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Annealing of maize starch

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Abstract

A comparison was made between the annealing characteristics of commercial waxy, normal and amylomaize starches. The waxy and normal starches responded similarly to annealing where improved registration of amylopectin double helices is proposed, with perhaps optimisation of hydrogen bonding along the full length of individual helices. This is attributed to similar amylopectin crystalline structure of these starches. It is considered unlikely that the free amylose (FAM) forms any significant numbers of amylose–amylose or amylopectin–amylose double helices throughout its length, but may randomly form some double helical regions in the crystalline shells created essentially by the amylopectin. Single amylose helices are also considered unlikely. Probably lipid complexed amylose (LAM) is excluded from the amylopectin crystallites and is primarily located in amorphous shell regions (between crystalline shells) and consequently affects hydration (and glass transition, Tg) of these regions. In amylomaize starches, however, the larger amylopectin chain lengths, broad gelatinisation endotherm, leaching characteristics and double helix content (by NMR) indicates that FAM does here play a significant part in the crystalline regions of the starch. Annealing of amylomaize crystallites probably reflects enhanced registration (and possibly compartmentalisation) of amylopectin–amylopectin, amylopectin–amylose and amylose–amylose helices.

Keywords: Maize starch; Annealing; Glass transition; Crystallisation; Gelatinisation

1. Introduction

In a recent publication, Tester, Debon and Karkalas (1998a) discussed annealing of wheat starch in terms of the initiation temperature, starch to water ratio and time. They found that annealing could be initiated 15°C below the onset of gelatinisation (T_o) although it was more marked the closer the temperature was set to T_o. At 20% moisture (by weight) the process could proceed although it was restricted unless the moisture content exceeded 60%. Increasing time also contributed to the amount of starch modification. Using ^{13}C-CP/MAS-NMR they found that the number of double helices remained constant post-annealing and it was proposed that annealing improved the crystalline register of double helices thereby ‘perfecting’ starch crystallites, rather than promoting the formation of additional double helices. This perfection of crystallites was possibly initiated by incipient swelling and the resulting mobility of amorphous α-glucans which facilitated ordering of double helices and, probably greater ordering of the amorphous regions themselves. The relative importance of the amorphous and crystalline regions of starch granules in annealing processes has also been addressed by Jacobs and Delcour (1998).

Some researchers have reported that annealing increases granular stability (Hoover & Vasanthan, 1994), or reorganises granular structure (Blanshard & Galliard, 1987; Krueger, Knutson, Inglett & Walker, 1987a; Krueger, Walker, Knutson & Inglett, 1987b), whilst others have discussed it in terms of crystallinity and perfection of starch crystallites and molecular reorganisations (Jacobs, Eerlingen, Clauwaert & Delcour, 1995; Jacobs, Eerlingen, Rouseu, Colonna & Delcour, 1998; Knutson, 1990; Larsson & Eliasson, 1991; Marchant & Blanshard, 1978, 1980; Nazakawa, Noguchi, Takahashi & Takada, 1984; Paredes-López & Hernández-López, 1991; Shi, Seib & Lu, 1991; Stute, 1992; Tester & Morrison, 1990b; Yost & Hoseney, 1986). More details concerning annealing (and heat moisture treatments) can be found in a recent review by Jacobs and Delcour (1998).

Although annealing of maize starches has been addressed elsewhere (Krueger et al., 1987a,b), interpretations of the annealing process as a function of amylose to amylopectin ratio have been difficult to make in this cereal starch. Part of the problem has been trying to decide if the amylose is

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Abbreviations: DP, degree of polymerisation; DP_w, degree of polymerisation by weight; DSC, differential scanning calorimetry; T_o, T_p and T_c, onset, peak and conclusion temperatures of gelatinisation; ΔH, enthalpy of gelatinisation; T_g, glass transition; ^{13}C-CP/MAS-NMR, ^{13}C-Cross Polarisation/Magic Angle Spinning-Nuclear Magnetic Resonance; FAM, free amylose; LAM, lipid complexed amylose

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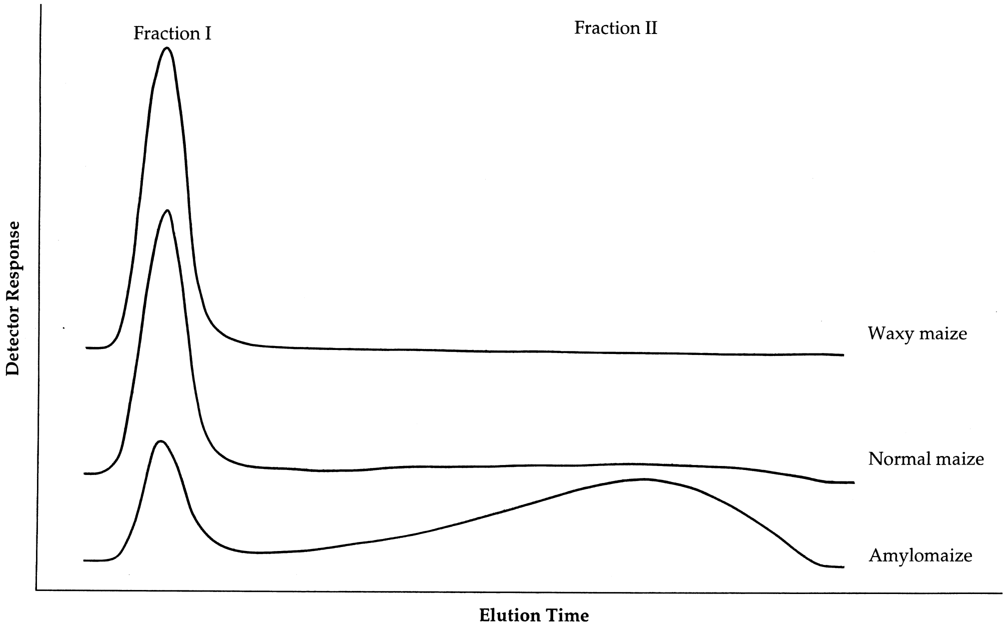

Fig. 1. Gel-permeation chromatography profiles of native maize starches. Column packed with Sepharose CL-2B.

dispersed within amylopectin crystallites and, if so, how. Knutson (1990) reported that although annealing of waxy and normal maize starches caused an increase in gelatinisation temperatures with little change in enthalpy, amylomaize showed a big increase in enthalpy with more distinct endotherms. He attributed this change to an interaction between amylose and amylopectin.

In view of the discrepancy which exists between the annealing characteristics of high amylose versus normal or waxy starches, the following study was undertaken to clarify the annealing mechanism.

2. Materials and methods

2.1. Starches

Waxy, normal and amylomaize starches were obtained from National Starch, Manchester, UK.

