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The events leading to the formation of ghost remnants from the starch granule surface and the contribution of the granule surface to the gelatinization endotherm

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Abstract

During the process of gelatinization, the external layers of starch granules form granule envelopes which degrade into ghost remnants. These envelopes contribute to the gelatinization endotherm of starch through the controlled dispersion of internal starch polymer. Ghost remnants are shown to be derived from the external layers of the granules for a range of starches with different amylose/amylopectin ratios. The ghost remnants are composed primarily of amylopectin and exhibit elastic/plastic properties. The surface amylopectin is shown to be structurally distinct from internal amylopectin. During the early stages of granule gelatinization, high amylopectin starch granules swell by 200% in size, where the outer layer of the granule forms an envelope surrounding the disrupted internal starch polymers. High amylose starches do not swell, but do form envelopes at high temperatures ($\geq$ 90°C). At a critical stress point the swollen envelope ruptures becoming a ghost, releasing the majority of the internal starch molecules, while a minority of the starch polymers remain trapped by the collapsed ghost. During the final stages of gelatinization the ruptured envelope degrades into ghost remnants. Manipulation of the starch granule surface, either genetically or chemically, would result in alteration of the gelatinization endotherm.

Keywords: Starch; Gelatinization; Granule surface; Ghosts; Microscopy

1. Introduction

Gelatinization of starch is vital for many industrial processes. Gelatinization is defined as the loss of crystallinity and simultaneous swelling of the granules. For our study we expand this definition to the loss of crystallinity and simultaneous swelling of the granules leading to complete dispersion of starch polymers. Understanding the mechanism of gelatinization is crucial to optimising modification and subsequent use of starch in industry. Recently, an in-depth structural study of starch granule gelatinization led to the description of a general pathway for the structural changes which take place as the granule is hydrated in excess water and exposed to heat (Atkin et al., 1998). This model takes account of many factors, such as amylose content, water content, chemical modifications, distribution of molecules within the granule and granule surface, which can all affect gelatinization. There have been several reported studies of the starch granule surface and of starch granule ghosts during and after gelatinization (Obanni & Bemiller, 1995; Obanni & Bemiller, 1996; Fannon & Bemiller, 1992; Seguchi, 1995; Derek et al., 1992; Baldwin et al., 1996; Baldwin et al., 1997). However, the structural composition of ghosts and the effect they have on granule structure during gelatinization remains unknown.

Several terms and definitions can be found in studies relating to starch granule ghosts. For the purpose of clarity we define the starch granule surface to be the outermost layer of the granule, the granule envelope to be the swollen surface which is intact and surrounds the majority of the internal starch polymers during gelatinization, and the starch granule ghost to be the remnants of the envelope after structural collapse where the majority of the starch polymers have been released.

Previous studies have used a variety of microscopical and physical techniques in order to examine the formation of starch granule ghosts. Light microscopy was used to provide an effective method of granule type identification (Obanni & Bemiller, 1995; Obanni & Bemiller, 1996). Scanning electron microscopy has been used to study the

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gelatinization process of starch granules in excess water (Jing-Ming & Sen-Lin, 1990) as well as to study the presence of granule remnants in gelatinized starch paste (Fannon & Bemiller, 1992). Structural analysis of starch granule ghosts was also carried out by chromatographic methods combined with solvent extractions to isolate lysophosphotidylglycerol (Seguchi, 1995). Raised nodules, 20–50 nm and 100–300 nm, were identified on the surface of ungelatinized potato starch granules by low voltage scanning electron microscopy and atomic force microscopy (Baldwin et al., 1997). The existence of ghosts within a population of damaged starch granules has been observed in a study suggesting a structural difference between the internal core and the external layers of starch granules (Adler et al., 1995). Granule residues and ghost structures have been analysed using X-ray diffraction and differential scanning calorimetry after enzymatic treatment illustrating that ghosts, formed from granule surfaces, have a disorganised molecular structure with higher content of protein than internal granule starch molecules (Derek et al., 1992).

The importance of starch granule ghosts has been underestimated in many of the physical analytical techniques which treat gelatinized starch as a uniform amylopectin structure with additional consideration given only to the presence of dispersed amylose and lipid molecules. The presence of large macromolecular structures within an otherwise homogeneous gel system will affect molecular processes, such as phase separation, and contribute to properties of starch gels. Such considerations are clearly of importance in industrial processing of starch. The aim of this study was to isolate and characterise starch granule ghosts and to determine the effects of such structures on starch granule gelatinization.

