid–amylose complexes and crystalline fatty acids. * = does not exist

Sample	Resonance and chemical shift					
	HOO**C**–CH_2– (~35.5 ppm)	HOOC–CH_2– **C**H_2– (~34.6 ppm)	–(**C**H_2)$_n$– (~33.6 ppm)	–**C**H_2–CH_2–CH_3 (~32.4 ppm)	–CH_2–**C**H_2–CH_3 (25.5 ppm)	–CH_2–CH_2–**C**H_3 (15.5 ppm)
Crystal lauric acid treated as the complex	73 ± 9	N/A	60 ± 3	122 ± 19	53 ± 5	1.0 ± 0.1
Crystal palmitic acid treated as the complex	N/A	N/A	208 ± 19	N/A	76 ± 7	1.4 ± 0.1
Lauric acid DP900-C12 complex	N/A	1.5 ± 0.2	*	1.1 ± 0.1 (–(CH_2)$_n$– complex at 32.4 ppm)	2.2 ± 0.2	2.2 ± 0.1
Palmitic acid DP900-C16 complex + free fatty acid	N/A	N/A	Cannot be measured (free acid) at 33.6 ppm	1.2 ± 0.1 (–(CH_2)$_n$– complex at 32.4 ppm)	2.9 ± 0.2	2.0 ± 0.1

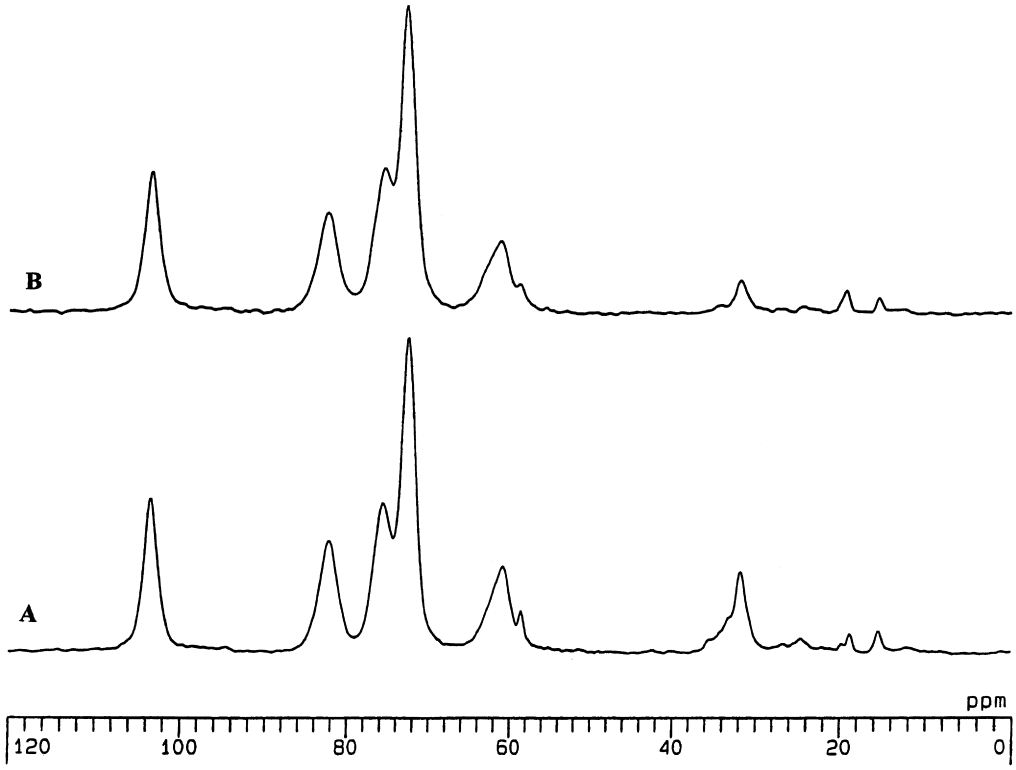

Fig. 3. ^{13}C CP/MAS NMR spectra (0–120 ppm) of: (A) amylose–palmitic acid complex and (B) amylose–lauric acid complex.

absence of caprylic acid under these conditions by chemical analysis. The relative intensity of the 31–32 ppm peak is greatest for the amylose–palmitic complex, although this is most probably because of the conditions of recording the spectra rather than that of quantitative significance.

Examining the amylose–palmitic acid complex (Fig. 3), one observes peaks at 33.6 and 32.4 ppm resonance. According to Earl and VanderHart (1979) the 34 ppm resonance corresponds to CH_2 sequences in a planar zig-zag arrangement for crystalline polyethylene. We have crystallized a pure palmitic acid sample under the conditions equivalent to those used to prepare the amylose–palmitic acid complex, but in the absence of amylose, and the main resonance at 34 ppm was observed. We conclude that these two resonances (31–32 ppm and 33–34 ppm) indicate the presence of the complex and free fatty acid, respectively. This is not the case for the amylose–lauric acid complex where a single 31–32 ppm resonance was observed.

