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THERMAL DISSOCIATION OF AMYLOSE–FATTY ACID COMPLEXES

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ABSTRACT

Complexes saturated with fatty acids of increasing chain length (10:0 to 20:0), prepared by neutralisation of an alkaline solution of amylose, were shown by differential scanning calorimetry not to contain uncomplexed fatty acids. Insoluble complexes, prepared by dilution with water of a solution of amylose in methyl sulphoxide, contained uncomplexed fatty acids. The dissociation temperature of the complexes increased with increasing chain length of the fatty acids, but the dissociation enthalpy was practically independent of the chain length. Dissociation of the complexes (originating from alkaline solution) at 135°, followed by annealing for 16 h at 90° and rapid cooling, increased the dissociation temperature. The results are discussed in the light of a stoichiometric relationship between fatty acids and amylose.

INTRODUCTION

The use of monoglycerides and other lipids for the control of the technological properties of processed foods containing starch is well established. Monoglycerides interact with the amylose component of the starch granules during gelatinisation and prevent its solubilisation[1]. This interaction involves[2] the formation of a helical inclusion complex whereby the monoacyl lipid molecules occupy the central axis of a long left-handed helix consisting, normally, of six glucosyl residues per turn, the distance between the helices being 0.8 nm. This structure appears to be identical with that of the familiar blue amylose–polyiodide product[2]. Fatty acids, fatty alcohols[3], lysophosphatidyl choline[4,5], and various other organic compounds[6] interact with amylose. The complexes are insoluble in aqueous media at a pH ≤ 7. This complex-forming ability of amylose with monoglycerides and related surface-active monoacyl lipids has been exploited in breadmaking to retard staling[7], in the manufacture of instant mashed potato granules to prevent stickiness[8], and in extruded starch-containing products to control texture[9,10]. The sodium, potassium,

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and calcium salts of fatty acids are permitted emulsifiers, up to 1.5% of the weight of the flour, in Dutch-type rusks[11].

Amylose–fatty acid complexes have been studied by X-ray crystallography[12], iodine-binding capacity[2], viscometry[13], optical rotation, surface tension, and n.m.r. spectroscopy[14]. Differential scanning calorimetry (d.s.c.)[15] and enzymic degradation techniques[15,16] have been used for the study of the thermal dissociation and digestibility, respectively, of complexes. The reported d.s.c. dissociation temperatures and enthalpies differ widely[13,15,17–19], presumably due to the formation of polymorphs. Moreover, multiple d.s.c. peaks[18,19] have been reported, depending on the temperature[18] at which the complexes have been pre-heated (annealed) as well as the water content[19] during calorimetry.

The exact quantity of lipid required to saturate the amylose helix has never been stated. Generally, investigators have used 10–25 parts of lipid per 100 parts of amylose for the formation of complexes[15,18,19], although the addition of an excess of complexing agent has also been reported[6,12,13]. Knowledge of the exact composition of the complexes is important for the calculation of their enthalpy of dissociation. Recently, a stoichiometric relationship was established between fatty acids of increasing chain length and amylose[20]. Under controlled experimental conditions, saturation of the amylose helix was achieved at a molar ratio of fatty acid/amylose which can be predicted from the chain length of the complexed fatty acid and the conformation of the helix. We have investigated the influence of the method of preparation on the composition and the thermal properties of the complexes. Insoluble complexes were prepared by neutralisation of an alkaline solution of amylose, to which fatty acids were added as their potassium salts, thus eliminating co-precipitation of uncomplexed lipids[15,19], a phenomenon associated with the presence of methyl sulphoxide, which is both a solvent and a complexing agent for amylose.

EXPERIMENTAL

Materials. — Potato amylose (A-9262) and fatty acids (>99% pure) were obtained from Sigma. Amyloglucosidase (208469, activity 6 U/mg, from *Aspergillus niger*) was obtained from Boehringer–Mannheim. All reagents were of analytical grade.

Preparation of complexes. — (*a*) *From alkaline solution.* A solution[20] of amylose in 0.01M KOH (pH 12) was used, which had a viscosity average molecular weight (M_w) of 2.9×10^5 and a d.p. of ~1800. Solutions of amylose (14 mL, 4 mg/mL), to which the required amount of fatty acid had been added[20], were neutralised with 0.1M HCl (1.4 mL) and adjusted to pH 4.6 by the addition of citrate buffer (15 mL, 460 mg of citric acid monohydrate and 840 mg of sodium citrate dihydrate in 100 mL of water). Samples were stored overnight at 25° and centrifuged for 30 min at 2000*g*, and the residues were freeze-dried.

(*b*) *From methyl sulphoxide solution.* To a solution of amylose (1 mL, 40

mg/mL) in anhydrous Me_2SO were added aliquots (0.7–2 mL) of a solution (2.4 mg/mL) of palmitic acid in Me_2SO, and the final volume was made up to 3.0 mL with Me_2SO. Citrate buffer (2 mL, pH 4.6) was then added, followed by water (25 mL). Each solution was kept for 15 min at 85° and then cooled, and the precipitated complexes were recovered by centrifugation and freeze-dried.

Differential scanning calorimetry. — A Mettler DSC30 with a TC10 thermal analyser-processor was used. Freeze-dried complexes (2–3 mg) were weighed into aluminium pans (25-μL capacity), water (15 μL) was added, and the contents were stirred with a needle. A pierced pan was used as a reference. The temperature programme was 10°→135° at 10°/min, followed immediately by rapid cooling. The calculation of the heat of dissociation of the complexes was based on the determination of the amylose contained in the pan on the completion of thermal analysis. The pan was opened with tweezers and placed in a 10-mL tube provided with a screw-cap. Water (1 mL) was added and the contents were vortex-mixed to give a homogeneous dispersion. 2M KOH (1 mL) was added and vortex-mixing was continued for 5 min. The mixture was neutralised with M HCl (2 mL) and the pH was adjusted to 4.6 with citrate buffer (1 mL). To a portion (1 mL) was added amyloglucosidase (1 mL, 2 mg/mL in citrate buffer pH 4.6), and the mixture was kept for 30 min at 60°. After cooling to 20°, water (8 mL) was added and the glucose was determined[21] in 1-mL aliquots (amylose = glucose × 0.9). The specific enthalpy of melting (heat of dissociation) of the complex was expressed as J/g of amylose in preference to J/g of complex, to account for possible variations in the content of lipid.

