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Polymer 41 (2000) 6361–6373

polymer

The effects of low temperatures on starch granule structure

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Received 20 July 1999; received in revised form 13 October 1999; accepted 8 November 1999

Abstract

Simultaneous small angle X-ray scattering, wide angle X-ray scattering and differential scanning calorimetry have been used to study the effects of sub-zero temperatures on the structure of hydrated starch granules. Results indicate that completely reversible compression of the starch lamellar structure occurs upon the freezing of water and ice formation. It is proposed that this compression, caused by the expansion of water upon freezing, results in preferential compression of the more 'open' amorphous lamellae which act as 'shock absorbers'. The lamellar repeat distance is reduced whilst periodicity is maintained. For solvents other than water, which do not expand on freezing, the behaviour is qualitatively different since no compression occurs. Freezing behaviour—as well as providing information about the micromechanical properties of starch granules—also has implications for cryo-microscopy studies of starch.

Keywords: Starch; Freezing; Small angle X-ray scattering

1. Introduction

Starch granules are found in various parts of plants—endosperm, leaf and root for instance. The granule is a natural source of polysaccharide polymers which are increasingly being used for a wide range of industrial purposes ranging from paper coatings to novel applications where biodegrability is seen as a positive advantage. Because it is a naturally derived source of polymer it can exhibit quite wide variations in its properties, and the botanical source is itself very important, as well as the environmental conditions during growth of the plant. Most wild starches contain two types of polysaccharide molecules: essentially linear amylose, and highly branched amylopectin, in the approximate ratio of 1:4 amylose:-amylopectin (although this is plant specific and can vary in both natural and genetically modified mutants). Both polymers are extremely high molecular weight, particularly amylopectin which may run to many millions. For such high molecular weights accurate determination is extremely difficult, and will not be attempted here. Also present in the natural granule are minor components such as proteins and lipids, which are present at very low levels and hence will not be discussed further here, although their role may be crucial both in diet and for other properties. In this paper three main sources of starch are used: potato, maize and waxy maize, all from commercial sources. Waxy maize is a naturally occurring mutant in which the amylose content is close to zero, whereas in the wild type maize it is around 30%. The starch is laid down in the plant in the form of granules, and it is structural changes within these granules which form the basis of the study presented here.

This study constitutes a detailed simultaneous small angle X-ray scattering (SAXS) and wide angle X-ray scattering (WAXS) and differential scanning calorimetry (DSC) investigation of the effects of sub-zero temperatures on starch granule structure. This work builds upon the preliminary experiments performed by Waigh et al., the results and interpretation of which are described elsewhere [1,2]. As identified by Waigh [2], the effects of low temperatures and freezing on the structure of starch granules are of interest for three main reasons:

- *Storage and damage*. The sub-zero storage of frozen starch based food products is common practice. Information about how starch structure, at the lamellar and molecular levels, is altered during freezing and freeze–thaw processing may prove to be of importance. Details about the stability of the starch granule to temperature extremes are also of general interest.
- *Characterisation*. Electron microscopy studies of starch granule structure are often performed at significantly sub-zero temperatures. Low temperatures are used to reduce the effects of probe beam damage, for instance in TEM studies [3,4]. The investigation of hydrated

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0032-3861/00/$ - see front matter
PII: S0032-3861(99)00813-7

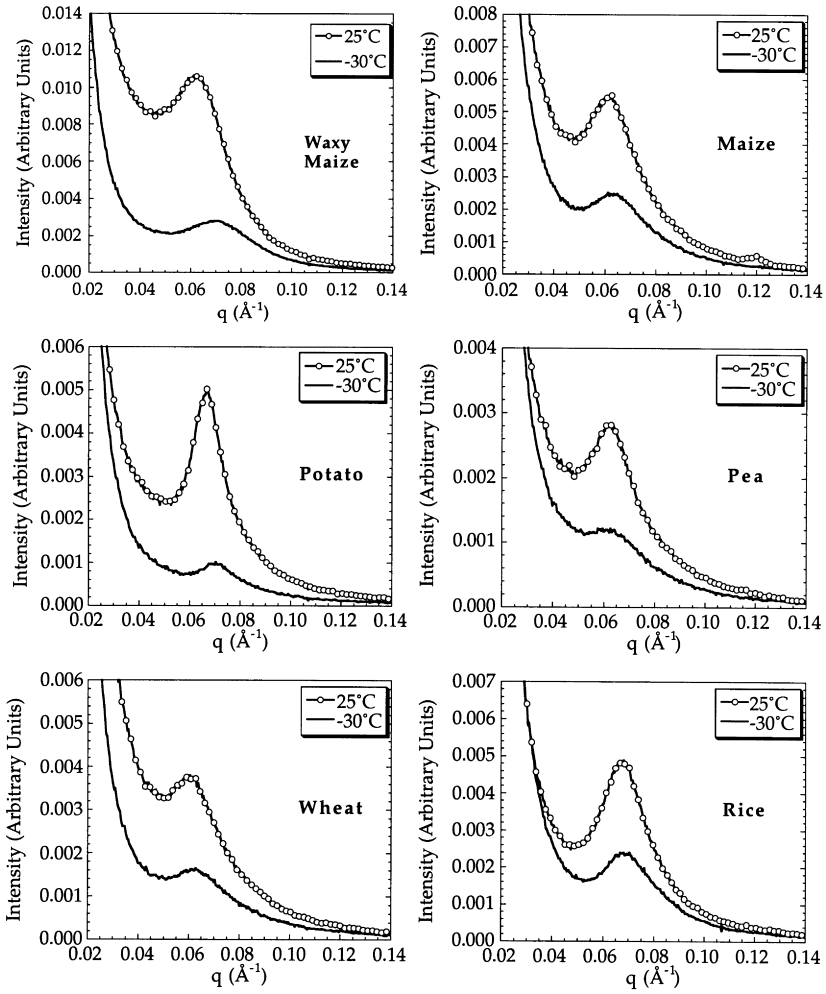


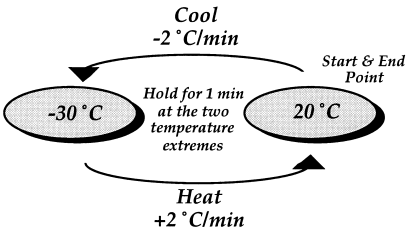
Fig. 1. SAXS from a range of hydrated starches at 25 and −30°C.

starch samples by other techniques such as nuclear magnetic resonance (NMR) spectroscopy often also requires significant cooling [5]. It appears probable that sub-granular structures detected at these low temperatures will be different from those found in native starch samples at room temperature. This requires further investigation.

- *Micromechanical properties*. Little is known about the mechanical properties of starch at the sub-granular level. In this context it is of interest to investigate such issues as whether the starch lamellar structure can be compressed without loss of granular integrity and how granular structure is altered when molecular motion is slowed and stresses are applied on the nanometre lengthscale.