In order to better understand the partial ordering of fatty acid–amylose complexes, samples of complexes with decreasing amounts of palmitic acid were studied. A set of five samples was prepared, containing 10, 7, 5, 2 and 1% palmitic acid (by weight). The recorded ^{13}C solid-state CP/MAS NMR spectra are shown in Figs. 4 and 5 for the "as-synthesized" (procedure in experimental section) and "humidified" samples.

The palmitic acid complex with amylose gives rise to a major resonance at ~32 ppm, and the 10, 7 and 5% spectra are similar, as can be seen in Figs. 4 and 5. (The 10% palmitic acid sample having a shoulder at ~34ppm was discussed above.) The 103 ppm resonance corresponding to the C(1) V-single helix of amylose is the only amylose polymorph present until the palmitic acid content decreases to 2% (Fig. 4D,E). There the 32 ppm resonance decreases sharply and the 103–100 ppm resonance shows the combined presence of V- and B-amylose signals. The intensity of the DMSO signal at 40 ppm suggests that a complex of amylose with DMSO may be present. The disappearance of the signal in the "humidified" spectra (Fig. 5) while leaving the V-signal at 103 ppm unchanged can be explained as follows: the water penetrating in the "humidifying" process can extract the DMSO and either replace it or not, leaving the helix intact. The resolution of the "as-synthesized" spectra changes with the fatty acid content. The resolution changes with decreasing fatty acid content from 5 to 1% with the latter spectrum (Fig. 4E), rather similar to "as-received" high amylose starches that contain a similar amount of fatty acid (LeBail, Morin & Marchessault, 1999).

The "as-synthesized" spectra (Fig. 4) are somewhat similar to the "humidified" spectra (Fig. 5) but the DMSO and ethanol resonances at 40 and 58 ppm have disappeared. The degree of order (resolution) is improved and the ratio of V-to-B, as seen for C(1), has changed for the 1 and 2% samples because of the increasing amount of B polymorph.

As mentioned before, there is a presence of the free fatty acid in the 10% sample (the 33.6 ppm shoulder seen in the NMR spectra (Figs. 3A and 4A)). Although, theoretically, 10% palmitic acid by weight is the required amount to