Investigation of heating of the complexes. — (*a*) *Effect of heat.* Complexes of lauric, palmitic, and arachidic acids were prepared by both the alkaline and the Me_2SO methods. One batch of the samples was kept for 30 min at 135° (to dissociate the complexes), and then for 16 h at 90°. The second batch was kept for 16 h at 90°. Each sample was then cooled rapidly and centrifuged, and the residue was freeze-dried and used for d.s.c. Each supernatant solution was extracted with ether (2 × 5 mL), heptadecanoic acid (0.09 mg) was added to the combined extracts, and the ether was evaporated in a stream of nitrogen. Each residue was treated with methanol–boron trifluoride for 1 h at 100°, and the fatty acid methyl esters were analysed[20] by g.l.c.

(*b*) *Extraction with anhydrous methanol.* Samples of palmitic acid–amylose complexes were prepared by the alkaline method. After centrifugation, methanol (8 mL) was added to the wet precipitate and the mixture was shaken for 1 h at 50°, 60°, or 70°. Each sample was allowed to attain room temperature and centrifuged, and the supernatant solution was evaporated to dryness. Fatty acids were determined as in (*a*). Each residue was freeze-dried prior to d.s.c.

(*c*) *Preparation at various temperatures.* Using the alkaline method, clear solutions containing amylose and the required quantity of lauric acid were kept variously, for 30 min, at 50°, 60°, or 70°. Calculated volumes of 0.1M HCl and citrate buffer (pH 4.6), both kept at the appropriate temperature, were then added,

and the mixtures were held at the relevant temperature for 30 min and then allowed to attain room temperature. Each precipitate was recovered by centrifugation and freeze-dried. Each supernatant solution was extracted with ether and the extract was analysed for free lauric acid by g.l.c.

RESULTS

Thermal properties of the complexes. — D.s.c. data were obtained for the insoluble amylose–fatty acid complexes, in suspensions containing ≥85% of water. Typical thermograms of complexes obtained by precipitation after neutralisation of alkaline amylose–fatty acid solutions, with added fatty acids below the level required for saturation of the amylose in solution, are shown in Fig. 1. Thermograms of amylose complexes with unsaturated fatty acids are shown in Fig. 2. Thermograms of complexes of amylose (AM) with palmitic acid (FA) prepared by the alkaline method with molar ratios[20] of FA/AM of 0.019 to 0.062 are shown in Fig. 3. When the saturation FA/AM ratio was exceeded, a small endothermic peak was observed

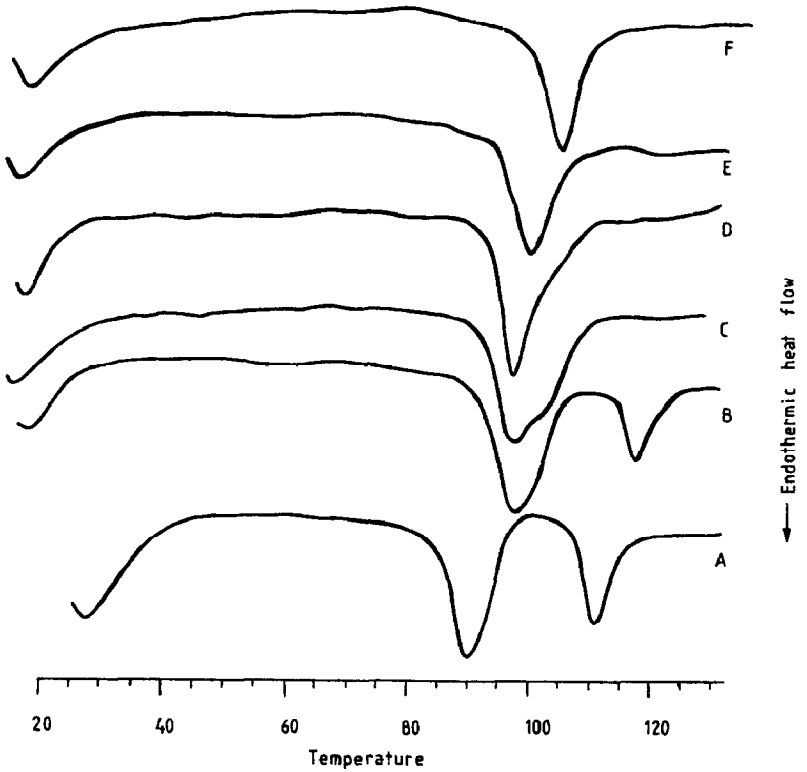

Fig. 1. Thermograms of amylose complexes, prepared by the alkaline method at 25°, with saturated fatty acids: A, decanoic; B, lauric; C, myristic; D, palmitic; E, stearic; F, arachidic. The level of added fatty acids was below the saturation requirement of the amylose.

near the melting point of palmitic acid (62.7°), suggesting that the excess of fatty acid was outside the helix of the complex, probably located in the interstices of the folded helices[22] of the complex. No uncomplexed fatty acid was detected when the FA/AM saturation molar ratio was not exceeded (Fig. 1). However, when oleic and linoleic acids (m.p. 10° and −6°, respectively) were used at ratios exceeding the saturation requirement of amylose, the free fatty acids could not be detected (Fig. 2) because their melting point was below the starting temperature of the heating programme. Decanoic acid (m.p. 31°) used above the saturation level of amylose was not detectable by d.s.c. because it remained in solution due to its relatively high solubility in water (15 mg/100 g at 20°).

For lauric, myristic, and palmitic acids, the amount of free fatty acid entrained by the precipitated complex (Table I) could be calculated as a percentage of added fatty acid, from the observed enthalpy due to melting and from published data for the specific enthalpy of melting[23]. Dissociation temperatures and enthalpies of complexes of saturated and unsaturated fatty acids are given in Tables I and II, respectively, together with the FA/AM molar ratios. The molar ratios at which free lauric, myristic, and palmitic acids could be detected by d.s.c. accorded with the values calculated for the saturation molar ratios (Table III), and with experimental data obtained by other techniques[20]. Within experimental error, the enthalpy of dissociation of the complexes remained essentially the same (29.6 ±2.1 J/g) regardless of the chain length of the fatty acids. For arachidic acid, the dissociation temperature was somewhat higher (33.3 ±0.9 J/g), and for oleic and linoleic acids it was