2.2. Methods

2.2.1. Physical measurements

Starch granule dimensions were measured with a Coulter Counter Multisizer counting in 256 channels (Tester, South, Morrison & Ellis, 1991), according to the general method of Morrison and Scott (1986). Starch swelling factors (SF) were determined at different temperatures on 50–200 mg samples of starch by blue dextran size exclusion (Tester & Morrison, 1990a). The onset (T_o), peak (T_p) and conclusion (T_c) temperatures and associated enthalpy (ΔH) of gelatinisation were determined by differential scanning calorimetry (DSC) on 3–4 mg of starch heated in 15 μl water (Tester & Morrison, 1990a). Because the gelatinisation enthalpy of the amylomaize starch contained a contribution from the amylose–lipid dissociation endotherm, this was subtracted based on the data of Karkalas, Ma, Morrison and Pethrick (1995). The number of double helices was calculated from ^{13}C-CP/MAS-NMR spectra according to Cooke and Gidley (1992).

2.2.2. Chemical analysis

Moisture content was determined as weight loss after heating at 130°C for 1 h. Nitrogen was determined by the standard Kjeldahl method, where protein was calculated as N × 6.25 (after correcting for lysophospholipid nitrogen, post lipid extraction). Starch phosphorus content was determined before and after lipid extraction according to Morrison (1964). Starch lipids were extracted in 1-propanol:water (3:1) at 100°C (Morrison & Coventry, 1985) and quantified as fatty acid methyl esters (FAME) by gas–liquid chromatography (GLC) according to (Morrison, Mann, Soon & Coventry, 1975; Morrison, Tan & Hargin, 1980). Lysophospholipid (LPL) content was calculated as $\mathrm{LPL} = 16.39 \times [\text{native starch } P - \text{defatted starch } P]$ (Morrison, 1988), free fatty acid (FFA) as $\mathrm{FFA} = 0.95 \times [\text{total FAME} - (\mathrm{LPL}/1.76)]$ (Morrison et al., 1975) and total lipid (TL) as $\mathrm{TL} = \mathrm{LPL} + \mathrm{FFA}$. Total α-glucan was determined according to Karkalas (1985). Leached α-glucan (during swelling, above) was also determined using the Karkalas (1985) method, (but omitting the α-amylase stage) as previously reported (Tester & Morrison, 1990a). Apparent (lipid-containing) and total amylose (lipid free) were determined colorimetrically (Morrison & Laignelet, 1983).

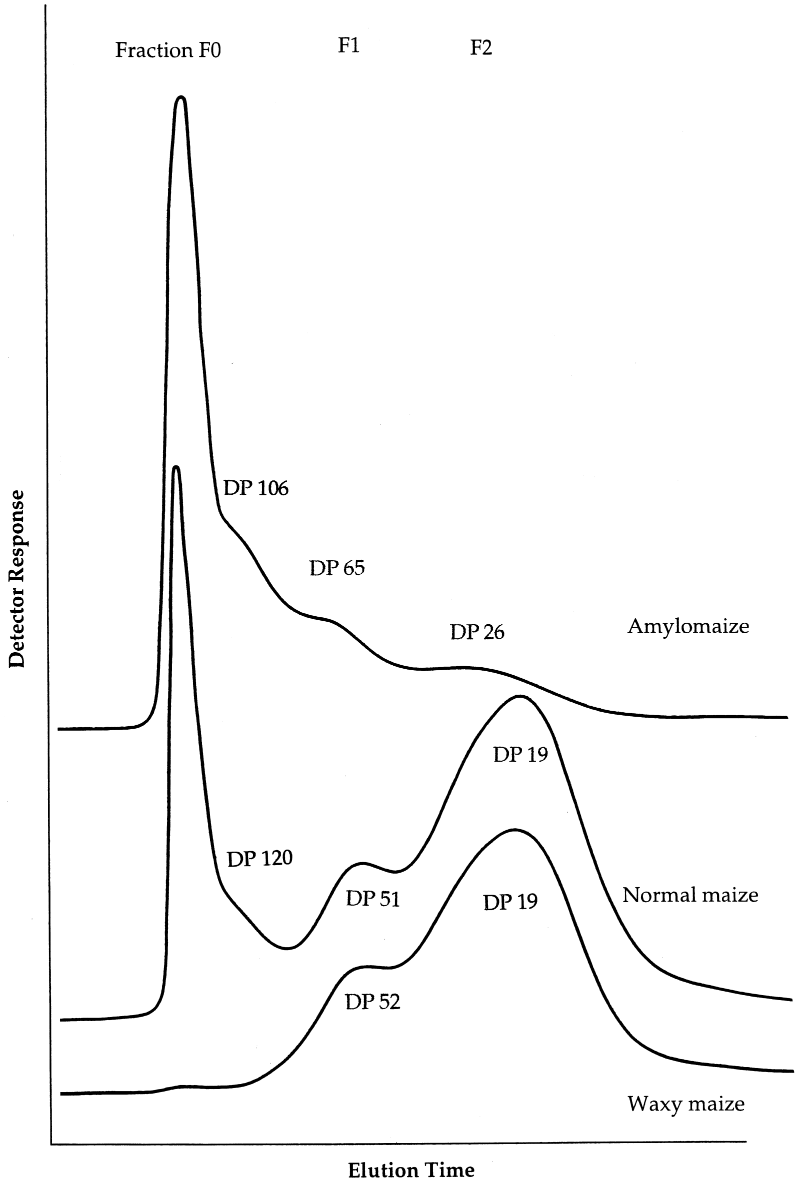

Fig. 2. Gel-permeation chromatography profiles of isoamylase-debranched maize starches. Column packed with Sephacryl S 200 HR.

2.2.3. Chromatographic analysis

The α-glucan chain distribution of solubilised native and isoamylase debranched starches was determined using the chromatographic system of Karkalas and Tester (1992), which incorporates continuous enzymatic hydrolysis and colorimetry. Native starches were fractionated on Sepharose CL-2B columns (100 cm × 1.6 cm id) and debranched samples on Sephacryl S 200 HR columns (100 cm × 1.6 cm id).