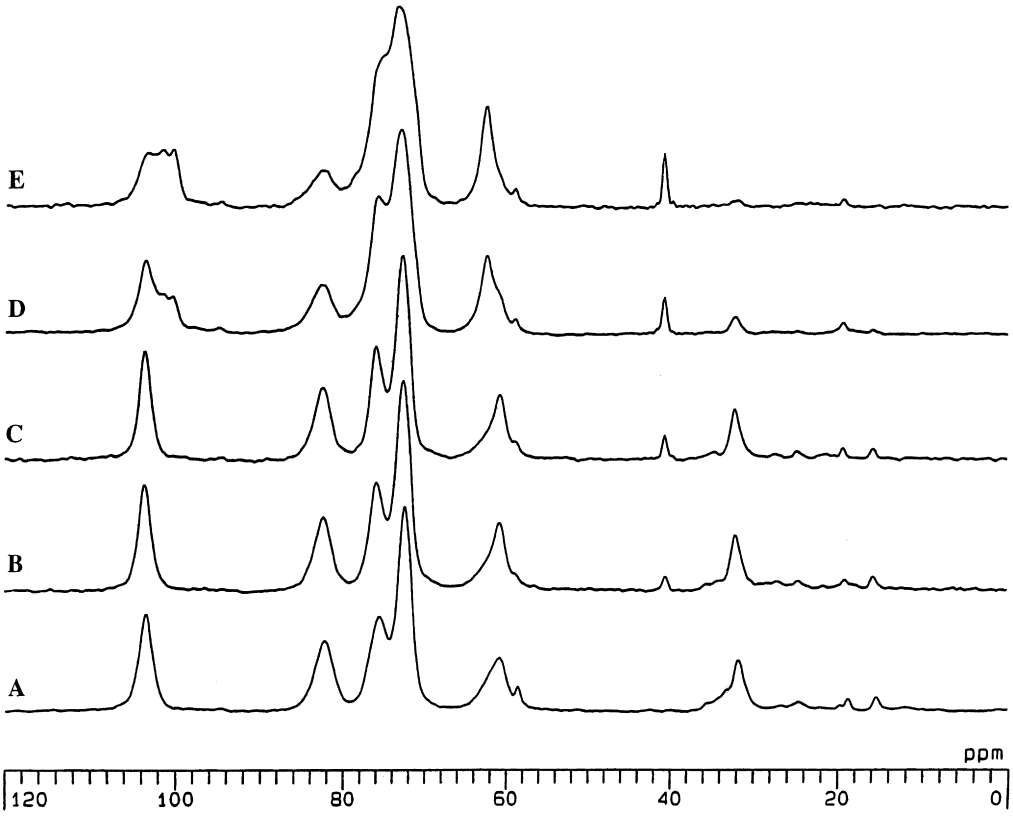

Fig. 4. Synthesized ^{13}C CP/MAS NMR spectra (0–120 ppm) of amylose–palmitic acid complexes: (A) 10%, (B) 7%, (C) 5%, (D) 2%, and (E) 1%.

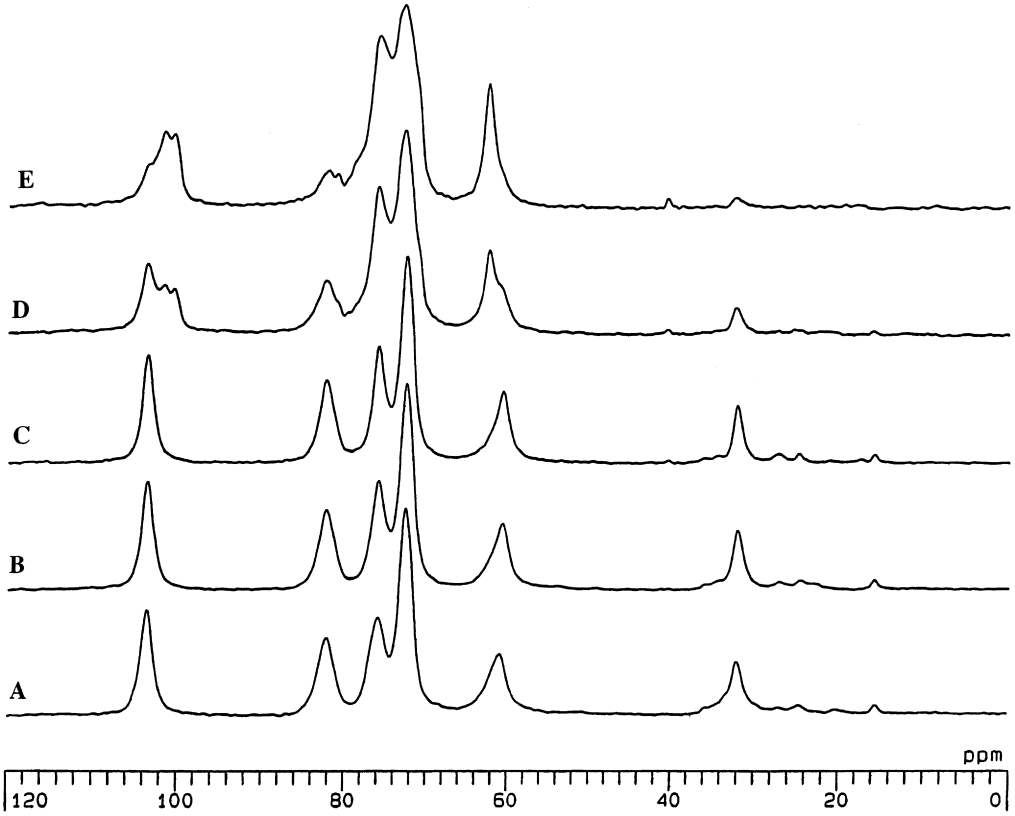

Fig. 5. Humidified ^{13}C CP/MAS NMR spectra (0–120 ppm) of amylose–palmitic acid complexes: (A) 10%, (B) 7%, (C) 5%, (D) 2%, and (E) 1%.