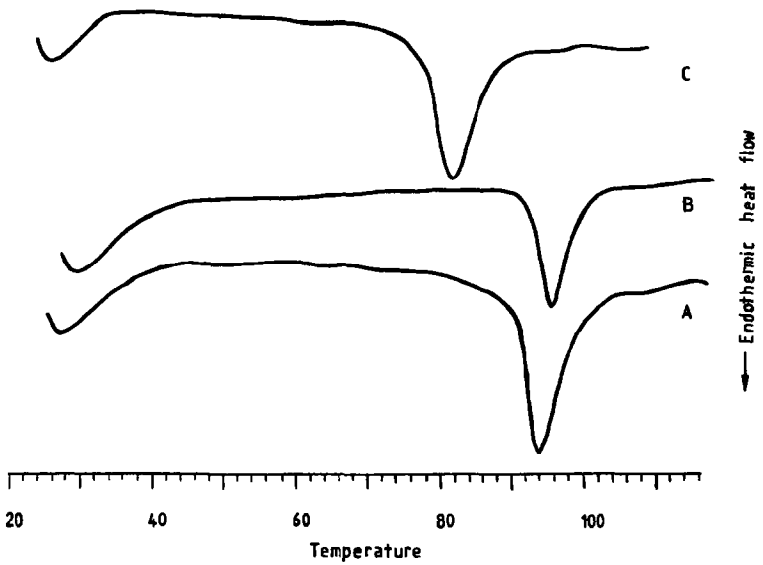

Fig. 2. Thermograms of amylose complexes, prepared by the alkaline method at 25°, with unsaturated fatty acids: A, oleic; B, elaidic; C, linoleic. The level of added oleic and linoleic acids exceeded the saturation requirement of the amylose.

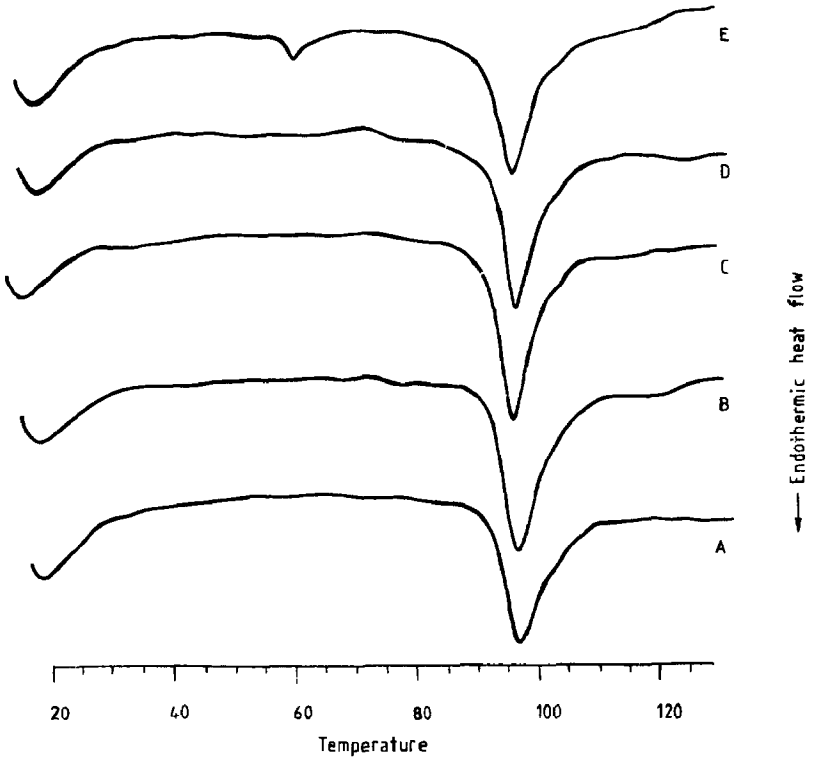

Fig. 3. Thermograms of amylose complexes, prepared by the alkaline method at 25°, with palmitic acid added at an increasing molar ratio (palmitic acid/amylose): A, 0.019; B, 0.025; C, 0.037; D. 0.049; E, 0.062.

lower (24.6 ±0.9 J/g). Moreover, for each fatty acid, the dissociation enthalpy of the precipitated complexes was relatively constant and almost independent of the amount of fatty acid added, suggesting that saturated complexes were removed preferentially from solution rather than partially filled amylose helices. Low dissociation enthalpies have been reported[5] for amylose–lysolecithin complexes, at low levels of lysolecithin, the preparation of which was attempted by mixing the dry reactants with water in the d.s.c. pans, heating to 127°, cooling, and carrying out d.s.c. The dissociation temperature (~150°) of retrograded amylose was not attained and, thus, only a fraction of the amylose present participated in complex formation.

The dissociation temperature (Table II) of complexes of unsaturated fatty acids decreased with the increasing number of double bonds and was highest for the *trans* isomer in accord with the results of other workers[17,18]. This finding could be due to imperfections in the helical structure because of the *cis* double-bonds, since the *trans* double-bonds give rise to an almost linear structure. Complexes made by mixing amylose and fatty acids in Me_2SO, followed by dilution with water to 10% of Me_2SO (v/v), invariably gave two peaks corresponding to the melting of

TABLE I

DISSOCIATION TEMPERATURE (T) AND ENTHALPY (ΔH) OF SATURATED FATTY ACID–AMYLOSE COMPLEXES[a]

Fatty acid	*Molar ratio*	T_1 *(degrees)*[b]	ΔH_1 *(J/g of amylose)*[b]	T_2 *(degrees)*[c]	ΔH_2 *(J/g of amylose)*[c]	*Free acid (%)*[d]
Decanoic	0.079	87.7 ±0.6	18.5 ±1.0	108.0 ±0.3	10.6 ±1.0	0
	0.106	87.8 ±0.7	19.4 ±0.8	108.8 ±1.0	9.2 ±0.6	0
	0.132	87.2 ±0.5	19.2 ±0.2	107.9 ±0.8	9.5 ±0.8	0
	0.159	87.3 ±0.6	21.0 ±1.8	108.2 ±0.6	9.2 ±0.8	0
Lauric	0.040	94.1 ±0.2	22.4 ±1.8	114.0 ±0.8	6.0 ±0.5	0
	0.053	94.6 ±0.8	23.2 ±0.9	114.5 ±1.0	5.9 ±0.8	0
	0.079	93.6 ±0.8	23.0 ±1.2	114.0 ±0.9	5.5 ±0.6	4
	0.106	96.0 ±0.7	23.1 ±1.5	114.6 ±1.2	5.6 ±0.7	15
Myristic	0.036	93.7 ±1.0	24.6 ±0.2			0
	0.048	94.2 ±0.7	29.1 ±0.8			0
	0.072	93.3 ±0.9	29.0 ±2.0			10
	0.096	93.0 ±1.0	28.2 ±0.2			32
Palmitic	0.019	94.1 ±0.7	30.0 ±1.0			0
	0.025	94.0 ±0.5	26.0 ±0.7			0
	0.037	94.4 ±0.8	29.7 ±0.4			0
	0.049	94.5 ±1.0	30.2 ±0.8			0
	0.062	94.4 ±0.7	29.0 ±1.6			15
Stearic	0.012	97.3 ±0.9	26.5 ±0.6			0
	0.018	98.0 ±0.7	29.3 ±2.0			0
	0.024	98.1 ±0.1	26.6 ±0.9			0
	0.030	98.3 ±0.6	29.7 ±1.8			0
Arachidic	0.020	101.2 ±0.2	32.4 ±1.2			0
	0.026	101.6 ±0.8	34.6 ±1.5			0
	0.032	102.0 ±0.6	33.3 ±2.2			0
	0.039	101.0 ±0.4	33.1 ±1.2			0