Table 1
Chain length distribution of isoamylase debranched maize amylopectin

	Waxy maize	Normal maize	Amylomaize
DP$_w$			
A + B1 chains (F2)	19.5	18.9	26.2
B2-B4 chains (F1)	51.6	51.1	64.6
All	26.5	26.3	48.6
Ratio F2/F1			
Weight basis	3.6	3.4	0.7
Molar basis	9.5	9.1	1.8

2.2.4. Annealing experiments

Maize starch samples (100 mg, in triplicate) were annealed in excess water (>96% by weight) for 72 h at 25 to 65°C (single step annealing) or 72 h at a temperature 10°C below the 'desired' annealing temperature followed by 72 h at the desired temperature (double step annealing), in screw top 10 ml Sovirel tubes. Toluene (10 μl) was added as a bacteriostatic agent. On conclusion of the experiments, all starches were recovered by centrifugation, washed with cold

Table 2
Amylose content of maize starches. ΔAM is total-apparent amylose

Maize	Colorimetric % (db)			GPC native starch % (α-glucan)		GPC debranched starch (α-glucan)		
	Apparent	Total	ΔAM	I	II	F0	F1	F2
Waxy	1.8 ± 0.0	1.7 ± 0.1	−0.1	100	0	0.5	21.6	77.9
Normal	24.1 ± 0.2	28.3 ± 0.2	4.2	66.8	33.2	28.4	16.4	55.1
Amylomaize	58.6 ± 0.6	63.1 ± 0.5	4.5	22.5	77.5	62.2	22.0	15.8

water and then acetone and air equilibrated at room temperature.

3. Results and discussion

3.1. General properties

The GPC elution profiles of the dissolved native starches are shown in Fig. 1, with the elution profiles of the debranched starches shown in Fig. 2. Unit chain distribution profiles for these debranched starches are presented in Table 1. The elution profiles of the native starches are comparable to data presented by South, Morrison and Nelson (1991); Yeh, Garwood and Shannon (1981). The small amylose content of the waxy starch is quite visible, as is the increasing size and polydispersity for the normal and especially the high amylose starch. Elution profiles for the debranched starches are comparable to data presented by South et al. (1991), while chain length distributions are in reasonable accord with other authors (Baba & Arai, 1984; Baba, Uemura, Hiroto & Arai, 1987; Cheetham & Tao, 1997; Curá & Krisman 1995; Fuwa, Glover, Miyaura, Inouchi, Konishi & Sugimoto, 1987; Hizukuri, 1985; Ikawa, Glover, Sugimoto & Fuwa, 1981; Shi, Capitani, Trzasko & Jeffcoat, 1998; Takeda, Shitaozono & Hizukuri, 1988; Takeda, Takeda & Hizukuri, 1993) although there is significant variation between these authors reflecting different fractionation methods used.

The weight average degree of polymerisation (DP_w) of debranched amylopectin in fractions F1 and F2 was 51.1–51.6 and 18.9–19.5 for the waxy and normal starches whereas the weight ratio F2/F1 was 3.6 and 3.4, respectively (Table 1). In amylomaize starch the average chain length was significantly higher, 64.6 and 26.2 for fractions F1 and F2, respectively, with the weight ratio reduced to 0.7. These longer chains correspond to the atypical chains which are 5–15 glucosyl residues longer than chains found in normal maize (Morrison & Karkalas, 1990). Extensive structural analysis of waxy, normal and amylomaize starches has been reported more recently (Shi et al., 1998) and is in reasonable agreement with the data presented here.

The amylose content of the maize starches is presented in Table 2. There is good agreement between the total (delipidated) amylose content determined by the colorimetric method and the amylose content (F0) calculated from the debranched starch chromatograms, although the amylose content calculated from the fractionated native starches was about 20% higher. The lipid content of the starches is presented in Table 3, which is within the range described by Morrison (1988). There was good correlation (albeit for a very small sample number) between Δ-amylose and FAME ($r = 0.9747$, $P < 0.05$). The correlation between Δ-amylose and LPL ($r = 0.9969$, $P < 0.01$) or FFA ($r = 0.9615$, $P < 0.05$) were both high, suggesting that both lipid fractions are complexed with amylose in native starches, the presence of which has been previously demonstrated by Morrison, Law and Snape (1993a) using NMR.

Granule dimensions for the maize starches are presented in Table 4. The average granule diameter decreased with increasing amylose content (11.3, 10.7 and 10.5 μm for waxy, normal and amylomaize starch, respectively) and were within the range reported by other authors (Morrison, 1992). Although granule size is subject to genetic control, environmental factors (especially temperature) have a major impact on dimensions (Tester, 1997) and hence it is hard to define genuine differences between genotypes unless they have been grown in the same location at the same time.

The gelatinisation characteristics of the starches determined by differential scanning calorimetry are presented in Table 5. The waxy and normal starches have comparable onset, peak and conclusion gelatinisation temperatures (62.6, 72.6, 84.4 and 62.9, 70.5, 81.1°C, respectively), where differences in the enthalpy of gelatinisation (15.6 and 12.6 J/g, respectively on a starch basis, corresponding to 15.8 and 18.1 J/g on an amylopectin basis) can be largely attributed to differences in the amylose to amylopectin ratio. The amylomaize has a characteristic broad gelatinisation endotherm with higher onset, peak (which is difficult to accurately define) and conclusion temperatures (67.7, 91.0 and 104.8°C) than the other starches. Similar gelatinisation temperatures have been reported elsewhere, although other workers often report a smaller temperature range for amylomaize starch than was found here (Hoover & Manuel, 1996; Knutson, 1990; Krueger et al., 1987a,b).

The enthalpy of gelatinisation for the waxy and normal starches was similar (15.8 and 18.1 J/g respectively, Table 5). If we assume that the crystallinity of amylopectin is primarily due to the size and number of A- and B1 chains (Hizukuri, 1986), the enthalpy calculated from Fraction 2

Table 3
Phosphorus and lipid content of maize starches

	Total P (mg/100 g, db)	Non-lipid P (mg/100 g, db)	FAME[a] (mg/100 g, db)	LPL[b] (mg/100 g, db)	FFA[c] (mg/100 g, db)	Total lipid[d] (mg/100 g, db)
Waxy	5.8 ± 0.3	2.4 ± 0.2	69 ± 4	55 ± 6	36 ± 7	91 ± 9
Normal	24.6 ± 1.2	2.6 ± 0.3	636 ± 39	360 ± 20	413 ± 44	773 ± 48
Amylomaize	36.9 ± 0.6	11.8 ± 0.8	862 ± 14	411 ± 17	597 ± 22	1008 ± 28

[a] Determined by GLC.
[b] LPL = 16.39(native starch P − delipidated starch P), according to Morrison (1988).
[c] FFA = 0.95(Total FAME − (LPL/1.76)), according to Morrison et al. (1975).
[d] Total lipid = LPL + FFA.

(F2) of the debranched amylopectin (Fig. 2) gives values of 20.0 and 22.9 J/g suggesting a comparable level of hydrogen bonding per helix. Cooke and Gidley (1992) found that if they extrapolated values of enthalpy obtained from waxy and normal starches to hypothetical starches containing 100% molecular order (double helices by ^{13}C-CP/MAS-NMR) they obtained enthalpy figures of 33.3 J/g, corresponding to 57.1 and 53.0 J/g for the waxy and normal starches if calculations were based on 100% crystalline order (X-ray diffraction). The similarity of the gelatinisation properties of waxy and normal maize starches is due to the similarities of the amylopectin structures, where there is comparable double-helical order on an amylopectin basis (but a ‘dilution’ of the amylopectin in the normal starch as discussed below) associated with the same polymorphic structure.