Starch, when hydrated to levels of greater than 30% (w/w) water, adopts a well-defined periodic lamellar arrangement, with an average lamellar repeat distance of approximately 9 nm developing [6]. This is indicated by the presence of a broad peak within SAXS patterns. Water is also incorporated into crystalline unit cells resulting in the appearance of characteristic A-, B- and C-type starch X-ray diffraction patterns. A representation of the hierarchical structure of hydrated starch granules, developed by Cameron [7,8] can be found in the papers of Donald and co-workers [9,10]. This model incorporates many aspects previously reported by earlier workers but, in the context of the work presented here, has the particular advantage that it permits detailed fitting of the small angle scattering curve, permitting quantification of changes that occur during different processing histories. For a fuller discussion of this model in the context of others extant in the literature, the reader is referred to Ref. [11]. The model fit is a six-parameter fit, all six parameters having a clear physical meaning as described in Refs. [8–10]; the meanings of each are listed in Table 2.

Waigh and co-workers [1,2] reported that cooling hydrated potato and waxy maize starches to sub-zero temperatures resulted in a pronounced drop in the intensity of the lamellar repeat marked by the prominent SAXS peak. This loss of scattered intensity was found to occur over a narrow temperature range. Treating the lamellar structure of starch as being analogous to a side-chain liquid-crystalline polymer, Waigh [2] proposed that this phenomena was



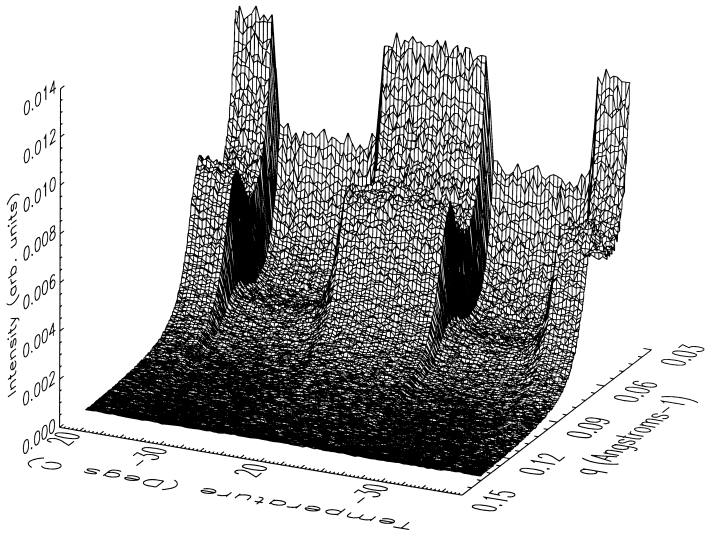


Fig. 2. Variation in SAXS during temperature cycling of hydrated waxy maize starch. Temperature profile starts (20°C) at the left-hand side of the plot.

caused by a loss of molecular mobility which occurs upon cooling below the glass transition temperature of the amorphous lamellar regions. It was argued that this loss of mobility leads to the collapse of the amorphous lamellae and the formation of a 'rippled' lamellar structure. Loss of long-range correlation due to lamellar collapse was cited as the physical cause of the loss of the SAXS peak.

The present study builds on the previous work, and constitutes a detailed simultaneous SAXS and WAXS and DSC investigation of the effects of sub-zero temperatures on starch granule structure. The results lead to the drawing of alternative conclusions about both the cause and structural basis of the transition.

2. Experimental

All starches used are of commercial origin, and as such there is no control over the precise amounts of amylose and amylopectin. However, since it is the change in response with temperature, and the magnitude of the changes between species, which is the focus of the paper, this is not thought to be a limiting factor.

2.1. Simultaneous SAXS/WAXS

Simultaneous SAXS/WAXS experiments described in this study were performed on station 8.2 at the Synchrotron Radiation Source at the Daresbury Laboratory, Cheshire, UK. The small angle detector camera length was set between 1.5 and 3.5 m, providing a range of q values from approximately 0.009–0.500 $Å^{-1}$. All samples were made up as starch slurries of approximately 40–45% (w/w) starch in the appropriate solution and pure solvent. For the study of plasticised starch, vacuum dried starch samples were solvated and plasticised with ethylene glycol (pure or mixed with water) and butane-1,4-diol, and also made up to 40% (w/w) starch in solvent at room temperature. All samples were investigated in aluminium DSC pans, supplied by TA instruments, which were modified in an attempt to avoid strong absorption of X-rays in the manner described by Bras [13]. The temperature of the samples was controlled using a Linkam hot stage, acting as a basic heat flux DSC.

All data were corrected with respect to the incident flux, the mass of the sample and the detector efficiencies prior to subtraction of the empty cell and liquid scattering. Corrected data were calibrated using the well characterised scattering from wet rat tail collagen (SAXS) and high density polyethylene (WAXS). More details about the experimental station specifications and operation can be found elsewhere [12–14].