[a]Prepared by the alkaline method at 25°, with increasing molar ratio of fatty acid/amylose. Means of four replicates ± standard deviation. [b]First dissociation temperature and enthalpy, respectively. [c]Second dissociation temperature and enthalpy, respectively. [d]As per cent of added fatty acid.

the uncomplexed fatty acid and the dissociation of the complex. With palmitic acid (Fig. 4), the dissociation temperature remained constant, whereas the dissociation enthalpy showed only a slight increase with increasing addition of fatty acid (Table IV). The dissociation enthalpy was lower than that of the complexes originating from alkaline solution, suggesting a less-ordered structure of the helix.

After the sample had reached maximum temperature (135°), followed by rapid cooling, reheating at 10°/min in the d.s.c. gave a single peak corresponding to the fatty acid released from the helix but no endotherm due to the complex (Fig. 5, thermograms A′–D′). However, the complexes with decanoic and lauric acid, each

TABLE II

DISSOCIATION TEMPERATURE AND ENTHALPY (ΔH) OF UNSATURATED FATTY ACID–AMYLOSE COMPLEXES[a]

Fatty acid	*Molar ratio (FA/AM)*	*Dissociation temp. (degrees)*	ΔH *(J/g of amylose)*
Elaidic	0.028	94.4 ±0.9	28.4 ±0.5
	0.037	94.2 ±0.3	30.2 ±2.0
	0.046	94.2 ±0.4	28.7 ±0.9
	0.056[b]	93.8 ±0.4	28.5 ±1.5
Oleic	0.028	91.2 ±0.5	24.1 ±0.9
	0.037	91.6 ±0.7	25.0 ±0.8
	0.046	91.2 ±0.6	25.1 ±1.0
	0.056	90.9 ±0.7	23.7 ±0.8
Linoleic	0.031	81.5 ±0.1	25.0 ±1.0
	0.039	80.4 ±0.8	25.6 ±1.2
	0.047	81.5 ±0.8	23.0 ±1.0
	0.055[c]	81.6 ±0.3	25.0 ±0.8

[a]Prepared by the alkaline method at 25°, with increasing molar ratio of fatty acid/amylose. Means of four replicates ± standard deviation. [b]Free fatty acid (11%) was detected in the complex at this molar ratio. [c]Free fatty acid was detected in the supernatant solution.

TABLE III

DATA FOR LIPID-SATURATED AMYLOSE HELICES

Lipid	*Carbon atoms*	*Chain-length[a] (nm)*	*Glucosyl residues[b]*	*Saturation molar ratio (lipid/AM)*	*Lipid (g/100 g of amylose)*	*Lipid (g/100 g of complex)*
Decanoic	10	1.48	11.1	0.090	9.6	8.8
Lauric	12	1.73	13.0	0.077	9.5	8.7
Myristic	14	1.98	14.9	0.067	9.5	8.7
Palmitic	16	2.23	16.7	0.060	9.5	8.7
Stearic	18	2.49	18.7	0.054	9.4	8.6
Arachidic	20	2.74	20.6	0.049	9.4	8.6
Monolaurin	12 + 3	2.16	16.2	0.062	10.4	9.4
Monomyristin	14 + 3	2.41	18.1	0.055	10.3	9.3
Monopalmitin	16 + 3	2.66	20.0	0.050	10.2	9.3
Monostearin	18 + 3	2.92	21.9	0.046	10.1	9.2

[a]Calculated according to ref. 20. [b]Per lipid molecule, for 6 glucosyl residues per helix and 0.8-nm helix spacing.

of which gave double peaks, did not appear to dissociate, and the peaks merged into a single peak on reheating (Fig. 6) (see below).

Thermal behaviour of heat-treated complexes. — According to Stute and Konieczny-Janda[18], amylose–lauric acid complexes heated in the presence of water

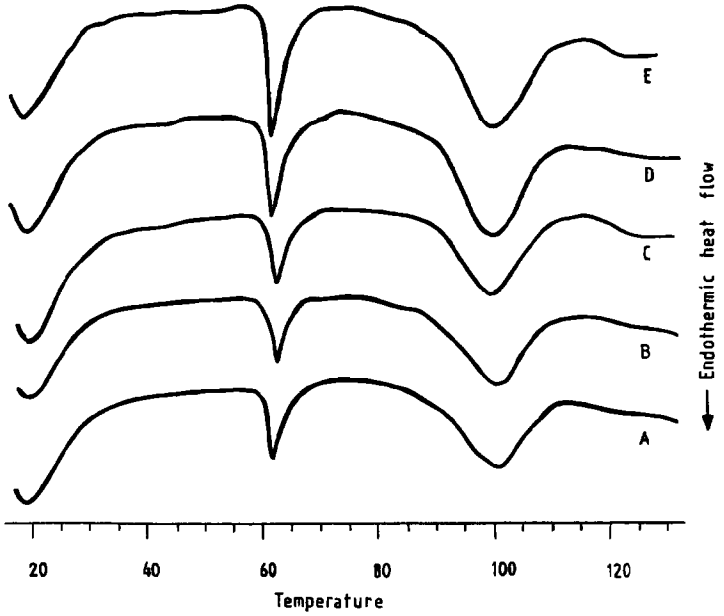

Fig. 4. Thermograms of amylose complexes, prepared by the Me_2SO method, with palmitic acid added at an increasing molar ratio (palmitic acid/amylose): A, 0.027; B, 0.039; C, 0.050; D, 0.066; E, 0.078.

at 80–100° and cooled rapidly showed a shift in the dissociation temperature from 98° to 115°. The complex with the high dissociation temperature was birefringent and gave an X-ray diffraction pattern typical of crystalline material. Amylose–palmitic acid complexes (in aqueous suspension containing 20% of dry complex) could be dissociated[18] by heating to 135° since, after rapid cooling, two peaks were obtained on d.s.c. The first peak corresponded to the melting of the free fatty acid and the second (at ~150°) was due to the dissociation of crystalline (retrograded) amylose.