The gelatinisation enthalpy of the amylomaize starch (amylose–lipid enthotherm corrected) was calculated to be 16.5 J/g, which is higher than other literature values, for example 7.4 and 8.6–10.5 J/g for Krueger et al., (1987b) and Knutson (1990), respectively. Whittam, Noel and Ring (1990) found that for B-type spherocrystals of linternised potato starch, there was an enthalpy of 35.7 J/g. By analogy with the B-type crystals of the amylomaize amylopectin fraction (37.8% of the α-glucan), the enthalpy due to hydrogen bonding of the amylopectin double helices would yield 13.5 J/g. This is, however, lower than the figure calculated from the DSC data generated in this study of 16.5 J/g—presumably due to differences of the lengths of chains in the double helices between potato and amylomaize amylopectin molecules. It is interesting that although amylomaize starch contains double helices discernible by ^{13}C-CP/MAS-NMR, the amount of crystallinity by X-ray diffraction is small, supporting the view that the enthalpy is primarily due to double helices rather than crystalline regions (Cooke & Gidley, 1992; Zobel, 1992).

Hizukuri (1985) and Shi et al. (1998) have investigated the relationship between the fine α-glucan structure of maize starches (containing different amylose to amylopectin ratios) and crystallinity. They concur that amylopectin extracted from waxy and normal starches (which exhibit A-type X-ray diffraction patterns) contains shorter average chain lengths and a larger proportion of short chains than amylopectin fractions extracted from B-type starches like amylomaize. This indicates that packing within the waxy and normal (A-type) starches is similar, but amylomaize (B-type) because of the longer amylopectin chains is different.

Shi et al. (1998) in reference to low amylopectin maize starch (LAPS), reported that the double helix content of the starch (which contains 90% amylose) was 33% as determined by ^{13}C-CP/MAS-NMR. Hence, amylose *must* account for some of the double helical content. The amylose double helices dissociation requires high temperature and hence the breadth of the gelatinisation endotherm. This does not, however, mean that amylose is solely within crystalline regions as Shi et al. report that there is a strong signal from their X-ray diffraction studies (2θ of 20°) suggesting a highly ordered crystalline structure of *amylose–lipid* complex.

Temperature dependent swelling and leaching data (excess water conditions) are presented in Fig. 3 and show clearly how the the dilution effect of amylopectin (by amylose) reduces swelling and in the case of the amylomaize starch, leaching of α-glucan. Similar swelling data for a selection of maize mutants is reported elewhere by Hoover and Manuel (1996) and Shi et al. (1998) and related work on gel structure from these starches by Case et al. (1998). Previous work (Tester & Morrison, 1990a) has shown that the α-glucan is primarily amylose, and especially free- (FAM) rather than lipid-complexed (LAM) material which tends to remain in the swollen granules (Morrison, Tester, Snape, Law & Gidley, 1993b).

There was a very high correlation between swelling and leaching for the maize starches, where $r = 0.9705$, 0.9876 and 0.9886 ($P < 0.001$) for the waxy, normal and amylomaize starch, respectively. This confirms the strong relationship between swelling and leaching (Tester & Morrison, 1990a,b).

The swelling factor ratio between the waxy, normal and amylomaize starches at 70 and 80°C is 13.8:3.4:1.0 and 14.2:3.2:1.0, respectively; equivalent to 5.2:1.7:1.0 and 5.3:1.6:1.0 when expressed on an amylopectin basis. This demonstrates that although the amylose fraction dilutes the swelling capacity of the amylopectin at these temperatures, the effect of amylose on swelling is more than simply a

Table 4
Dimensions of maize starch granules using a Coulter Counter Multisizer

	Modal diameter (μm)	Mean diameter (μm)	Mean surface area (μm^2)	Mean volume (μm^3)	Specific surface area (m^2/g)
Waxy maize	12.8	11.3	468	1,143	0.292
Normal maize	7.4	10.7	428	1,024	0.299
Amylomaize	10.4	10.5	395	909	0.311

dilution. This confirms that the amylose (or part of the amylose fraction at least) also *actively* restricts swelling (Morrison et al., 1993b) as discussed below.

The active restriction of swelling by amylose—as FAM—could be by ‘cross-linking’ of amylopectin crystallites. However, although the amylose and amylopectin fractions are co-synthesised during granule biosynthesis, the thermodynamic favourability of the short exterior chains of amylopectin forming double helices together probably precludes this happening to a great extent—although it could well occur intermittently in places. However, the ease of FAM leaching (see below) during swelling also suggests that the FAM is randomly distributed throughout the granules with fairly limited anchorage by complexing with amylopectin. Indeed, the FAM fraction could interfere with the formation of amylopectin crystallites (although not helices) and consequently facilitates swelling (Morrison et al., 1993b) of these regions by causing some destabilisation. This may be corrected by annealing (below).

Unlike FAM, LAM does restrict swelling (Morrison et al., 1993b). It seems logical that the amylose–lipid inclusion complexes (which form spontaneously during biosynthesis (Morrison et al., 1993b)) are excluded from amylopectin crystallites and form their own ordered regions. Probably then, the LAM is in the amorphous shell regions (alternating with amylopectin crystalline shells) as a semi-discrete fraction. This material is consequently difficult to leach from the granules (and the appropriate amylose–lipid dissociation temperature of greater than c. 95°C must be attained to facilitate this). The restriction placed on swelling by this fraction would not, therefore, be due to cross-linking of amylopectin crystallites (preventing their dissociation) but by restricting hydration of the amorphous regions. This control of amorphous region hydration would in turn (assuming as we do that gelatinisation and swelling proceeds from the amorphous to the crystalline regions) restrict the hydration of crystalline regions and dissociation of double helices therein.

The ratio of leached α-glucan for the swollen waxy, normal and amylomaize starches at 70 and 80°C (2.9:5.0:1.0 and 2.7:2.4:1.0, respectively) is (reflecting swelling factors) moderated by the gelatinisation properties (T_c–T_o, Table 5). However, the restriction placed on the leaching of α-glucan (essentially amylose) must, as mentioned above, reflect the extent of amylose entrapment within granules—especially as the amylose content increases to the level found in the amylomaize starch. Increasing LAM content, as postulated above, restricts granule expansion by restricting hydration of amorphous regions (which elevates gelatinisation temperatures). This indirectly restricts FAM leaching, although it is believed that the FAM fraction is radially dispersed throughout the granules, in some cases (especially amylomaize) forming double helices with other amylose molecules or amylopectin.