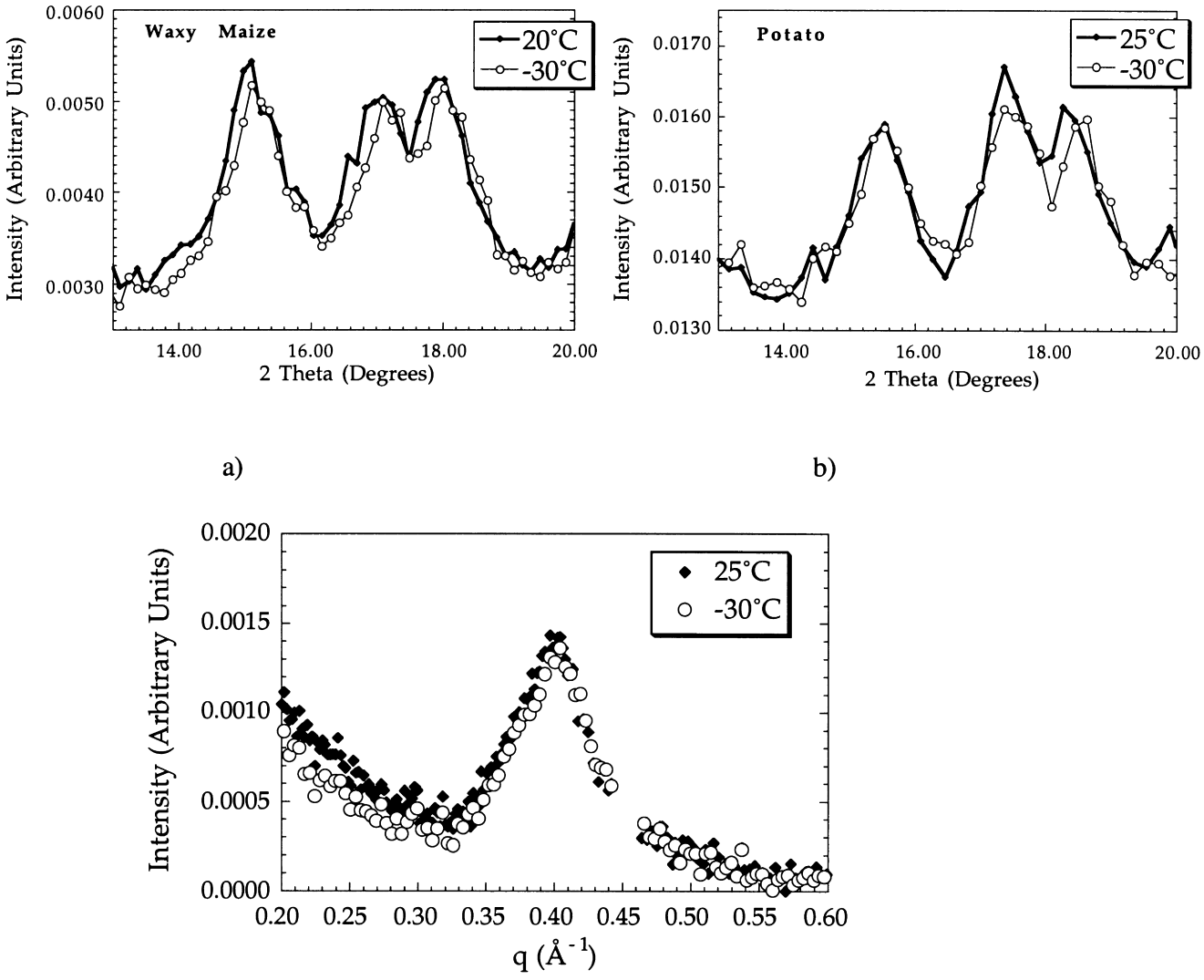


Fig. 3. Variation upon cooling to −30°C in the WAXS patterns of waxy maize and potato starch (top) and the 100 inter-helix reflection of potato starch (bottom).

2.2. Differential scanning calorimetry

A Perkin–Elmer power compensated DSC-7 equipped with an Intracooler II was used for all experiments, with an empty sample pan being used as a reference in all cases. Temperature and enthalpy parameters were calibrated using the melting transition of indium. The majority of samples were investigated in standard Perkin–Elmer aluminium 40 μl sample. Perkin–Elmer 60 μl Large Volume Capsules (LVCs) which could withstand internal pressures of up to 240 atm., were used at high temperatures where the possibility of solvent loss was greatest. Sample masses were 10–25 mg.

3. Results

Fig. 1 illustrates the form of the small angle scattering transition observed upon rapidly quenching six hydrated starches from 25 to −30°C. Although the precise form of the transition varies between starch cultivars, in all cases the SAXS peak exhibits a pronounced drop in intensity and a shift to higher q values.

Real time SAXS/WAXS was recorded during dynamic cooling and reheating of a variety of starch cultivars. As an example the variation in SAXS from waxy maize starch is shown in Fig. 2. The dynamic temperature cycle profile used is also shown.

Fig. 2 shows that the intensity of the SAXS peak drops abruptly upon cooling. The intensity remains at this low level upon further cooling, before abruptly returning to its original intensity upon reheating. It was found that the behaviour of all starches was qualitatively identical. In all cases, upon cooling a slight reduction in peak intensity was found to precede a *step change* drop in intensity, occurring over a temperature range of less than 0.5°C. All starches investigated exhibited transition temperatures in the range −6 to −14°C. Cooling further below the transition

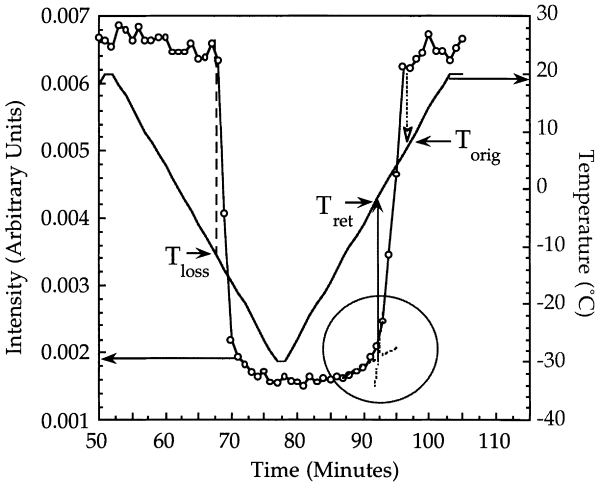


Fig. 4. A plot of the variation of the intensity of the SAXS peak during a temperature ramp. The temperature profile is shown by the zigzag line as a function of time, the temperature being read off the right-hand axis. The peak intensity, in arbitrary units, can be found at any given time in the cycle from the left-hand axis. From this plot, three key temperatures can be read off: the temperature, T_{loss} at which the intensity first falls; the temperature T_{ret} at which the intensity first starts to climb on reheating; and the temperature T_{orig} at which the intensity returns to its original value.

temperature did not lead to significant additional changes in SAXS intensity. In a separate experiment, small angle scattering was found to be unchanged after rapidly freezing and reheating hydrated starch samples in a cycle of 30 repetitions. Such extensive freeze–thaw processing does not permanently affect room temperature starch lamellar structure.

WAXS data, collected simultaneously with the SAXS data showed that, unlike SAXS, hydrated starch diffraction patterns (both A- and B-type) were not significantly affected by cooling to sub-zero temperatures. Low temperatures do not appear to alter in any way the starch crystalline unit cell or the degree of order and packing. Representative WAXS data from hydrated waxy maize (A-type, Fig. 3a) and potato (B-type, Fig. 3b) starches at 25 and −30°C are shown in Fig. 3. Also shown in Fig. 3c is the effect of sub-zero temperatures on the 100 (5.5° 2θ) inter-helical diffraction peak from potato starch.[1] It can be seen that the inter-helix distance is unchanged, as shown by the invariance of the peak position, and there is no evidence for peak broadening, which would be indicative of crystalline distortion and helical 'splaying'. It appears clear that the observed SAXS transition is caused by a change which predominantly only influences structure on the lamellar length scale or above, in the direction of the lamellar perpendicular.

Returning to consideration of the loss and return of the SAXS peak, various temperatures can be identified for the transition (T_{loss}, T_{ret} and T_{orig}). An explanation of the meaning of these temperatures in relation to the SAXS peak intensity plots is shown in Fig. 4. Table 1 presents their values during temperature cycling of waxy maize and potato starches. It can be seen that, whilst SAXS peak intensity is lost over a very narrow temperature range, peak intensity returns over a temperature range of approximately 10–12°C. Upon reheating, the return of SAXS peak intensity is initiated at a temperature some 6–8°C above that at which it is lost upon cooling. The peak does not fully return until a temperature significantly above 0°C.

Table 1
Temperatures defining the loss and return of the SAXS peak for waxy maize and potato starch. Uncertainties derive from experimental scatter as well as the difficulty in determining unique temperature values from the given data

	T_{loss} (°C)	T_{ret} (°C)	T_{orig} (°C)
Waxy maize	−11 ± 2	−3 ± 2	+7 ± 2
Potato	−9 ± 1	−3 ± 2	+8 ± 2

As well as the drop in peak intensity upon cooling, all starch samples investigated exhibit an increase in the q position of the SAXS peak at the SAXS transition.

As well as changes in starch crystalline structure, WAXS was also used to investigate changes in the state of the water component of the hydrated starch system. Fig. 5 illustrates the appearance of sharp crystalline peaks in the WAXS pattern from hydrated potato starch at the same temperature as the SAXS intensity drops (−9.85 ± 0.25°C). The diffraction peaks moving from left to right in Fig. 5a were respectively indexed as the 010/100, 002 and 011/101 reflections from ice (type I) crystals.[2] Fig. 5b shows data recorded during temperature cycling of hydrated waxy maize starch. Fig. 5c plots the temperature dependence of the SAXS and crystalline ice diffraction peak intensities.