2. Material and methods

2.1. Materials

The starches used were waxy maize (Cerestar Ltd), potato (Cerestar Ltd) and Hylon VII (National Starch and Chemicals: Starch Division). For further details see ref. Atkin et al. (1998).

2.2. Methods

2.2.1. Preparation of starches during gelatinization

A constantly agitated 5% (w/v) aqueous solution of each starch type was heated to 40°C. The solution was maintained at this temperature for 15 min. after which several aliquots were removed. The remaining solution was heated to 70°C where, after another 15 min. several more aliquots were removed. The solution was finally heated to 100°C and sampled after another 15 min. Once an aliquot was removed from the solution, it was placed in an Eppendorf tube in ice. The rate of heating from room temperature to 70°C was approximately 4°C m^{-1}. From 70°C to 100°C the rate of temperature increase was approximately 1.5°C m^{-1}. All starch samples were prepared in deionised water.

2.2.2. Separation of the ghosts from the dispersed free starch during gelatinization

Once in cooled Eppendorf tubes, the samples were centrifuged at 1000 r.p.m. for 4 min. using an Eppendorf 3200 mini-centrifuge. The supernatant was discarded and the pellets, which contained ghosts, were washed with 1 ml of deionised water. The samples were then centrifuged for 4 min. and the supernatant was discarded. This was repeated three times to ensure the removal of the majority of dispersed free starch molecules.

2.2.3. Light microscopy of starch granules and ghosts

Whole dry granules of each starch type were spread on a glass slide and stained with iodine vapour. Starch granules in 5% (w/v) solution at room temperature and after heating to 40°C, 70°C and 100°C, were also observed after iodine vapour staining. Birefringence was determined using crossed polarising filters.

2.2.4. Preparation of sections of starch granule ghosts

The isolated ghost samples were fixed using 2.0% glutaraldehyde in ethanol. Glutaraldehyde was used for the fixation of the major component of the ghost, the carbohydrate. Osmium tetroxide was not used for the fixation of minor lipid component of the ghost because of interference with the enzyme-gold labelling. Once fixed, the samples were dehydrated in ethanol and embedded in Nanoplast Resin(R. Bachuber, W. Germany). Thick (200 nm)sections of embedded starch granule ghosts were prepared with a Reichert OMU3 ultramicrotome and collected on uncoated and poly-L-lysine coated glass slides for light microscopy. Thin (60–90 nm) sections were also prepared and collected on copper grids for transmission electron microscopy.

2.2.5. Periodic acid/Schiff's reagent (PAS) staining of starch granule ghosts

Sections mounted on uncoated glass slides were treated with the PAS reagent in order to reveal the total carbohydrate content of the ghosts. In parallel, a control reaction was carried out using 2,4-dinitrophenyl-hydrazine to block the binding of Schiff's reagent to aldehydes.

2.2.6. Enzyme gold labelling of the embedded starch granule ghosts

Sections mounted on poly-L-lysine coated glass slides were treated with a pullulanase-gold enzyme complex which enables visualisation of α-1,6 linkages present in

branched amylopectin molecules (Beesley, 1989). Contrast of the bound enzyme-gold complex was increased with silver enhancement reaction.

2.2.7. Recording of light micrographs

Sections were observed using a Nikon Diaphot-TMD inverted light microscope. Light micrographs were recorded on T-Max 100 film and printed on Ilford Multigrade III photographic paper.

2.2.8. Uranyl acetate/lead citrate staining of thin sections of starch granule ghosts

Sections collected on copper grids were stained with 2% aqueous uranyl acetate solution and Reynold's lead citrate solution for ultrastructure determination.

2.2.9. Freeze-etching of starch granule ghosts samples

Washed starch granule ghosts, from 40°C, 70°C and 100°C, were diluted through a range of concentrations (1, 5 and 10 mg ml^{-1}). A 0.25 μl aliquot of each sample preparation was removed using a micropipette and rapidly spread on a piece of freshly cleaved mica (~5 × 5 mm). This was immediately plunged into a pre-cooled cryogen consisting of 35% methyl butane in liquid propane using an ultra rapid freezing technique (Robards & Abeysekera, 1992). Frozen samples were transferred to a Cressington CFE-50 Freeze-Etch Unit at a pressure $< 2 \times 10^{-7}$ mbar, and etched at $^{-}$65°C for 30 min. Etched samples were rotary shadowed at $^{-}$150°C and coated with 0.4 nm platinum at a 7° angle and 12.0 nm carbon at a 90° angle. Replicas were removed by floating on distilled water and mounted on copper grids.