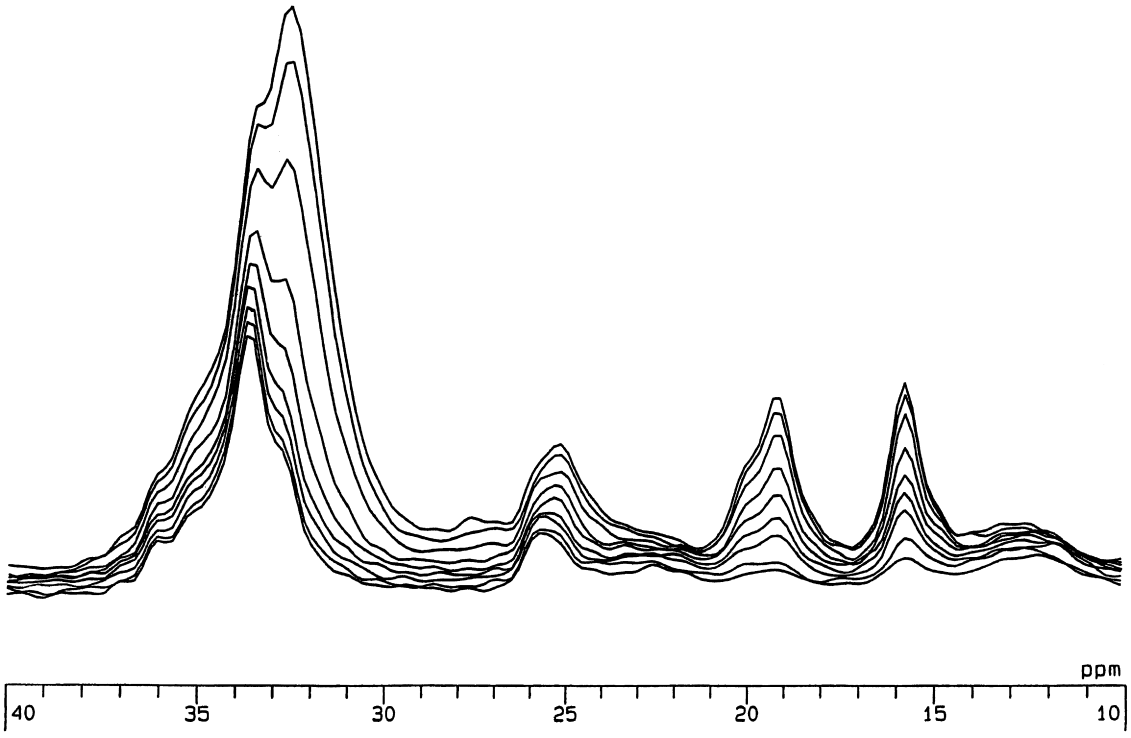

Fig. 6. ^{13}C CP/MAS T_1 NMR spectra (10–40 ppm) of amylose–palmitic acid complex measured at 100 ms (top), 200 ms, 400 ms, 800 ms, 1.2 s, 1.6 s, 2 s, 3 s, and 4 s (bottom).

complex all the amylose, it is unlikely, because of kinetic and steric factors, that complexation would be 100%. The excess free fatty acid can be trapped by the amylose without being complexed (between the long polymer chains) during the synthesis. This is less likely to happen when there is no excess of fatty acid (7% by weight or less).

The B-amylose is detected in the 1 and 2% spectra because there is not enough fatty acid to seed 100% V-crystallization. The size of the B-amylose peak is noteworthy when reducing the amount of fatty acid to less then 5% (Fig. 4D,E) or by humidifying the sample (Fig. 5D,E). PeakFit analysis (PeakFit™, v3.0[1]) of the V-to-B ratio for the 2 and 1% samples are: 1% "as-synthesized"— 48:52, 1% "humidified"— 29:71, 2% "as-synthesized"— 75:25, 2% "humidified"— 54:46. These observations, together with the change in resolution of the spectra, can be explained as follows: decreasing the fatty acid content hinders the crystallization of V-type amylose, but below 5%, palmitic acid V- and B-polymorphs compete and a mixture of single and double helices are formed. This competition has the effect of reducing the overall crystalline order in the sample.

Crystallographic studies with models of the amylose fatty acid complexes are needed to better understand the extent to which studies such as these truly mimic the role of fatty acids in baked products. The V-polymorph of amylose, if truly complexed with the fatty acid as shown in our model (Fig. 8), should be detectable by model crystallographic studies, which have been ongoing elsewhere (Steiner & Saenger, 1998).

3.4. T_1 experiments

The relaxation study was based on the amylose–lipid relaxation spectra shown in Figs. 6 and 7 for the palmitic and lauric acid complexes, respectively. The samples studied were "as-purified" from ethanol, although H_2O vapour humidified samples yield very similar spectra. Table 1 summarizes the T_1 data for the lauric and palmitic samples as complex and as pure crystalline material. The relaxation delay (T_1) for the complexes was calculated from the spectral data with the PeakFit program (PeakFit™ v3.0) using the Voigt method (a linear combination of Gaussian and Lorentzian curves).

The chemical shift for the fatty acid as a complex with amylose (Snape et al., 1998) is approximately 32.4 ppm. The reported T_1 is 0.8–0.9 s for the complexed fatty acid, and 5 s for a fatty acid–starch physical mixture (Snape et al., 1998) (equivalent to the free fatty acid). Our results (Table 1) for the air dry materials, are inconclusive for the 33.6 ppm peak and 1.2 s for the 32.4 ppm peak in the palmitic acid complex, and 1.1 s for the peak at 32.4 ppm for the lauric acid complex.