The concept that amylose (FAM) forms double helices (primarily within the amylomaize starch) across the granule is supported by the work of Shi et al. (1998) and our own measurements in this study where the native amylomaize starch was found to contain 38% double helices (on an ‘as is’ basis, equivalent to 43% dry basis, Table 5) which is more than the amylopectin content of the starch (37.8%, based on GPC fractionation, Table 2).

3.2. Annealing

The effect of single and double step annealing on the gelatinisation temperatures of the waxy and normal maize

Table 5
Gelatinisation characteristics (DSC) of maize starches. T_o, T_p and T_c represent onset, peak and conclusion temperatures of gelatinisation. ΔH and ΔH AP represent enthalpy of gelatinisation on a starch or amylopectin basis, respectively, where amylopectin fraction is determined from GPC data

	Gelatinisation temperatures (°C)				
	T_o	T_p	T_c	ΔH (J/g)	ΔH AP (J/g)
Waxy maize	62.6 ± 1.2	72.6 ± 0.2	84.4 ± 0.9	15.6 ± 0.5	15.8 ± 0.5
Normal maize	62.9 ± 0.5	70.5 ± 0.4	81.1 ± 0.8	12.6 ± 0.2	18.1 ± 0.4
Amylomaize	67.7 ± 1.3	91.0 ± 1.6	104.8 ± 2.2	17.7 ± 0.5[a] (16.5 ± 0.4)[b]	

[a] Enthalpy of gelatinisation including contribution from amylose–lipid complexes.

[b] Enthalpy of gelatinisation excluding contribution from amylose–lipid complexes.

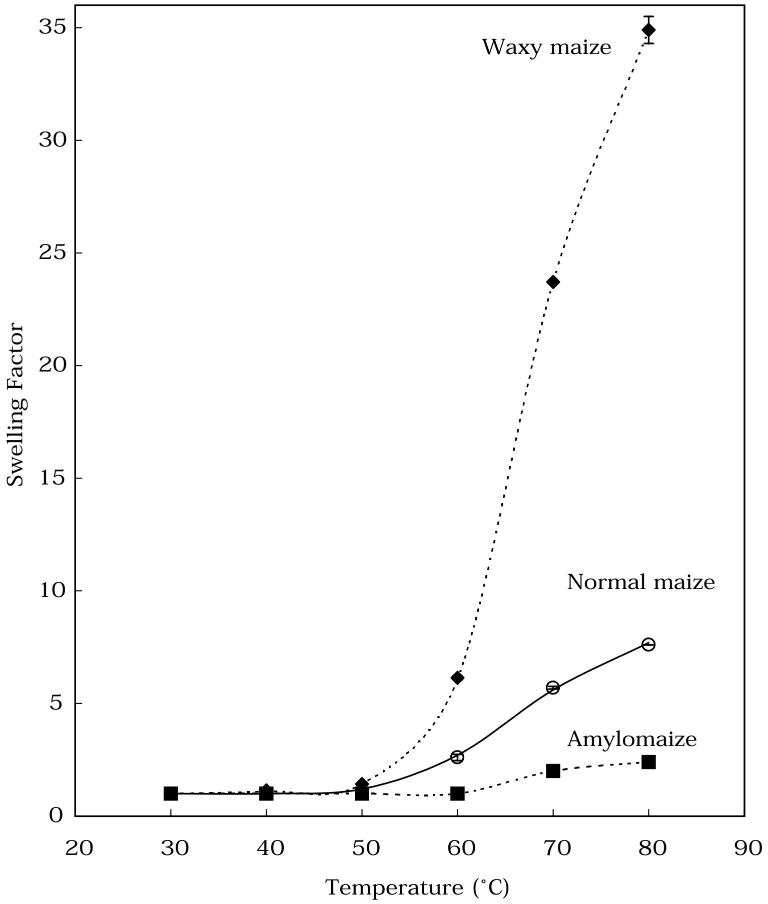

Fig. 3. Swelling curves of maize starches incubated in excess water at temperatures up to 80°C.

starches are presented in Fig. 4. Although there was a small increase of T_o between 25 and 45°C, a significant linear increase only occurred between 45 and 65°C, where the maximum shift was obtained at 65°C. At this temperature, the temperature increase (ΔT, with respect to the native starches) was in the following order: $T_o > T_p > T_c$. This corresponded to $14.3 > 6.6 > 5.8$°C and $14.9 > 9.5 > 7.5$°C for waxy and normal maize starches, respectively. As a consequence the range T_p–T_o was significantly reduced from 10.0 to 2.3°C and 7.6 to 2.3°C for the waxy and normal maize starches, respectively. Similar data have been reported by Knutson (1990) for the different gelatinisation temperatures and by Larsson and Eliasson (1991) for T_o (but not for T_p or $T_{c,}$ presumably because they were studying intermediate moisture systems). Krueger et al. (1987a) found that if starches extracted from four normal dent and one commercial maize cultivar were annealed in excess water at 50°C, the starches tended towards the same maxima of onset and peak temperature (69 and 72°C, respectively) which is in accord with the data obtained from this study (67–69 and 73.5°C, respectively).

The effects of annealing—both single and double step—on the gelatinisation endotherm of the waxy and normal maize starches are shown in Fig. 5. For single-step ‘annealing’ at 65°C partial gelatinisation occurred because T_o of the native starches exceeded this temperature, resulting in a decrease rather than constancy/increase of gelatinisation enthalpy as anticipated for unrestricted annealing. The sequential increase in T_o by double step annealing, however, increases T_o above 65°C and hence there is only slight gelatinisation (for the normal maize starch). Apart from where gelatinisation occurs, the enthalpy of the normal maize starch is the same before and after annealing. The same applies for the waxy starch although the double step annealing at 55 and 65°C increased enthalpy by 1.1 and 1.2 J/g ($P < 0.01$), respectively. Similar data have been reported by Krueger et al. (1987a) and Larsson and Eliasson (1991).