Fig. 5 shows that for waxy maize starch and in all other cases for all starch cultivars investigated (data not shown):

- SAXS peak intensity drops at the same temperature as ice forms.
- Initiation of SAXS peak intensity return correlates with the onset of ice melting.
- SAXS peak intensity returns to its original level at the same temperature at which ice melting is complete (WAXS intensity returns to the room temperature baseline).

3.1. Solvents other than pure water

Initially, granules in non-aqueous solvents show no peak in the SAXS pattern. The evolution of the peak required increased temperature and/or time to permit solvation to takes place. This solvation is discussed elsewhere [15], and will be the subject of a separate paper. The experiments on freezing of interest here were performed on fully

[1] The 100 reflection is observed in the SAXS pattern with the experimental geometry used in this work. It is a systematic absence of the A-type starch unit cell.

[2] Crystallographic data taken from Eisenberg [18].

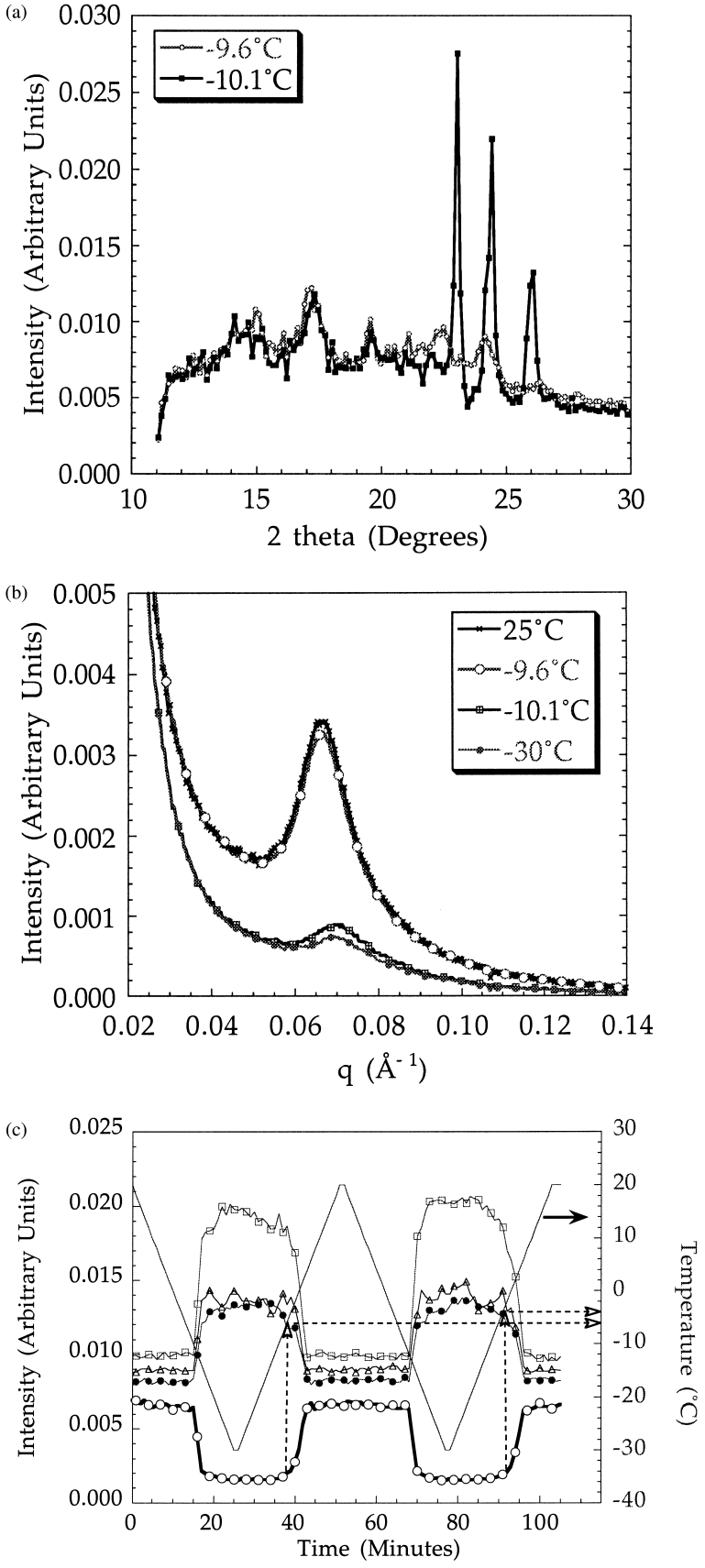


Fig. 5. Coincidence of: (a) crystalline peak appearance in WAXS pattern and the SAXS transition; (b) for hydrated potato starch; (c) variation in the intensity of the SAXS peak (-○-) and ice peaks for waxy maize starch (- ○ -) and ice peaks for waxy maize starch ((- △ -) ice 002 peaks; (- ● -) ice 011/101 peak; and (- □ -) ice 010/100 peak) during a temperature cycle shown by the zigzag line. Data are displaced vertically for clarity. Dashed lines designated T_{ret} (see Fig. 4).

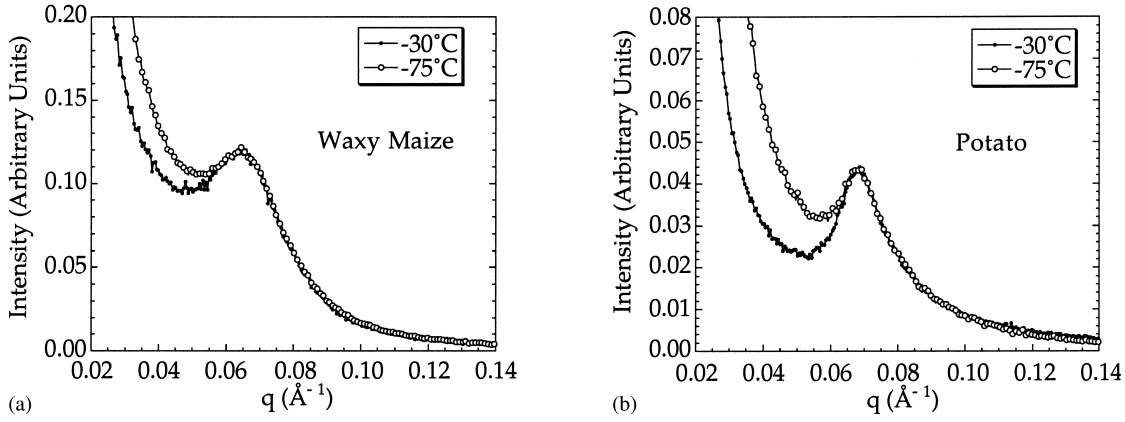


Fig. 6. Variation in SAXS upon cooling waxy maize and potato starches plasticised with ethylene glycol.