2.2.10. Recording of transmission electron micrographs

Sections stained with uranyl acetate/lead citrate and replicas of ultra-rapid frozen ghosts were examined using a Jeol 1200 EX transmission electron microscope operated at 80 kV. Micrographs were recorded on Kodak 4489 Film and printed on Ilford Multigrade III photographic paper.

3. Results

3.1. The pathway of formation of starch granule ghosts during gelatinization as determined by iodine staining

The formation of starch granule ghosts during gelatinization of waxy maize, potato and Hylon VII starches is illustrated in Fig. 1(a–l). All the starches examined appear as single granule units at room temperature [Fig. 1(a–c)]. The majority of waxy maize and potato granules are swollen at 40°C [Fig. 1(d,e)], while still remaining as single granule units. Granule envelopes become distinguishable only at higher temperatures: 70°C for potato and waxy maize starch [Fig. 1(g, h)] and at 100°C for Hylon VII starch [Fig. 1(l)]. The temperatures at which the envelopes become defined structures, coincide with the maximum granule swelling observed during gelatinization of both waxy maize and potato starches. This also explains why only a small proportion of the Hylon VII starch envelopes appear because swelling of the majority of granules only begins at temperatures higher than 100°C.

The diameters of the swollen waxy maize ghosts [Fig. 1(j)] and potato starch ghosts [Fig. 1(k)] are at least 200% the diameter of their dry granules. In contrast, Hylon VII starch ghosts, visible at 100°C [Fig. 1(l)], appear to be of similar size to the intact dry Hylon VII granules [Fig. 1(c)]. Tubular shaped envelopes or ghosts, originating from the tubular granules, were not observed within the Hylon VII population. The ghosts of all the starch types can be seen to envelope and retain some small starch particles [Fig. 1(h,j) arrows]. After gelatinization, the ghosts do not show birefringent order as determined by polarised light microscopy.

3.2. The molecular composition of the starch granule ghosts determined by the PAS reaction and pullulanase-gold complex binding

PAS staining showed the envelopes and ghosts to be composed of carbohydrate [Fig. 2(a–e)]. The binding of the pullulanase-gold complex to granule envelopes and ghosts illustrates that this carbohydrate is densely branched and, therefore, mainly amylopectin [Fig. 2(f–j)]. The variation in envelope and ghost sizes observed can be accounted for by the natural variation of a population containing starch granules at different developmental stages, different degrees of swelling and containing sections of grazing granule surfaces and ghosts as well as complete cross-sections [Fig. 2(a–j)].

3.3. The effect of temperature on the granule surface and ghost structures

The increase in temperature from 70°C to 100°C causes both waxy maize and potato granule envelopes and ghosts to decrease in thickness from 2.5 μm and 3.5 μm, by 60%–75% and 50%–60%, respectively [Fig. 2(a–d)]. The waxy maize starch envelopes appear to be broken down internally leaving a structure composed of many cavities [Fig. 2(a), small arrow]. With an increase in temperature, the envelopes give rise to ghosts forming a web–like network of amylopectin [Fig. 2(b)]. The potato starch envelopes and ghosts at 70°C contain layers of carbohydrate [Fig. 2(c), small arrow and Fig. 3(c–e)]. These layers are also observed with the pullulanase-gold label, although the contrast between the layers is not as clear as with PAS staining [Fig. 2(h)]. Raising the temperature to 100°C reduces the frequency and intensity of the layering in ghosts