In a recent publication, Tester et al. (1998a) have discussed annealing in relation to wheat starches. These authors found that the number of double helices determined by ^{13}C-CP/MAS-NMR and the enthalpy of gelatinisation (and hence hydrogen bonds stabilising double helices) was essentially the same pre- and post-annealing. They concluded that annealing enhances the ordering of double helices in crystallites (intially facilitated by hydration of amorphous regions) rather than facilitating formation of double helices per se. The process of annealing would, therefore, involve hydration and expansion of amorphous regions (which are in a rubbery state due to $T_{\text{annealing}}$ being

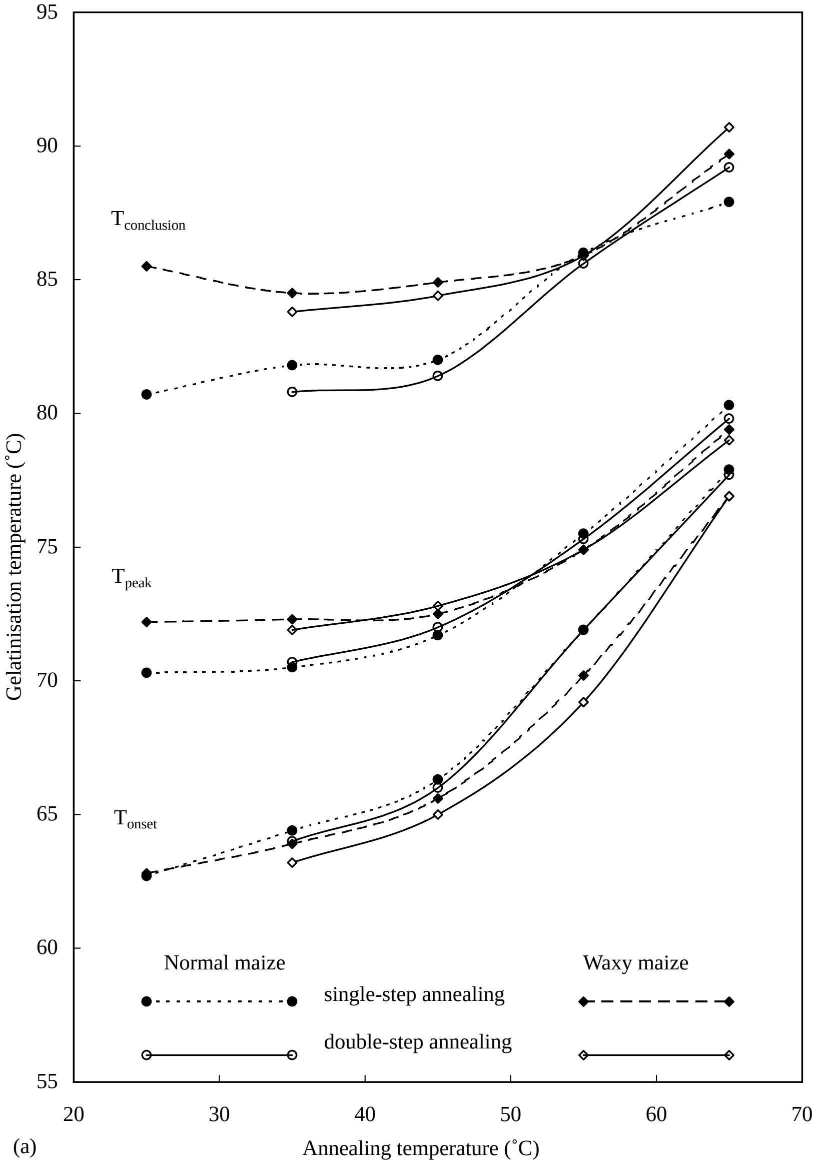

Fig. 4. Effect of single-step and double-step annealing (in excess water for 72 hours) at various temperatures on the gelatinisation temperatures of maize starches. (a) Waxy and normal maize starches; (b) amylomaize starch.

$>T_g$), and initiate re-organisation of the spatial location of double helices with respect to each other in crystalline regions. The mobility of the amorphous regions being positively dependent on annealing temperature, subject to sufficient hydration of these regions, is most effective around T_o (but not above this temperature as gelatinisation is initiated).

In a related paper concerning the effects of growth temperature on starch biosynthesis and properties in potatoes, Tester, Debon, Davies and Gidley (1998b) have discussed growth temperature induced ‘annealing’ in the context of enhanced order (rigidity) of amorphous regions of amylopectin which restrict hydration and hence gelatinisation, ordering (registration) of double helices to form more perfect crystallites, helix aggregation and the possibility that increased growth temperature causes an increase in double helix length (without an increase in α-glucan chain length or significant increase in hydrogen bonding).

An increase in the length of the helices during annealing (or as a consequence of elevation of growth temperatures) would, it has been hypothesised, be due to optimisation of intrahelical bonding where uncoiled glucose residues (probably at the end of helices) become integral parts of the extended helices and consequently elevate gelatinisation temperatures, as predicted from model systems (Moates, Noel, Parker & Ring, 1997; Safford et al., 1998). Elevated growth temperatures may also result in helices being initiated closer to branch points than at lower temperatures as the entropic contribution per residue will be higher, thus resulting in shorter non-ordered regions for the same molecular architecture.

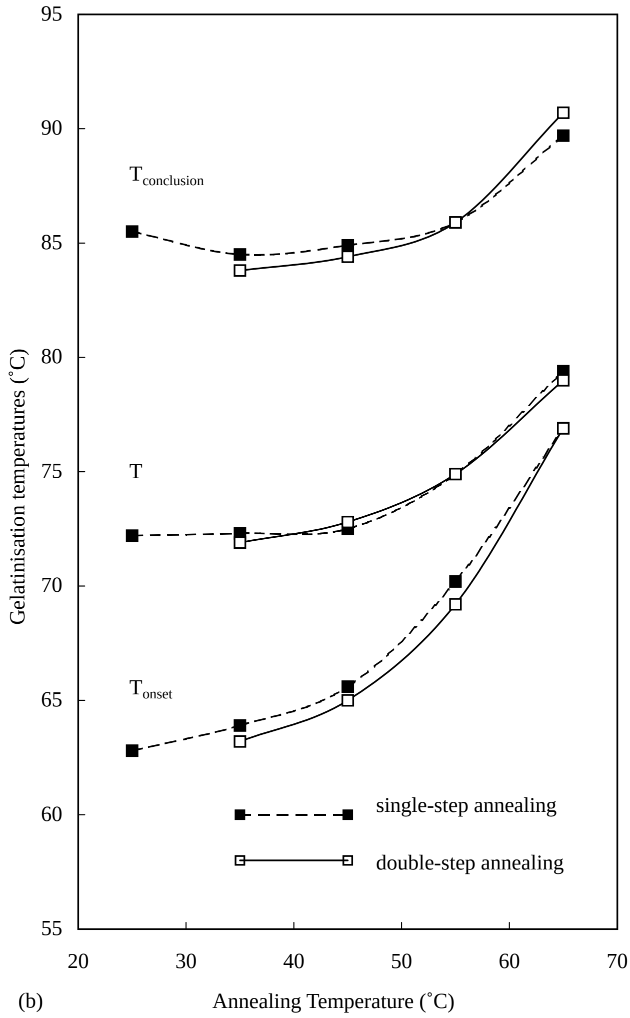

Fig. 4. (*continued*).