For the lauric acid complex, our calculation used data from the 50 ms–1.6 s spectra. For the palmitic acid complex

[1] PeakFit™ v3.0 software©, Labtronics Inc. 75 Rickson Avenue, Guelph, Ontario, Canada N1G 3B6.

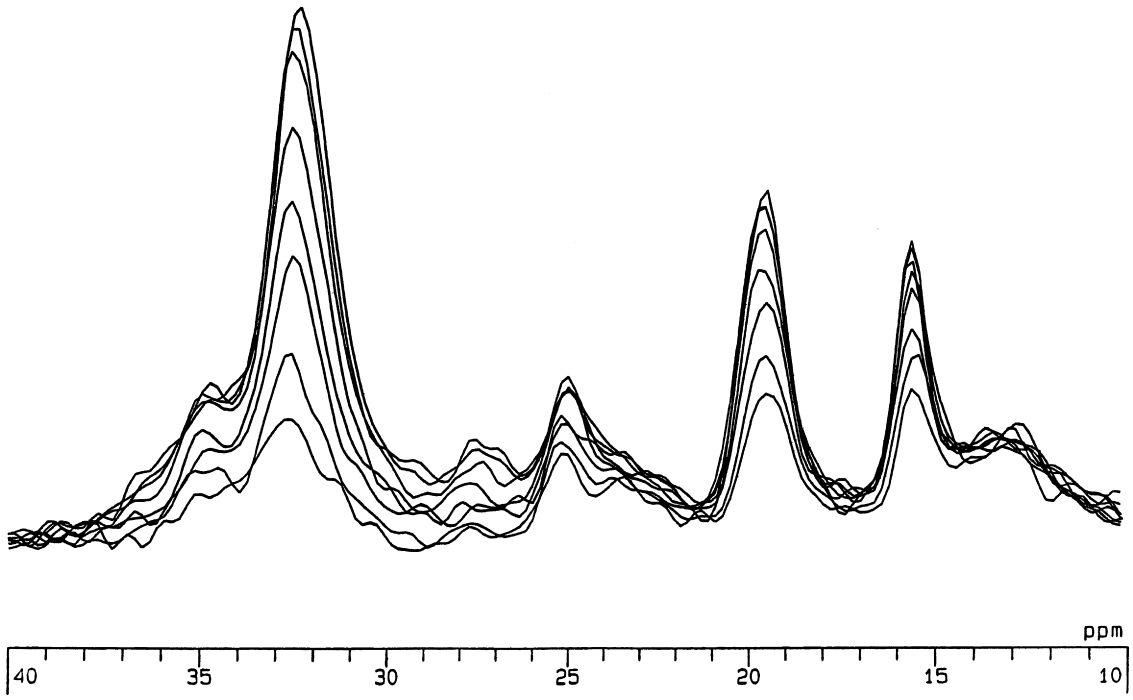

Fig. 7. ^{13}C CP/MAS T_1 NMR spectra (10–40 ppm) of amylose–lauric acid complex measured at 50 ms (top), 100 ms, 200 ms, 400 ms, 600 ms, 800 ms, 1.2 s, and 1.6 s (bottom).

the calculation used data from 100 ms–2 s only. We exclude the data from the 3 and 4 s spectra in order to get a meaningful result (Those two last spectral data use τ values that are 2.5 times and more that of the calculated T_1, and the intensity of the peak is below 10% of the original 50 ms data). An attempt to calculate the non-complex fatty acid at 33.6 ppm failed, even with higher τ values (up to 20 s). The relaxation delay is either very long (similar to the crystalline data, in the hundreds of seconds region), or the very low intensity signals (small amounts of non-crystalline material) do not give as good a representation of reality.

These results are consistent with our characterization data indicating a high level of complexation for C12 samples, and confirm the results in the literature, which did not actually show the spectra from which these data were derived (Snape et al., 1998). The presence of two distinct peaks for the palmitic acid (Fig. 6) compared with the one peak for the lauric acid (Fig. 7), suggests that the palmitic acid complex has either two different types of fatty acids (e.g. complexed and non-complexed), or the complexation is not perfect, part of the fatty acid is outside the V-amylose helix. As for the lauric acid, the fact that only one peak is observed having the same T_1 relaxation value as reported by Snape et al. (1998), suggests a near fully complexed fatty acid, i.e. the "stem" minus the carboxyl head is entirely inside the V-helix.