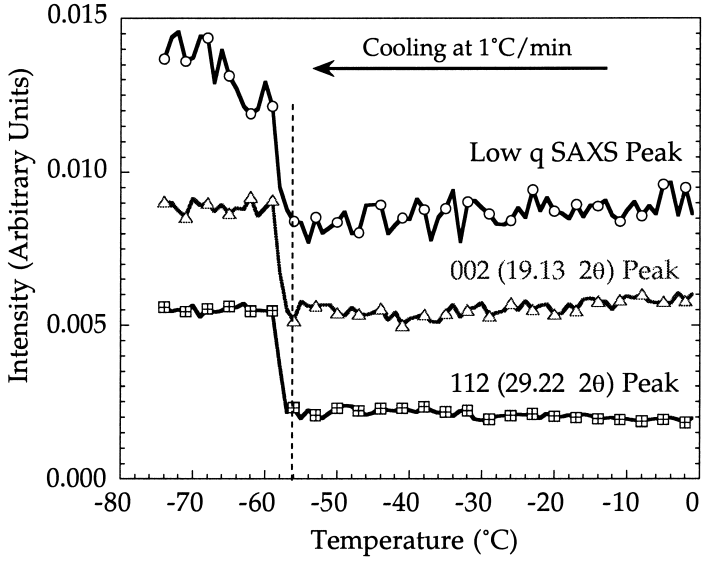


Fig. 7. Variation in the intensity of low q (0.023 Å^{-1}) scattering and crystalline ethylene glycol peaks for maize starch in ethylene glycol cooled at 1°C min^{-1}.

solvated and plasticised slurries. Static temperature cycle data is shown in Fig. 6 for waxy maize and potato starches plasticised with ethylene glycol.

As with hydrated starch samples, the change in SAXS, which in this case is characterised by the 'raising up' of the scattering at low q, occurs as a step change transition, over a temperature range of less than 0.5°C (data not shown). Importantly, the SAXS transition was found in all cases to be coincident with freezing of the constituent solvent—ethylene glycol or butane-1,4-diol. Fig. 7 shows the step change increase in the intensity of SAXS in the low q region and the coincident appearance of the crystalline ethylene glycol peaks.[3] The low q SAXS data were obtained by recording the variation at a q value of 0.023 Å^{-1}, this value being arbitrarily chosen as being a point on the curve which varied sharply in intensity at the SAXS transition. Exactly the same form of transition and coincident solvent freezing was found with butane-1,4-diol. Only the temperature at which the transition was found to occur differed between solvents: -56 ± 4°C for ethylene glycol and -14 ± 3°C for butane-1,4-diol.

Finally, the effect of the addition of small amounts of ethylene glycol to water (this depresses water's freezing and melting point temperatures[4]) was explored. The results are shown in Fig. 8, which is analogous to Fig. 4, representing the temperatures at which the sharp drop in SAXS intensity is observed for additions of 5 and 10% ethylene glycol. It can be seen there is a systematic depression in the

[3] Ethylene glycol crystallises in an orthorhombic unit cell, with space group $P2_12_12_1$.

[4] It is appreciated that adding such a co-solvent will also affect the density change upon freezing.

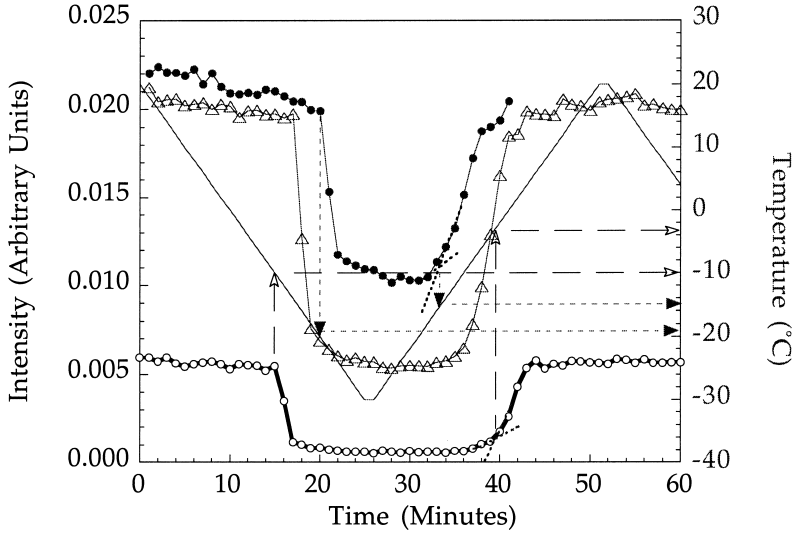


Fig. 8. Variation in the SAXS peak intensity of waxy maize starch in pure water (- ○ -) and 5% (- △ -) and 10% (- ● -) w/w ethylene glycol solutions. T_{loss} and T_{ret} are designated by dashed lines for pure water and dotted lines for 10% ethylene gylcol. The temperature profile is indicated by the zigzag line, with heating and cooling rates of 2°C min^{-1}, and a hold of 1 min at each extreme.

transition temperature for loss of SAXS intensity, correlating with the depression in freezing point.

4. Discussion and interpretation

4.1. Modelling scattering profiles

SAXS patterns obtained from a range of starch cultivars were fitted using the six parameter paracrystalline model function developed by Cameron and Donald [7,8,16]. This model treats the starch structure as being composed of three distinct regions: crystalline and amorphous lamellae, which combined constitute the semi-crystalline growth rings, and an amorphous background, which is equated with the amorphous growth rings. Extensive descriptions of this three-phase model are given in the original publications of Cameron and Donald (see, e.g. Refs. [7,8]). Modelling was carried out to determine the extent of any variations upon cooling in the average lamellar repeat distance, d, the fractional lamellar crystallinity, φ, the width of the distribution of lamellar sizes, β, and the electron density differences between the three regions of the starch granule. The model is very insensitive to the precise value of the parameter N, which represents the number of semi-crystalline repeats within each semi-crystalline growth ring. Model fitting was carried out with the NAG least squares fitting routine, E04JAF.

Fits to the SAXS pattern recorded at 25 and −30°C from maize and waxy maize in water, are shown in Fig. 9. Table 2 presents the best-fit structural parameters as well as summarising the meaning of these parameters. As waxy

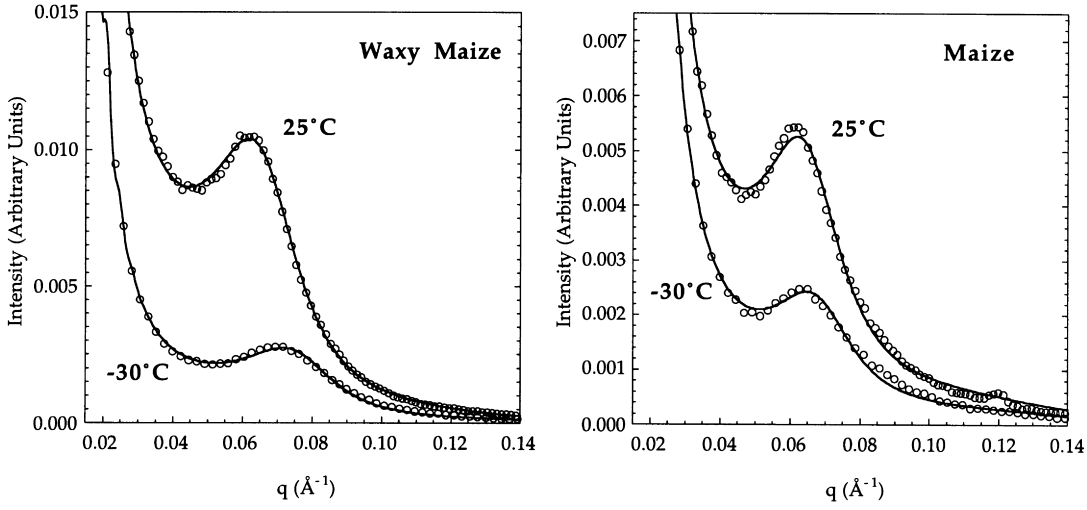


Fig. 9. SAXS profiles (open circles) and the associated model fits (solid lines) from waxy maizes (left) and maize (right) starches at 25 and −30°C.