TABLE IV

DISSOCIATION TEMPERATURE AND ENTHALPY (ΔH) OF PALMITIC ACID–AMYLOSE COMPLEXES[a]

Molar ratio	*Dissociation temp. (degrees)*	ΔH *(J/g of amylose)*[b]	*Free acid*[c] *(%)*
0.027	97.2	19.2	53
0.039	96.6	20.7	
0.050	96.5	21.3	30
0.066	97.0	22.0	
0.078	96.5	22.5	38

[a]Prepared by the Me_2SO method, at various molar ratios of fatty acid/amylose. [b]Data in duplicate. [c]As per cent of added fatty acid.

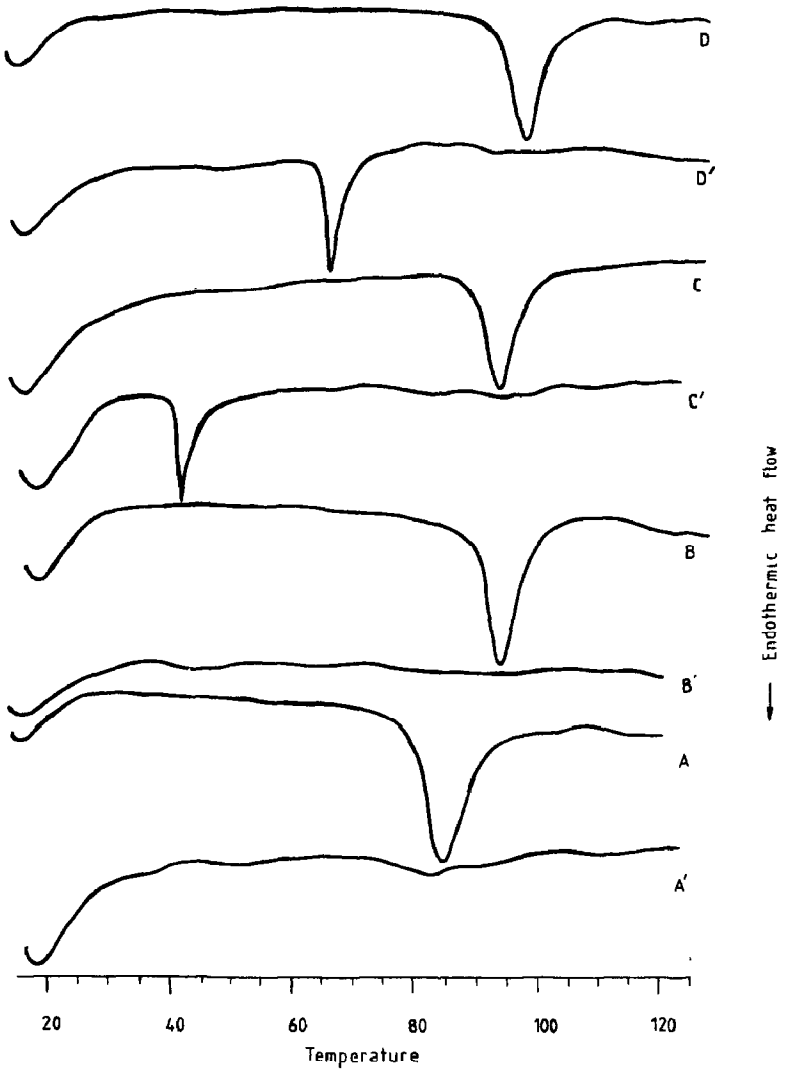

Fig. 5. Thermograms of amylose complexes, prepared by the alkaline method at 25°, with fatty acids: A,A', linoleic; B,B', oleic; C,C' elaidic; D,D', stearic. A–D complexes heated in the d.s.c. to 135° at 10°/min and cooled rapidly; A'–D', same samples reheated to 135° at 10°/min.

The results of similar experiments performed during the present work are shown in Figs. 7–9 for the complexes containing lauric, palmitic, or arachidic acid. The freeze-dried complexes containing lauric or palmitic acid, obtained by the alkaline method, when heated at 90° and then cooled rapidly, gave two peaks (Figs. 7 and 8), whereas a single peak was obtained on heating first to 135° followed by heating at 90° and rapid cooling. The complex containing arachidic acid (Fig. 9) gave a single peak at 90°, but dissociation occurred at 135°, giving rise to a large

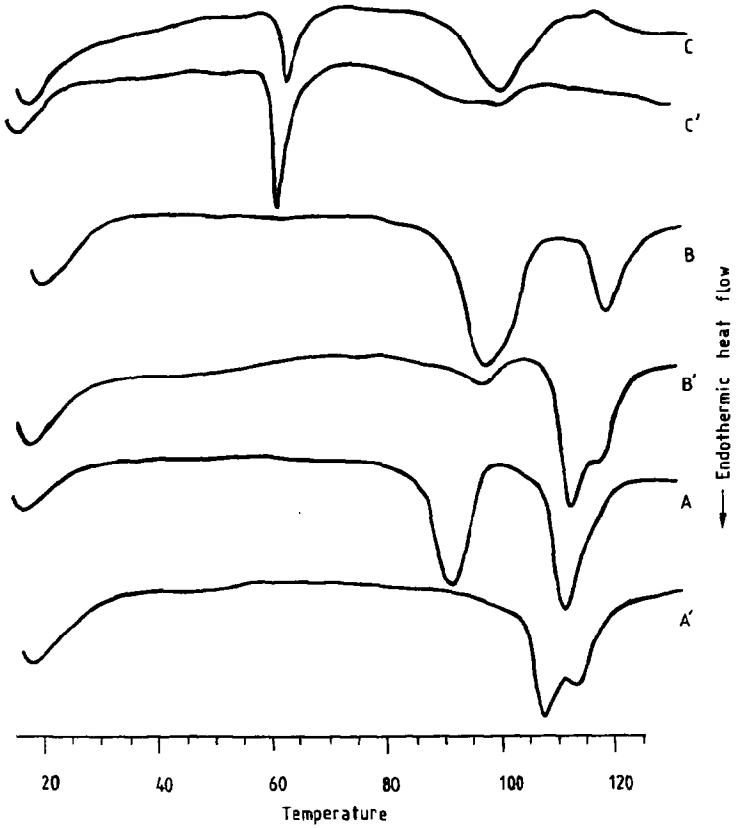

Fig. 6. Thermograms of amylose complexes, prepared by the Me_2SO method, with fatty acids: A,A', decanoic; B,B', lauric; C,C', palmitic. A–C complexes heated in the d.s.c. to 135° at 10°/min and cooled rapidly; A'–C', same samples reheated to 135° at 10°/min.