Computer modelling of the amylose–fatty acid complex demonstrated that the "all-*trans*" segment for the fatty acid can fit into the helix (Godet et al., 1993a). Accordingly, we suggest a model for the complex where the fatty acid is not completely inside the helix, but partly outside, either free or complexed. When the complex forms, the amylose polymer winds around the fatty acid and although the "all-*trans*" conformation is energetically favoured (global minimum), the energy difference between that of "all-*trans*" and a semi-random "*trans–gauche*" conformation is small, hence a kinetic compromise is likely. The result is an imperfect helix with the fatty acid partly inside, partly out, depending

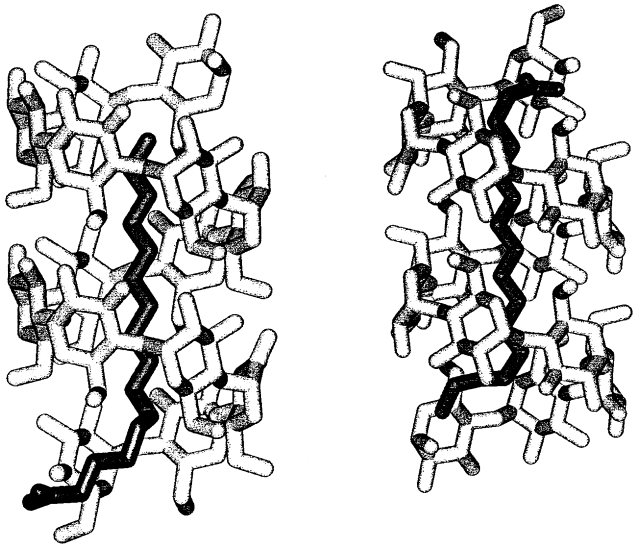

Fig. 8. A model of fatty acid complexed with V-type amylose. Two possible fatty acid conformations where the "*trans–trans*" part is inside the helix, and the "*gauche–trans*" part is outside.

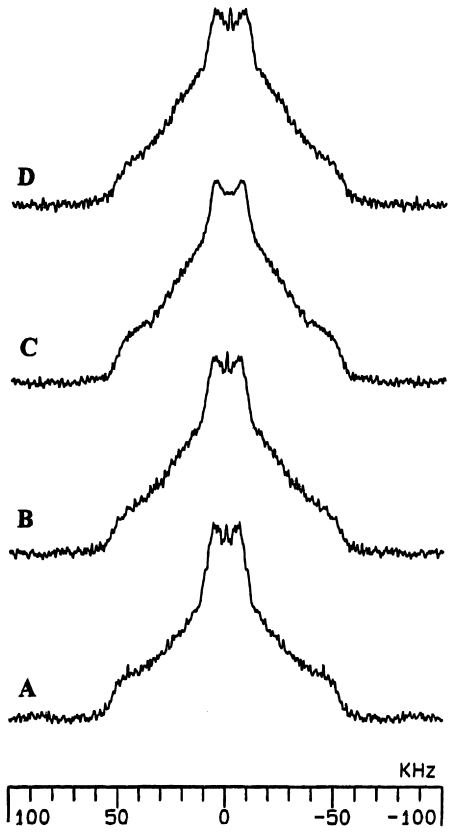

Fig. 9. Temperature dependence deuterium NMR spectra of amylose–lauric acid complex measured at: (A) 23°C, (B) 35°C, (C) 50°C and (D) 65°C.

on crystallization conditions and the necessity of placing the carboxyl head outside the V-helix. Fig. 8 shows models of partial inclusion of the aliphatic segment of fatty acid in the cavity of the V-amylose helix; two possible locations of "*trans–trans*" segments and "*trans–gauche*" segments along the fatty acid are shown.

3.5. Deuterium NMR

Fig. 9 shows a typical deuterium NMR spectrum for the amylose–lauric acid complex, which was recorded at several temperatures. Evidence for what may be a very small amount of free lipid is the minor isotropic resonance in the center (Fyfe, 1983; Horii, 1998). The pyramidal shape of the spectrum is the expected pattern for the fully deuterated fatty acid (Fyfe, 1983). The narrow Pake doublet, corresponding to the CH_3 group, remained the same at temperatures as high as 65°C, which is well above the melting temperature of lauric acid (M.P. = 40°C), but below the observed melting point of the complex (approx. 120°C). We recommend the recording of the deuterium NMR spectrum above and below the melting point of the fatty acid as a demonstration of the presence of the amylose–fatty acid complex. When some free lipid is present, a collapse of the multiple peak doublets above the melting point of the pure lipid is observed, as in Fig. 10 for the amylose–palmitic acid complex (M.P. = 62°C). In Fig. 10, the series of spectra on the right side were recorded after the 70°C spectrum on the left. Clearly the complex is only partially disordered by the thermal treatment above the melting temperature of the fatty acid and the complex, as shown by the shoulder in the temperature-dependent spectra (Fig. 10). Deuterium spectra of "all-deuterated" crystalline palmitic acid at different temperatures (23°C up to 70°C) was measured (spectra not shown). Above the melting point of the fatty acid (70°C), the collapse of the multiple Pake doublets was observed, this time with no shoulders, confirming that the shoulders observed in the amylose–palmitic acid complex spectra belong to the complex.