Table 2
Variation in the six independent structural parameters determined from the model fitting of waxy maize and maize starches at 25 and −30°C. The bottom section gives the definitions of the various structural parameters used in the modelling of SAXS from starch

	Waxy maize		Maize	
	25°C	−30°C	25°C	−30°C
d (Å)	85.0 ± 0.3	76.7 ± 0.8	88.1 ± 0.5	84.6 ± 0.7
N	22 ± 2	22 ± 2	22 ± 2	22 ± 2
β	0.39 ± 0.01	0.40 ± 0.01	0.34 ± 0.01	0.34 ± 0.01
φ	0.64 ± 0.01	0.71 ± 0.02	0.76 ± 0.01	0.78 ± 0.02
$\Delta\rho$ [a]	1.00 ± 0.02	0.60 ± 0.07	1.00 ± 0.03	0.70 ± 0.08
$\Delta\rho_u$	0.00 ± 0.05	−0.2 ± 0.1	−0.24 ± 0.06	−0.5 ± 0.1
ρ_c	Electron density of the crystalline lamellae			
ρ_a	Electron density of the amorphous lamellae			
ρ_u	Electron density of the amorphous growth rings			
$\Delta\rho$	Electron density difference between the crystalline and amorphous lamellae $\rho_c-\rho_a$			
$\Delta\rho_u$	Electron density difference between the two amorphous regions $\rho_u-\rho_a$			
d	Average lamellar repeat distance			
N	Average number of lamellar repeats in a semi-crystalline stack			
φ	Fraction of the lamellar repeat distance which is crystalline			
β	Width of the lamellar size distribution functions			

[a] The value of $\Delta\rho$ is scaled so that $\Delta\rho$ at the initial temperature is equal to 1.0.

maize starch exhibits a far greater variation in peak position than other starches upon cooling it is used as an (extreme) example in all data. Maize starch is used here as an example of the other starches, which all exhibited very similar trends upon cooling.

Model fitting of the SAXS patterns indicates that the value of d decreases upon passing through the SAXS transition. This reduction suggests that the lamellar system is compacted upon freezing. In the case of waxy maize starch d drops from approximately 8.5 to 7.7 nm, a decrease of approximately 10%, whereas maize and other starches show a less pronounced decrease. The value of β, the measure of the width of the distribution of lamellar sizes, is effectively unchanged in both waxy maize starch and other cultivars. The invariance of β is interpreted as a lack of significant variation in the distribution of average lamellar repeat distances, centred about the mean value, d. The fraction of the total repeat distance which is crystalline, φ, is seen to increase upon freezing. This is consistent with lamellar compaction occurring during freezing, with the amorphous lamellae being compacted to a greater extent than the crystalline lamellae (as would be expected on the basis of crystalline regions having a higher modulus). As before, waxy maize starch is seen to exhibit more pronounced variation than maize. Reduction in the two density contrast terms, $\Delta\rho$ and $\Delta\rho_u$, upon freezing supports the idea of preferential compaction of the amorphous lamellae. Reduction in both of these parameters is consistent with an increase in the density of the amorphous lamellae, ρ_a, with this density change being greater than any change in the densities of the crystalline lamellae or amorphous growth rings. An increase in ρ_a can be attributed to compaction of the amorphous lamellae at constant mass. Again, it is found that after a gradual continuous decrease, $\Delta\rho$, $\Delta\rho_u$ drop as a step change at the SAXS transition temperature. This proposal of amorphous lamellar compaction, is in accord with the work of Morgan et al. [17], where it was claimed that the amorphous regions of starch granules may be expected to act as 'shock absorbers' upon the application of compressive forces. Compaction of the amorphous lamellae as the primary mechanism for the loss of SAXS peak intensity is also supported by the observed correlation between the extent of shift in SAXS peak position brought about upon freezing and the reduction in peak intensity.

This analysis can be pushed further. Since the SAXS peak arises from electron density contrast between the crystalline and amorphous lamellae, $\Delta\rho$, using the $\Delta\rho$ values shown in Table 2, the contribution to the decrease in SAXS peak intensity which can be attributed to reductions in $\Delta\rho$ (and bearing in mind that intensity is proportional to $\Delta\rho^2$) can be evaluated very simply:

$$\text{Maize}: \quad (\Delta\rho^2_{25°C}/\Delta\rho^2_{-30°C}) = 1/0.7^2 = 2.0$$

$$\text{Waxy Maize}: \quad (\Delta\rho^2_{25°C}/\Delta\rho^2_{-30°C}) = 1/0.59^2 = 2.9$$

Comparing these values with the measured reduction in peak intensity based on the SAXS patterns recorded at 25 and −30°C for these two starches (shown in Fig. 1), it is found that there is very good correlation for maize starch, but not as good for waxy maize. For maize starch the predicted drop in peak intensity is 2.0, as compared with the experimentally determined drop of 2.2. Thus in the case of maize starch effectively all of the drop in SAXS peak intensity can be accounted for by variation in scattering density contrast alone. For waxy maize starch, the drop in SAXS peak intensity produced by variation in $\Delta\rho$ is seen to be significantly less than that actually observed (2.9 as compared to approximately 4.6).

This suggests that there may be additional structural changes occurring during the freezing of waxy maize starch as compared to other starch cultivars, such as maize. This would be consistent with the trend for β, the degree of paracrystalline distortion, to increase upon freezing waxy maize starch (although this effect is very small). One possible additional structural change may be that originally postulated by Waigh et al. [1], in which the starch lamellar structure becomes irregularly 'buckled'. This we no longer believe to be the dominant mechanism since, if such a structural change were predominantly responsible for loss of peak intensity, then it would be expected that the width of the distribution of lamellar repeat distances (as represented by β) would increase, and the SAXS and 100 crystalline inter-helical peaks would broaden due to a reduction in lamellar and helical order. Experimental evidence as presented in Table 2 and Fig. 3 does not support either of

these expectations. β is unaffected upon freezing and peak broadening is seen not to be significant. However, although lamellar 'buckling' and loss of periodicity may not be the major mechanism by which the SAXS peak intensity is lost in the majority of starch cultivars, it may still play a minor role—and its contribution could be most significant for waxy maize for reasons discussed below.