peak due to the free fatty acid and a small peak due to the complex. Complexes prepared by the methyl sulphoxide method had a lower temperature of dissociation and contained entrained, uncomplexed fatty acid (Figs. 7 and 8, thermograms B and B'). These complexes appeared to be less affected by heat treatment than those prepared by the alkaline route, presumably because they had already been partially annealed by heating to 85° during their preparation. No shift in the endothermic peak was observed after heating to 135°. Dissociation enthalpies are given in Table V. The uncomplexed fatty acids, present in the supernatant solutions, increased in the order lauric, palmitic, arachidic in the ratios ~1:10:100. The amount of uncomplexed acids precipitated with the complex also increased in the same direction, probably reflecting the sequence of melting points. Due to the rapid rate of cooling, after maintaining the complexes at 135°, the fatty acids crystallised faster than they could complex with amylose, so that only a small fraction of the

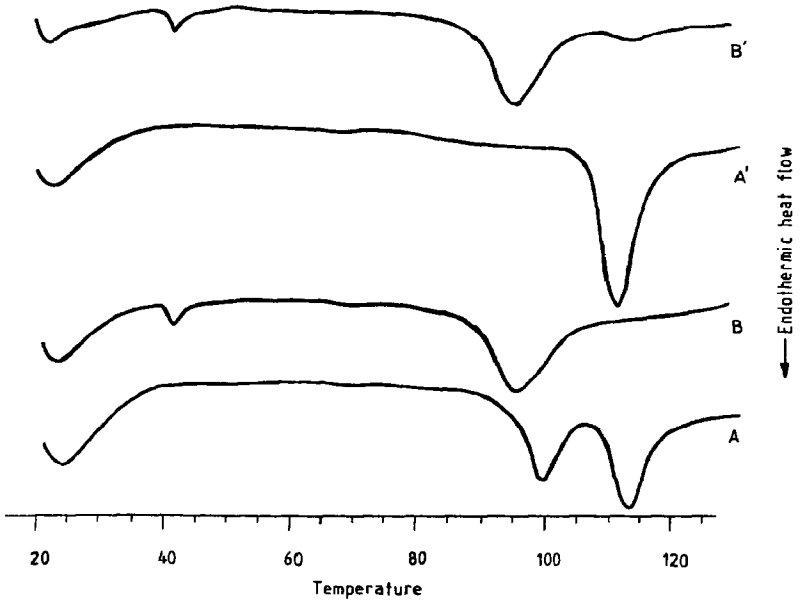

Fig. 7. Thermograms of amylose complexes with lauric acid: A, prepared by the alkaline method at 25° and heated for 16 h at 90°; B, prepared by the Me_2SO method and heated for 16 h at 90°; A', prepared as A but heated for 30 min at 135°, then for 16 h at 90°; B', prepared as B but heated for 30 min at 135°, then for 16 h at 90°.

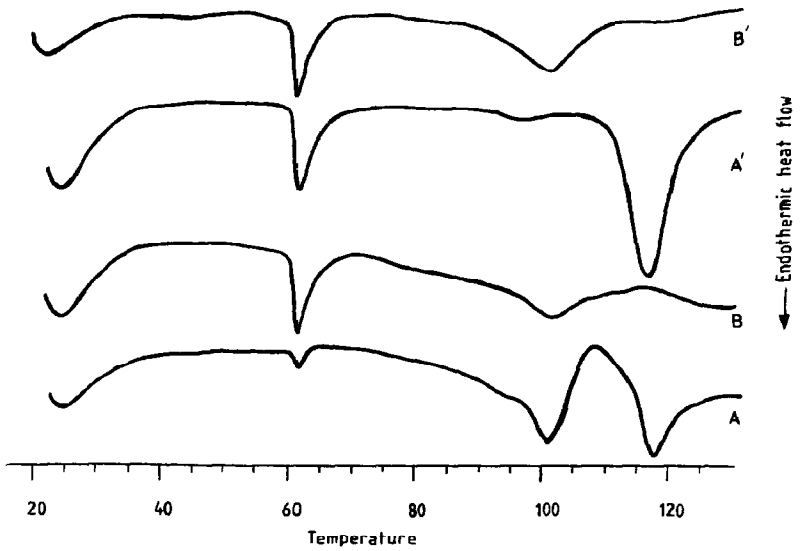

Fig. 8. Thermograms of amylose complexes with palmitic acid: A,A' and B,B', see Fig. 7.

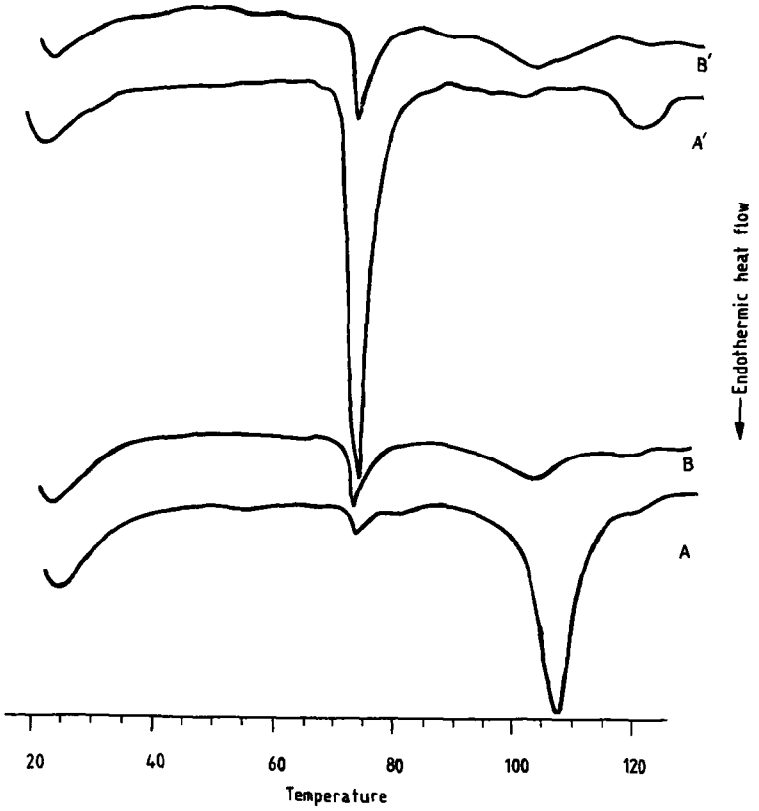

Fig. 9. Thermograms of amylose complexes with arachidic acid: A,A′ and B,B′, see Fig. 7.