Knutson (1990) has discussed A-type crystallites (including wheat, waxy maize and maize starches) in the context of them being relatively stable (because they are densely packed), and consequently affected far less by annealing than B-type crystallites. In the A-type crystallites, the amylose and amylopectin fractions cause and exist in essence in separate domains. If we accept that the separate domains exist and that exterior chains of amylopectin dictate the theoretical enthalpy of fully ordered (at 100% double helical order) amylopectin (Cooke & Gidley, 1992; Moates et al., 1997), this would explain why the enthalpy (and extrapolated enthalpy) of annealed wheat starch with an average external chain length of amylopectin (ECL, incorporating A- and B1-chains) of 18.0 (Tester et al., 1998a) is lower than annealed maize and waxy maize starches (ECL 18.9 to 19.5, respectively) as presented in Table 6.

Imperfections in the amylopectin crystalline regions due to sub-optimal registration of the crystallites or helical length, are largely removed during annealing of the waxy and normal starches. This process makes it harder for amorphous regions to be hydrated as temperature is increased, and the trigger temperature for gelatinisation (hydration of amorphous regions with any associated glass transition of the amorphous material in these regions) to proceed is also increased. Hence the gelatinistion temperatures are increased and swelling is restricted.

Amylomaize responds quite differently to annealing than waxy or normal maize starches. Gelatinisation temperatures as a function of annealing temperature are presented in Fig. 4. T_o increases rapidly above 45°C while T_p is unaffected by annealing up to 65°C and ‘increases’ significantly only at 75°C—which is in fact due to partial gelatinisation. The end of the gelatinisation endotherm at T_c remains virtually unchanged, which also suggests that amylose–lipid complexes (with a dissociation temperature of 105.6–C) remain unchanged.

After annealing, the enthalpy of gelatinisation for the amylomaize starch increased very significantly between 20 and 65°C, but decreased between 65 and 70°C (Fig. 5). The magnitude of the increase is far beyond that which would be anticipated from waxy and normal starches. Single step annealing was, perhaps surprisingly, more effective at elevating ΔH than double step annealing. The increase in ΔH cannot be explained on the basis of annealing of amylose–lipid complexes, as T_c remained constant. We also assume that all of the lipid is complexed (Morrison et al., 1993a,b) and consequently the endotherm does not reflect the formation of more complexed amylose.

Knutson (1990) also found an increase in enthalpy of amylomaize starch after annealing and suggested that B-type starches have a higher degree of ‘intermingling’ of amylose and amylopectin. This would allow for more extensive interactions between amylose and amylopectin during annealing and consequently an increase in the gelatinisation enthalpy. In addition, Knutson proposed that B-type starches are more energetically disposed towards reforming (during annealing) to give A-type polymorphs although Stute (1992) and Hoover and Vasanthan (1994) showed that no changes in the X-ray diffraction pattern were detectable after pronounced annealing of B-type (potato) starches.

The number of double helices of the native amylomaize starch (38%, Table 7) supports the data of Shi et al. (1998) and the previously discussed view that amylose must account for some of the double helices as it exceeds the amylopectin content of the amylomaize starch (Table 2). The increase in the amount of double helical material post-annealing (Table 7) broadly reflects the increase of enthalpy of the single and double-step annealed starches (Fig. 5).

Annealing has a dramatic effect on the crystalline nature of amylomaize starch which is different to waxy and normal starches. This, we believe, is due to a number of effects. The DSC endotherm reflects the amount of hydrogen bonding (within double helices) in the starch. Where there is constancy or a very small increase in the endotherm, we

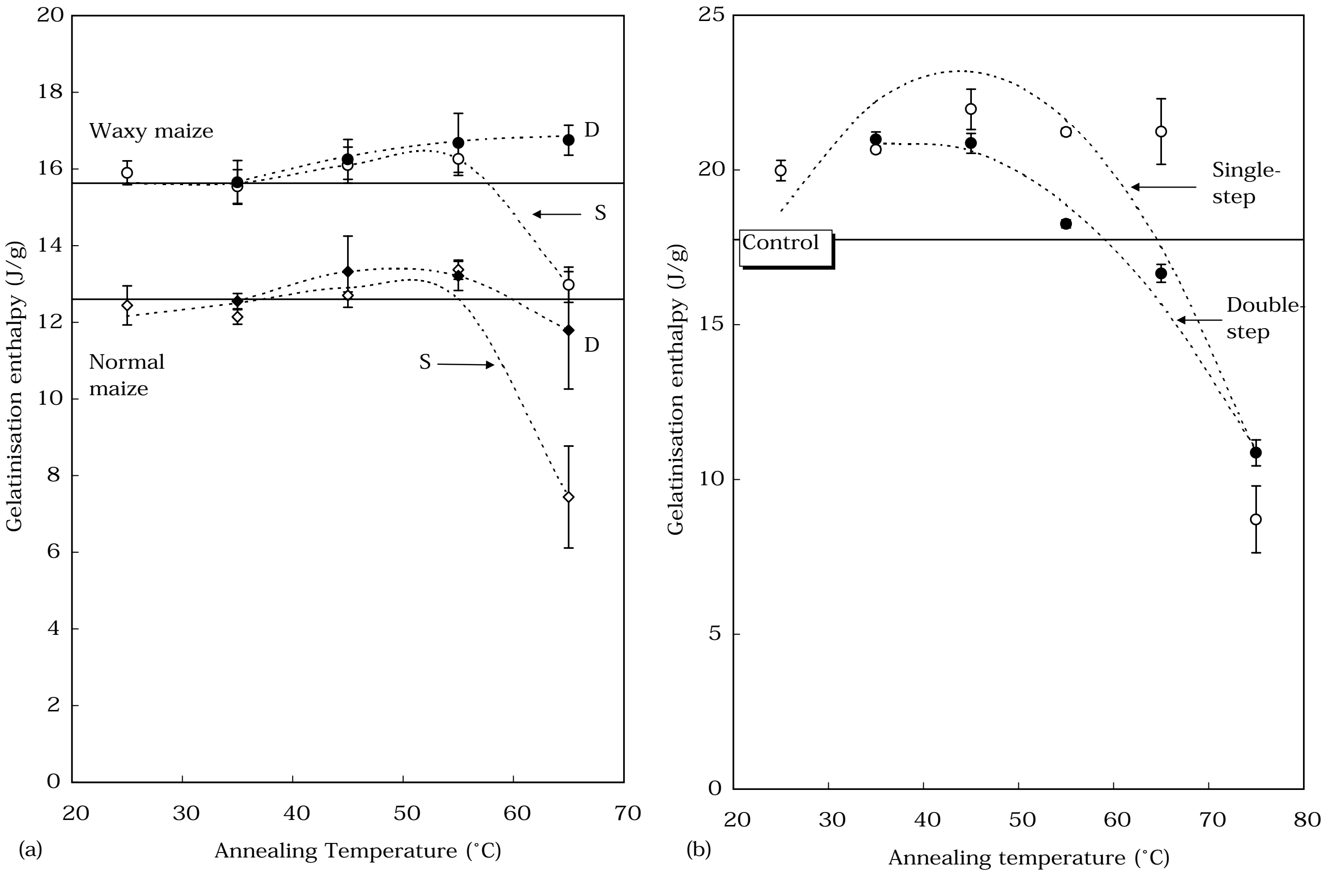

Fig. 5. Effect of single-step and double-step annealing (in excess water for 72 hours) at various temperatures on the gelatinisation enthalpy of maize starches. Solid lines represent the control values. (a) Waxy and normal maize starches; (b) amylomaize starch.