Another experiment (Fig. 11) on the amylose–palmitic acid complex involved changing the recycle delay time from 500 ms to 60 s. At a recycle delay of 500 ms, the spectrum has only the fastest relaxing component (the complex) and at a recycle delay of 60 s the outer part (less-mobile part) (Fyfe, 1983; Horii, 1998) is clearly shown, indicating that there is more than the complex in this material, probably free fatty acid also. On the other hand, the amylose–lauric acid complex (spectrum not shown) showed no change upon changing the recycle delay, suggesting only one component is present (the complex).

Attempts to study deuterium spectra of specifically labeled amylose–palmitic acid complexes (labeled at C2 and C9,10) were unsuccessful because of poor signal to noise. This failure is most probably related to the small amount of deuterium in the overall complex. Future efforts in this regard will focus on larger blocks of selected deuteration in the fatty acid.

4. Conclusions

The fatty acid inside the amylose single helix enhances the cohesion of the helix structure, and increases the crystallinity of that polymorph type. Upon humidifying the complexed sample, the normal preference for the B-type amylose arrangement over the V-type is prevented. The existence of the V-type amylose as a complex with the fatty acid prohibits its conversion to the B-form, even if the sample is less than fully complexed (e.g. 5% fatty acid).

Both the relaxation and the deuterium experiments showed that the complexed fatty acid relaxes faster than the free one. The fast relaxation of the fatty acid in the complex could be explained because of the intimate contact between the fatty acid and the complex atoms.

Support for the disordered complex model (Fig. 8) is provided by comparing the T_1 results for the V-amylose carbons as shown by Horii, Yamamoto, Hirai and Kitamaru (1987) with our data for the complexes. Horii's ^{13}C T_1 of V_a and V_h amylose are listed in Table 2, along with our data for