4.2. Causes of lamellar compaction

In all cases investigated the formation of ice has been found to be coincident in temperature with the SAXS transition, and the melting of ice correlates with the return of SAXS peak intensity. Upon freezing, due to the reduced density of ice as compared to liquid water (0.92 and 1.00 g cm^{-3}, considering a temperature of -20°C and effectively free water, respectively [17]), the volume occupied by 'freezeable' water will increase. It is therefore proposed that freezing of water external to the lamellae leads to *compression* of the amorphous lamellae. At constant mass this compression would account for the increase in the electron density of the amorphous lamellae. Whether the site of the freezing water is within the amorphous growth rings, or external to the granule, is not clear. However, no sign was ever seen for two freezing temperatures in the DSC, which might be expected if there were two types of water within and external to the granule, so it is possible that the distinction is academic (or that the water in the amorphous growth rings of the granule itself never freezes over the temperature range we investigated).

Two other possible causes of lamellar compaction can be envisaged, both of which involve contraction and collapse of the lamellar structure as opposed to externally induced compression. The first alternative explanation is that water may migrate from the amorphous lamellae upon freezing due to thermodynamic reasons, such as osmotic pressure variations and the enthalpic bonus derived from crystallisation. Collapse of the amorphous lamellae would be the result of the loss of plasticising solvent. The second alternative, which was described at the beginning of this paper, is that the lamellar structure collapses upon cooling below the glass transition temperature (T_g) of the amorphous lamellar regions [2].

These two alternative possible causes of lamellar compaction and SAXS peak intensity loss are objected to, as they would be expected to result in loss of periodicity, peak broadening and lamellar 'buckling'. It has already been shown that there is no evidence for lamellar collapse and periodicity loss upon freezing the majority of starch cultivars. On the contrary, the view that it is a compression-driven SAXS transition is also supported by two other experimental findings, concerning the behaviour in solvents other than pure water.

Fig. 8 shows that varying the freezing and melting point transition temperatures of water (achieved by adding small amounts of ethylene glycol to bulk water) does not lead to a separation of the SAXS transition temperature from the freezing temperature of the water. The SAXS peak intensity is always found to drop at the point at which ice forms, and increases again when the ice melts, no matter what the transition temperatures actually are. If a glass transition were the cause of lamellar compaction, it would be expected that the SAXS transition and ice formation temperatures would be separated upon freezing and melting point temperature depression.

Expansion of water upon freezing being the cause of the compression is also supported by the behaviour observed upon cooling starch plasticised with non-aqueous plasticisers such as ethylene glycol, glycerol and butane-1,4-diol, which increase (rather than decrease) in density upon freezing leading to a contraction. Fig. 6 illustrates that the form of the SAXS transition which is coincident with the freezing of ethylene glycol (and butane-1,4-diol although this is not shown) is very different from that observed in hydrated starch. Whereas with water a large drop in SAXS peak intensity and a shift of the peak to higher q values is found, neither phenomena are observed upon freezing ethylene glycol. This supports the view that if solvent expansion does not occur, the lamellar regions are unaffected by the freezing. If the SAXS transition was due to the cooling below of a T_g, or the migration of solvent from the amorphous lamellae, then lamellar collapse and a drop in SAXS peak intensity would still be expected. Instead, an increase in the intensity of scattering at low q is observed, with there being no apparent effect on the position of the SAXS peak.

This increase in intensity of low q scattering suggests that there is a shift of the amorphous growth ring peak to higher q values, indicative of a constriction of these amorphous growth ring regions. This in turn is consistent with solvent freezing within the amorphous growth ring, with an accompanying reduction in solvent volume and consequent decrease in volume of the whole region. There will be few (although some) connecting molecules between the amorphous and semi-crystalline growth rings, so contraction of the amorphous growth rings would not be expected to produce significant expansion of the semi-crystalline growth rings. This finding also supports the contention that water may freeze within the amorphous growth ring regions of hydrated starches at the SAXS transition, but cannot be regarded as firm proof.

4.3. A model for the low temperature behaviour of starch

Based upon the experimental data presented, a model of the freezing of hydrated starch is now proposed. The model is represented schematically in Fig. 10:

- The small but continuous decrease in SAXS intensity observed at sub-zero temperatures is attributed to a reduction in entropy and molecular mobility within the amorphous regions of the starch granule. As a result of

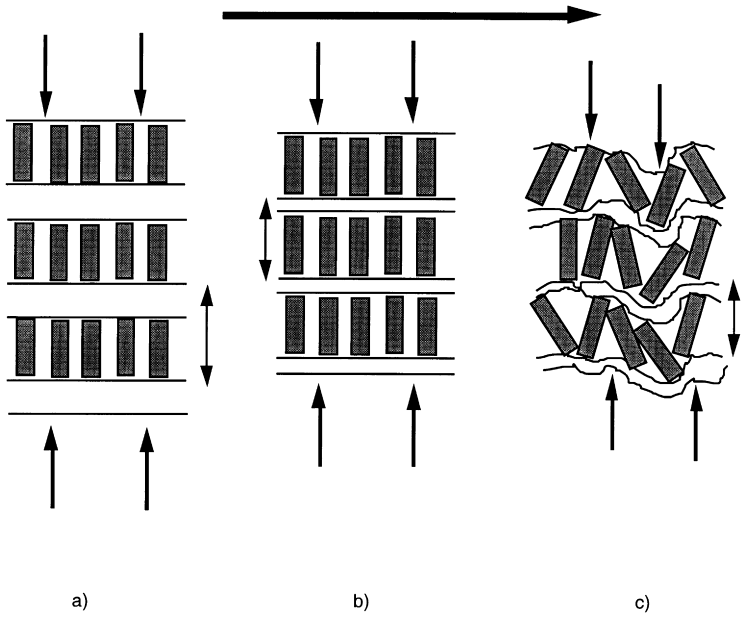


Fig. 10. Schematic representation of the processes occurring on the lamellar level during the freezing of starch: (a) Water external to the semi-crystalline growth ring freezes. This leads to volume expansion in regions external to the lamellae. The lamellae are placed in compression. (b) Compression leads to preferential compaction of the amorphous lamellae, and the repeat distance d is reduced. The electron density difference $\Delta\rho$ is also reduced so the SAXS peak intensity falls. (c) Upon reaching maximum compaction of the amorphous lamellae, further stress may be accommodated by lamellar 'buckling'. This leads to an increase of the distribution of lamellar sizes and the SAXS peak broadens.

this loss of mobility, polymer chains within the amorphous regions contract, leading to tighter coiling. This results in a continuous increase in density within amorphous regions and a consequent continuous reduction in the lamellar scattering density contrast.