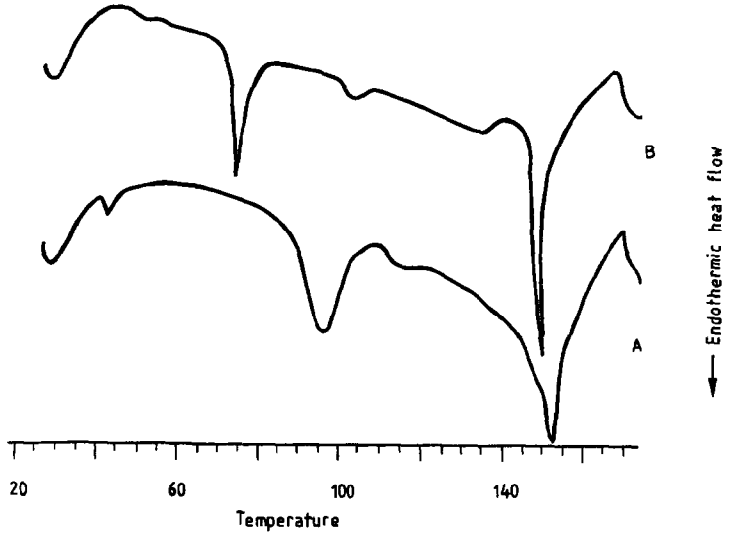

Fig. 10. Thermograms of amylose complexes, prepared by the Me_2SO method, with: A, lauric acid heated for 30 min at 135°, then for 16 h at 90°; B, arachidic acid prepared and treated as A.

TABLE V

DISSOCIATION TEMPERATURE (T) AND ENTHALPY (ΔH) OF FATTY ACID–AMYLOSE COMPLEXES PREPARED AS DESCRIBED IN THE TEXT AND HEATED AS SHOWN[a]

Fatty acid	*Dissociation temp. (degrees)*	ΔH *(J/g of amylose)*	*Free acid (%)*[b]	*Adsorbed acid (%)*[c]	*Molar ratio (FA/AM)*
Lauric[d]					
KOH 90°	(T_1) 98.5 ±1.0	(ΔH_1) 8.1 ±0.3	0.1	0	0.048
	(T_2) 111.5 ±0.2	(ΔH_2) 11.5 ±0.7			
Me_2SO 90°	94.0 ±0.5	22.6 ±1.1	0.0	[e]	0.039
KOH 135°	110.2 ±0.5	30.5 ±1.2	0.1	0	0.048
Me_2SO 135°	92.5 ±0.5	19.5 ±0.6	0.0	[e]	0.039
Palmitic[d]					
KOH 90°	(T_1) 99.5 ±1.0	(ΔH_1) 17.4 ±0.8	0.8	14 ±1	0.048
	(T_2) 115.1 ±0.7	(ΔH_2) 7.9 ±0.6			
Me_2SO 90°	98.6 ±0.5	13.8 ±1.0	0.2	[e]	0.041
KOH 135°	114.1 ±0.3	28.3 ±0.2	0.8	46 ±2	0.048
Me_2SO 135°	97.2 ±1.2	12.5 ±0.6	0.9	[e]	0.041
Arachidic[d]					
KOH 90°	105.2 ±0.1	35.0 ±0.7	0.6	11 ±1	0.030
Me_2SO	100.5 ±0.5	18.6 ±1.0	0.5	[e]	0.025
KOH 135°	121.3 ±0.2	21.0 ±2.0	7.2	[f]	0.030
Me_2SO 135°	100.0 ±0.5	16.0 ±1.2	0.8	[e]	0.025

[a]Means of four determinations ± standard deviation. [b]As per cent of added fatty acid extracted from the supernatant solution with ether and determined by g.l.c. [c]As per cent of added fatty acid calculated from the thermogram showing an endothermic peak for the free fatty acid. [d]Method of preparation of complexes (KOH or Me_2SO) and corresponding heating temperature (see Experimental). [e]Not calculated because free and adsorbed fatty acid would not be distinguished. [f]Large value beyond the measuring range of the d.s.c. instrument.

TABLE VI

DISSOCIATION TEMPERATURE (T) AND ENTHALPY (ΔH) OF LAURIC ACID–AMYLOSE COMPLEX[a] PREPARED AT VARIOUS TEMPERATURES

Temperature of preparation (degrees)	T_1 *(degrees)*	ΔH_1 *(J/g)*	T_2 *(degrees)*	ΔH_2 *(J/g)*	$\Delta H_1 + \Delta H_2$ *(J/g)*	*Free acid (%)*
50	93.3 ±1.0	27.2 ±0.6	111.5 ±0.5	0.6 ±0.2	27.8	0.0
60	93.3 ±0.8	22.4 ±0.7	112.6 ±0.7	2.0 ±0.2	24.4	0.0
70	94.5 ±0.5	15.8 ±0.6	111.0 ±0.2	7.6 ±0.2	23.4	0.1

[a]Molar ratio (FA/AM) = 0.046.

fatty acid with a high melting point could form a complex. Other workers[18] attributed this phenomenon to the diminished solubility of long-chain fatty acids. Thermograms of partly dissociated complexes with a peak at ~150° due to crystalline amylose are shown in Fig. 10.

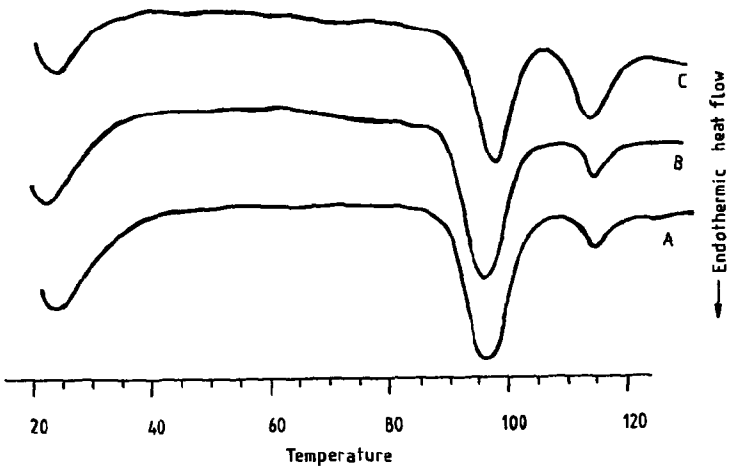

Fig. 11. Thermograms of amylose complexes with lauric acid, prepared by the alkaline method, at A, 50°; B, 60°; C, 70°.