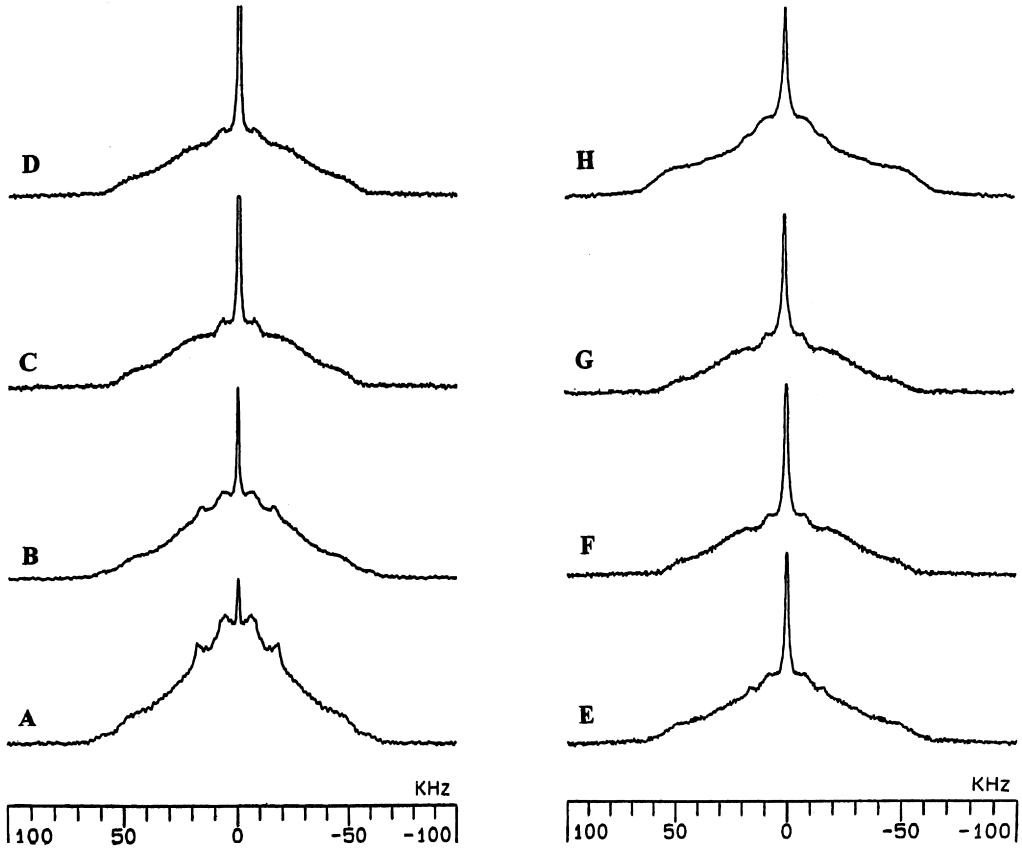

Fig. 10. Temperature dependence deuterium NMR spectra of amylose–palmitic acid complex measured at: (A) 23°C, (B) 50°C, (C) 65°C, (D) 70°C, (E) 50°C, (F) 125°C, (G) 140°C, and (H) 23°C. Spectra (A), (B), (C), (D), (F) and (G) were taken upon heating and spectra (E) and (H) upon cooling.

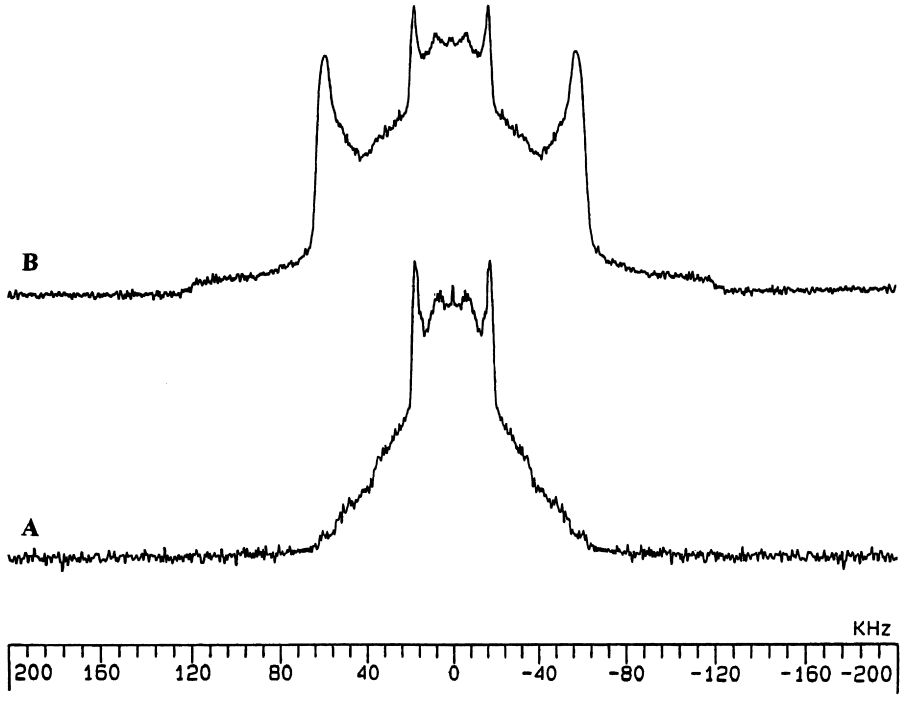

Fig. 11. Deuterium NMR spectra of amylose–palmitic acid complex measured at different recycle delay times. (A) 500 ms and (B) 60 s.

Table 2
T_1 results (in seconds) for V-amyloses and Hylon VII, compared with amylose–palmitic acid complex

Sample	C1 (103 ppm)	C4 (83 ppm)	C2,3,5 (75–72 ppm)	C6 (61 ppm)
V_h-amylose[a]	11.1	11.2	8.6 8.4	0.9
V_a-amylose[a]	7.1	5.8	5.9	0.7
Palmitic acid–amylose complex	29 ± 1	24 ± 1	18 ± 1 18 ± 1	2.5 ± 0.1
Hylon VII (air dry)	22 ± 1	13 ± 1	16 ± 1 15 ± 1	4.9 ± 0.4
Hylon VII (100% humidity)	15 ± 1	7.1 ± 0.4	12 ± 1 11 ± 1	2.8 ± 0.2

[a] Data from Horii et al. (1987).

the DP900 amylose–palmitic complex and Hylon VII. The difference in the relaxation rate for the V-amylose carbons compared with the same carbons in a V-amylose–fatty acid complex is significant, suggesting that the amylose is restrained in mobility as a result of complexing.

The differences between the T_1 results of the "pure" V-amylose and the complexed one, suggest that the fatty acid may act as a pseudo cross-link molecule between the helixes. The model shown in Fig. 8 does not convey this concept. The well-known "anti-bread staling" effect of fatty acids in baking technology may be related to this model. Removal of the mobility of amylose, even with a small amount of fatty acid, could restrain amylose retrogradation (Champenios, Colonna, Buleon, Della-Valle & Renault, 1995), which is the starch chemist's terminology for crystallite formation.

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