- Bulk water freezes. This is possibly accompanied by the freezing of water within the large-scale amorphous growth rings.
- Due to the reduced density of ice as compared to liquid water, the volume occupied by water external to the lamellae increases. Expansion of water places the semi-crystalline growth ring under compression. Ice formation within the amorphous growth ring regions may account for their apparent lack of compression, as well as making the transmission of stress over large granules easier to visualise, but is not necessary to account for the overall compression.
- The amorphous lamellae are compressed, whilst the crystalline lamellae remain largely unchanged, due to their higher degree of order and rigidity. This is in accord with the claim of Morgan et al. [17], that the amorphous regions of starch granules may be expected to act as 'shock absorbers' upon the application of compressive forces. It is also consistent with the general way in which amorphous regions within lamellar systems 'protect' regions of crystallinity by preferential compression upon impact.
- At the limit of lamellar compression, excess compression may be accommodated by lamellar 'buckling', which leads to the formation of a non-correlated, rippled structure within the semi-crystalline growth ring. The resulting increase in the degree of disorder and aperiodicity produces further loss of peak intensity. It is thought that 'buckling' only occurs to any significant degree in waxy maize starch, which exhibits the greatest extent of lamellar compression. It is postulated that extensive lamellar compression may be a necessary precursor of 'buckling'.
- The reverse of this whole process occurs upon reheating. In this case, the shape of the SAXS transition is less sharp than that observed upon cooling, as the lamellar structure relaxes back to its pre-frozen state upon removal of the compression force. Relaxation begins at the point at which ice melting is initiated.
- It is proposed that the anomalous freezing behaviour of waxy maize may be due to the lack of amylose within the granule. There is evidence of correlation both between the amylose content and the drop in SAXS peak intensity, as well as the amylose content and the amount of the shift of the SAXS peak to higher values of q (smaller d). These correlations are shown in Fig. 11. Starch cultivars with lower amylose contents are found to exhibit a greater drop in peak intensity and a greater shift in peak position. It is hypothesised that the presence of amylose within the amorphous lamellae in some way restricts the amount of lamellar compression possible. Entanglements between linear amylose chains and amylopectin helices may act as a form of temporary physical cross-link, making the amorphous lamellae relatively rigid and incompressible. Another possible scenario is that the presence of

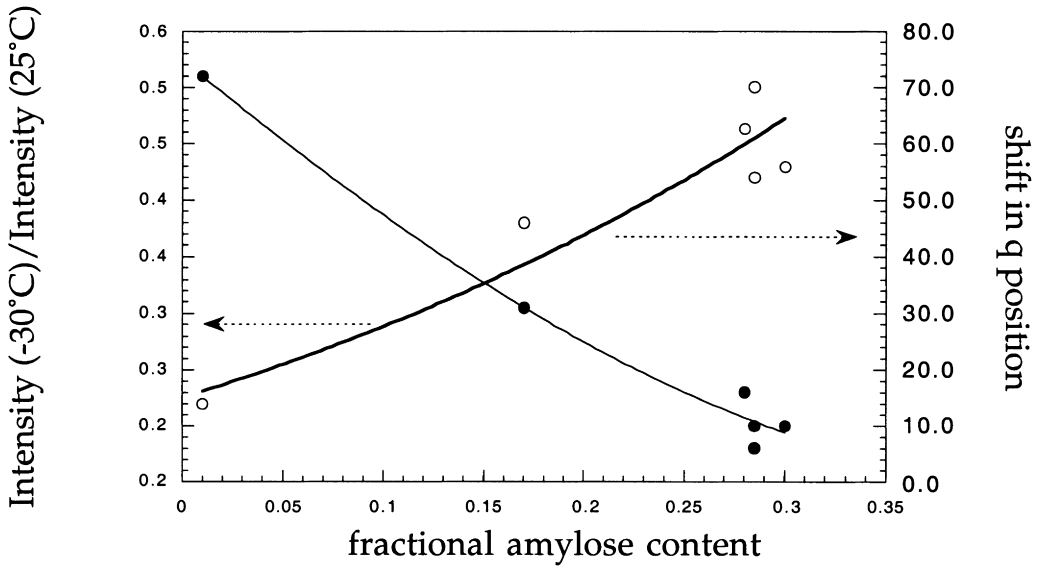


Fig. 11. Variation in the shift in q position (○) and relative peak intensities (●) at −30 and 25°C as a function of amylose content. Lines are a guide to the eye. The amylose content are standard values taken from literature and tabulated in Table 3.

amylose within amorphous granular regions may act, by a dilution effect, to reduce the coupling between amylopectin branches in neighbouring lamellae, reducing the long-range transmission of stress throughout the granule. Both of these hypotheses are consistent with the generally accepted idea that amylose is primarily contained in the amorphous regions of the starch granule [19,20].

Considering the reasons motivating this study of starch freezing, a number of points can be made.

Starch storage and damage: Although the effects of long-term deep-freeze storage or effects on the whole granule length scale were not assessed, it has been found that changes to the starch lamellar structure which occur during freezing are completely reversible upon subsequent reheating. There are no apparent detrimental effects to starch structure on the lamellar level, even after extensive freeze–thaw processing.

Microscopy: Experimental evidence leads to the conclusion that the average lamellar repeat distance observed at sub-zero temperatures is smaller than is present in the native granule at room temperature. Determination of sub-granule starch structure by cryo-microscopy techniques, although being essentially adequate, may not be without artefacts. It is conjectured that the amorphous lamellae may be smaller, by up to a third, than in native starch granules at room temperature. Lamellar compression would lead, in particular, to an underestimation of the size of the larger scale semi-crystalline growth ring by any cryo-microscopy technique.

Table 3

Starch source	Amylose content (%)
Waxy maize	1
Potato	23
Rice	28.5
Topioca	17
Pea	30
Wheat	28.5
Maize	17

Micromechanical properties of starch granules: results presented indicate that the starch lamellar structure can be reversibly compressed, with the application of external stress. The 9 nm lamellar repeat distance can be reduced, by preferential compression of the more ‘open’ amorphous lamellae, whilst still maintaining lamellar periodicity. The return of the 9 nm spacing upon reheating substantiates the claim made by Waigh [2] that this repeat distance is that which is energetically favourable at room temperature.

5. Conclusions

A model of the freezing of starch in water has been proposed which interprets SAXS and WAXS and calorimetry data in terms of structural changes on the lamellar level within starch granules. It is proposed that expansion of water external to the lamellae within starch granules upon freezing and ice formation places the semi-crystalline lamellar structure under compression. Amorphous lamellae are preferentially compressed upon freezing with the crystalline regions remaining largely unaffected. The amorphous lamellae act as a form of ‘shock absorber’ upon the application of compressive forces [17], protecting crystalline integrity. Lamellar ‘buckling’ and loss of correlation in the direction of the lamellar perpendicular may occur as a secondary process, especially after extensive lamellar

compaction, such as is observed in waxy maize starch. Due to the finding that low-amylose starches are affected to a greater extent by the freezing process, it is proposed that amylose plays a role in determining the response of the lamellar structure to applied stress.

For solvents, which do not expand upon freezing, the compression of the amorphous lamellae does not occur. On the contrary, there is a shift in the low q scattering as the volume of the amorphous growth ring shrinks.

Looking at practical implications of the freezing behaviour of starch granules it is seen that, at least in the short term, there are no detrimental effects to starch structure on the lamellar level, even after extensive freeze–thaw processing. At significantly sub-zero temperatures, the amorphous lamellae may be smaller, by up to a third, than at room temperature. Any cryo-microscopy technique is liable to underestimate the size of the large-scale semi-crystalline growth rings.

Acknowledgements

Dr B.U. Komanscheck formerly of the SRS at the Daresbury Laboratory is gratefully acknowledged for organisation and technical assistance during experimental beamtime. Dr T.A. Waigh is thanked for many invaluable discussions and pioneering ideas and he, along with Drs R.E. Cameron and P.J. Jenkins are acknowledged for motivating the physical study of starch structure and functionality in the first place. Finally, the authors would like to thank the Biotechnology and Biological Sciences Research Council and Nestec York Ltd for financial support of this project and CASE sponsorship of P. A. P.

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