Fig. 11 shows the thermograms of amylose–lauric acid complexes obtained at 50°, 60°, or 70° followed by slow cooling. The increase in the size of the peak at 115° accords with published data[18]. The enthalpies of dissociation are given in Table VI.

G.l.c. showed that palmitic acid was not extracted when amylose–palmitic acid complexes, prepared by the alkaline method, were not freeze-dried but treated with methanol (8 vol.) for 1 h at 50°, 60°, or 70°; and there was no change on d.s.c. of the freeze-dried precipitate. Thus, methanol did not displace palmitic acid from

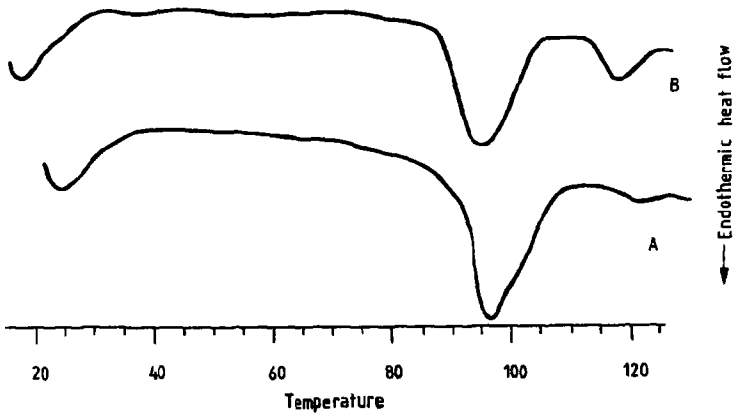

Fig. 12. Thermograms of amylose complexes with palmitic acid, prepared by the alkaline method at 25°, and extracted with methanol: A, wet precipitate for 1 h at 50°; B, freeze-dried precipitate for 1 h at 20°.

the complex. When the original freeze-dried complexes (~15% moisture content) were treated with anhydrous methanol for 1 to 4 h at 20°, the subsequent thermograms contained two endothermic peaks (Fig. 12), at 94° (identical to but broader than that of the untreated sample) and at 118°. The sum of the enthalpies of the two peaks was equal to that of the control. Presumably, anhydrous methanol removed part of the water from the complex and gave a more compact structure. X-Ray analysis[22] showed that wet amylose–2-propanol complexes had helices composed of 7 glucosyl residues, whereas methanol-treated complexes had helices with 6 glucosyl residues per turn. The crystalline polymorphs[22] had orthorhombic and hexagonal symmetries, respectively. The more compact hexagonal symmetry was the result of the dehydrating effect of the methanol.

DISCUSSION

The results in Table VII indicate that there are considerable differences in the reported[13,15,17,18] dissociation temperatures of the complexes, which may reflect the methods of preparation. Complexes prepared from media containing Me_2SO invariably show a peak for the free lipid as in Fig. 4. The d.s.c. data[15,19] obtained when Me_2SO was used as the solvent for amylose revealed uncomplexed lipids. When half the quantity of monoglyceride was used (*i.e.*, 5 mg/100 mg of amylose), uncomplexed monoglyceride was still indicated by the thermograms[15] and it is suggested that Me_2SO and lipids compete for occupancy of the helix.

TABLE VII

DISSOCIATION TEMPERATURE (°) OF FATTY ACID–AMYLOSE COMPLEXES REPORTED BY VARIOUS AUTHORS

Reference	*Decanoic*	*Lauric*	*Myristic*	*Palmitic*	*Stearic*	*Arachidic*
This work	88/108	95/114	94	94	98	102
Morrison[17]	66	84	94	109	115	119
Whittam *et al.*[13]	82	86	89	95	103	
Stute and Konieczny-Janda[18]	93	98		103	100/120	
Eliasson and Krog[15]		85[a]	90[a]	99[a]	104[a]	

[a]Corresponding monoacyl glycerides.

In the present work, the enthalpy of dissociation is expressed as J/g of amylose and not as J/g of complex. The determination of amylose contained in the d.s.c. pan is recommended for accurate results. However, if part of the determined amylose remains uncomplexed, the dissociation enthalpy will appear low. There are significant differences in the reported[15,17–19] dissociation enthalpies (ΔH) of complexes, which are difficult to explain. In principle, lipid-saturated amylose com-

plexes should have essentially the same dissociation enthalpy, since it will depend primarily on the hydrogen bonds between adjacent helices. Precipitated complexes consist[22] of lamellar crystals in which the helical chain of complexed amylose is perpendicular to the lamellae with a thickness, or folding length, of ~10 nm. In an ideal model of a complex, lipids should occupy the longitudinal axis of the amylose helix as an array of paired dimers linked by hydrogen bonding at the carboxyl groups (for fatty acids) or glycerol (for monoglycerides). The hydrocarbon chains are immobilised through hydrophobic interactions with the interior of the helix[6]. Therefore, monomeric lipids involved in complex formation cannot exist in a crystalline form or contribute significantly to the dissociation enthalpy of the complexes. It is suggested that complexes of amylose with fatty acids (C_{10-20}) should not differ materially in their enthalpy of dissociation. The data in Table I and those of Eliasson and Krog[15] support these ideas. The enthalpy of melting of fatty acids ranges from 163 J/g for decanoic acid to 227 J/g for arachidic acid, whereas that of the amylose–fatty acid complexes is ~30 J/g of complexed amylose (Table I).

The appearance of two endotherms for complexes containing decanoic or lauric acid and for palmitic acid after annealing at 90° may be due to the formation of a more-compact helix, *i.e.*, a transition from 7 to 6 glucosyl units per helix. A process of partial melting followed by recrystallisation[19] seems unlikely. The appearance of double dissociation peaks was observed[19] for complexes with a moisture content of <70%, whereas the present work and that of Stute and Konieczny-Janda[18] clearly demonstrated that double peaks are possible at moisture contents of 80–85%. Biliaderis *et al.*[19] reported also that, at a water content of <50%, the enthalpy of melting of the complexes was significantly reduced, but provided no explanation.

Thus, the thermal properties of the polymorphs of amylose–fatty acid complexes depend largely on the solvent used, the temperature at which the reactants are mixed, and the rate of cooling during preparation of the complexes. Further work is required to establish whether these factors also determine the number of glucosyl residues per helical turn of the complex.

A conformational rearrangement of amylose–fatty acid complexes formed by the alkaline route at room temperature, which occurs on heating at 90° or their dissociation at 135°, may be of technological significance in bread-making and in the preparation of extruded products containing starch, since temperatures in the region of 90 to 150° are normally associated with these processes.

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