attribute this to constancy of helical numbers, but improved registration of the existing helices and perhaps the 'completeness' of the helices along their lengths (Tester et al., 1998a). In other words, annealing mechanisms in waxy and normal starches enhance the inter- (registration) and intra-helical interactions with little effect on enthalpy (as few extra intra-helical hydrogen bonds/double helices are formed).

The increase in enthalpy for the amylomaize starch, which is greater for single rather than double step annealing (Fig. 5), can be explained as follows. In the amylomaize starch the crystallites contain 'strains' induced by variations in helical structures and packing—comprising amylopectin–amylopectin, amylopectin–amylose (FAM) and amylose–amylose (FAM–FAM) interactions. Additionally, the amylose may occur as unstable single helices 'trapped' within amorphous regions as indicated by NMR (Gidley & Bociek, 1985,1988a,b;) and X-ray crystallography (Zobel, 1988,1992).

Associations in native amylomaize starches are by no means optimised in terms of potential helix numbers and lengths. During single-step annealing, however, the strains are diminished and additional double helical material is formed—both new helices and elongation of incomplete

Table 6
Pseudo-equilibrium values of gelatinisation enthalpies and thermal transition of annealed A-type starches

	ΔH^a (J/g)	ΔH(AP)^b (J/g)	Extrapolated ΔH (100% double helical order)^c (J/g)	T_p (°C)	ECL (A- and B1-chains)
Wheat	10.3–11.4	14.6–16.4	24.9	70	18.0
Waxy maize	16.7–16.8	16.8–16.9	33.3	79	19.5
Maize	12.1–13.4	16.9–18.7	33.3	80	18.9

[a] Enthalpy of annealed wheat (Tester et al., 1998a), waxy maize and maize (Fig. 5) starches.
[b] Enthalpy calculated on an amylopectin basis.
[c] Extrapolated order based on data of Cooke and Gidley (1992).

Table 7
Number of double helices by NMR for the native and annealed (single and double step) amylomaize starches

Starch	Amount of double helices, %
Native amylomaize	38 ± 2
Single step annealed	42 ± 2
Double step annealed	43 ± 2

pre-existing double helices. Relatively unstable single helices of amylose reform as double helix formation is favoured. The 'demixing' of amylose and amylopectin during annealing has been discussed elsewhere (Kalichevisky & Ring, 1987) and appears entirely logical. Compartmentalisation of the amylopectin and amylose is favoured during single step annealing but they are not entirely optimised. The increase in double helices is apparent from the NMR data (Table 7).

Double step annealing enhances double helix formation *and* compartmentalisation of the amylopectin and amylose (FAM, as the LAM is already compartmentalised). The apparent decrease in enthalpy by the dual step process is because amylopectin–amylopectin double helices and perhaps amylopectin–amylose double helices only are detected by the DSC in this temperature range. However, amylose–amylose double helices, now segregated into amylose (FAM) crystalline regions (akin to retrograded amylose) have an endotherm above the low temperature endotherm (well above 100°C for retrograded amylose). The very high dissociation temperature of this material (in water) does, however, make it difficult to quantify. In other words, the dual step annealing process effectively produces 'resistant starch' as a consequence of the segregation of amylose and amylopectin.

If we consider how the number of double helices (NMR) relates to enthalpy (DSC) of native and physically damaged starches (Morrison, Tester & Gidley, 1994) this gives an additional insight into what happens when amylomaize is annealed. With this in mind, the relationship between enthalpy and amount of double helices is approximately linear where a doubling in amount of double helices causes a doubling of enthalpy. If this can legitimately be extrapolated to the amylomaize starch, where the enthalpy increases by at least 25% post-annealing (at 50°C), this implies that the amount of double helices has also increased by 25%. This tends to be supported by the NMR data generated in this study where the amount of double helices increases by 11% after single step annealing and 13% after the double step.

4. Conclusions

For the waxy and normal maize starches, the similarity in gelatinisation characteristics and response to annealing can be explained on the basis that:

1. Amylopectin structure is essentially the same.
2. Amylose dilutes the amylopectin fraction—which is responsible for the gelatinisation enthalpy—and consequently reduces the enthalpy of the normal compared to the waxy starch.
3. The amylose fraction (mainly normal starches) is segregated into material which is lipid bound (LAM) and lipid free (FAM). The LAM fraction has a dissociation temperature in the range 96–104°C and does not directly interfere with the dissociation of amylopectin crystallites.
4. FAM may form double helices with amylose and amylopectin chains, although the high amylopectin content, short exterior chains of amylopectin compared to long amylose chains and favourability of the short exterior chains of amylopectin spontaneously forming double helices during biosynthesis make this unlikely in waxy and normal starches. The converse may in fact be true to some extent, whereby the FAM prevents optimisation of amylopectin double helix formation during biosynthesis. The FAM fraction is probably interspersed radially throughout the starch granule.
5. LAM is probably located in the amorphous shells of starch granules where it can restrict hydration of these regions and hence prevent the trigger of granule swelling and gelatinisation (assuming that hydration and swelling of these regions destabilises the crystalline domains).
6. The amylose and amylopectin fractions are in waxy and normal starches, therefore, segregated although they are co-synthesised. Upon annealing in unrestricting water, the amorphous regions hydrate (and proceed through a glass transition) and allow amylopectin exterior chains—which are not optimally registered (packed together) or completely double-helical along their lengths (especially at the ends)—to optimise their helical associations and structure. Hence, the number of double helices pre- and post-annealing remain constant but registration is optimised. These events cause the gelatinisation temperatures to increase, but with small (if any) increase in gelatinisation enthalpy.
7. For the native amylomaize starch, the data generated in this study indicate that the crystalline regions comprise amylopectin–amylopectin, amylopectin–amylose and amylose–amylose double helices, with perhaps some single (relatively unstable) helices.
8. Annealing of amylomaize starches facilitates compartmentalisation of intermixed amylose and amylopectin chains into amylose–amylose and amylopectin–amylopectin double helices.

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