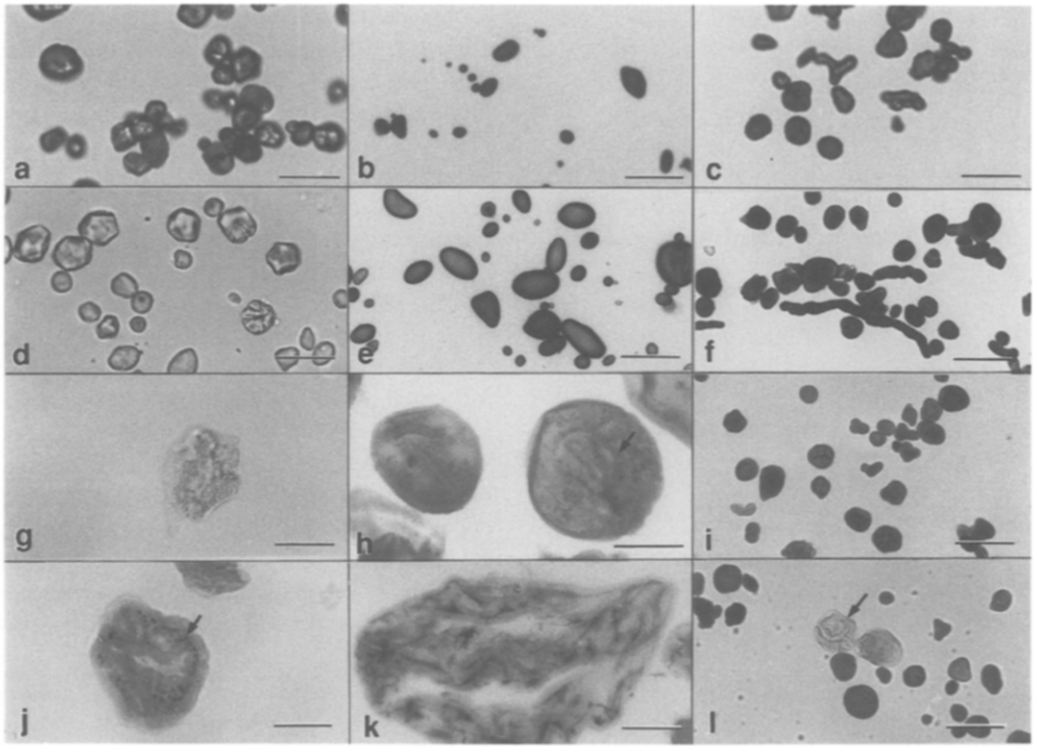

Fig. 1. The formation of starch granule ghosts determined by iodine staining in a range of starches at increasing temperatures during gelatinization. (a) Dry waxy maize granules illustrating intact unswollen granules, bar = 25 μm. (b) Dry potato starch granules, bar = 100 μm. (c) Dry Hylon VII starch granules, bar = 25 μm. (d) Waxy maize at 40°C showing intact starch granules, bar = 25 μm. (e) Potato starch at 40°C showing intact starch granules, bar = 100 μm. (f) Hylon VII starch granules at 40°C showing both regular and tubular granules, bar = 25 μm. (g) Waxy maize at 70°C showing a swollen granule with visible ghost structure, bar = 25 μm. (h) Potato starch at 70°C showing swollen envelope with trapped internal starch particles indicted by arrow, bar = 100 μm. (i) Hylon VII starch at 70°C showing slightly swollen granules, bar = 25 μm. (j) Waxy maize at 100°C showing a ruptured envelope with trapped internal starch particles indicated by the arrow, bar = 25 μm. (k) Potato starch at 100°C showing a collapsed envelope with wrinkled, folded surface, bar = 100 μm. (l). Hylon VII starch granules at 100°C with some visible envelopes (arrow), bar = 25 μm.

as observed by the PAS stain and pullulanase-gold label [Fig. 2(d, i)]. The layers appear to be independent to granule rings, seen during gelatinization of whole granules, as they are too irregular in size (0.45–2 μm).

Also visible at 70°C with both the PAS stain and the pullulanase-gold label are small particles (< 500 nm size) associated with the ghosts [Fig. 2(c, h), large arrows]. The frequency of the particles is greatly reduced as the temperature is increased [Fig. 2(d, i)]. The PAS reaction for Hylon VII starch granules and ghosts at 100°C showed a wide range of structures illustrating banding, disordered centres and thinning ghost structures [Fig. 2(e)]. The pullulanase-gold labelling illustrated a range of amylopectin intensity with different granules and ghost structures [Fig. 2(j)].

3.4. The architecture of the starch granule envelopes and ghosts illustrated by light microscopy on whole granules stained with iodine and sectioned granules stained with the PAS reagent

Envelopes of the swollen starches frequently appear to have localised regions of folds and wrinkles. This is most common in waxy maize starch [Fig. 3(b), arrows]. The ghosts, formed from ruptured starch envelopes, curl on to themselves creating large folds which entrap undispersed internal starch polymers [Fig. 3(a–e)]. Potato starch ghosts appear to contract and curl to a greater extent than waxy maize granule ghosts. Hylon VII ghosts appeared to be wrinkled [Fig. 1(l), arrow].

3.5. The amylopectin composition in starch granule ghosts illustrated by uranyl acetate/lead citrate staining

Thin sections of the layered ghosts, when examined by transmission electron microscopy after uranyl acetate/lead citrate staining, appeared to be made of fine fibre networks [Fig. 4(a)]. The fibre frequency increases from the internal side of the ghost towards the outer surface [Fig. 4(a, b)]. An amorphous material which separates the layering within the ghosts is also present [Fig. 4(c), small arrows).

3.6. The effects of gelatinization on the macromolecular architecture of waxy maize starch granule envelopes and ghosts

Transmission electron microscopy in combination with the frozen replica technique was used to

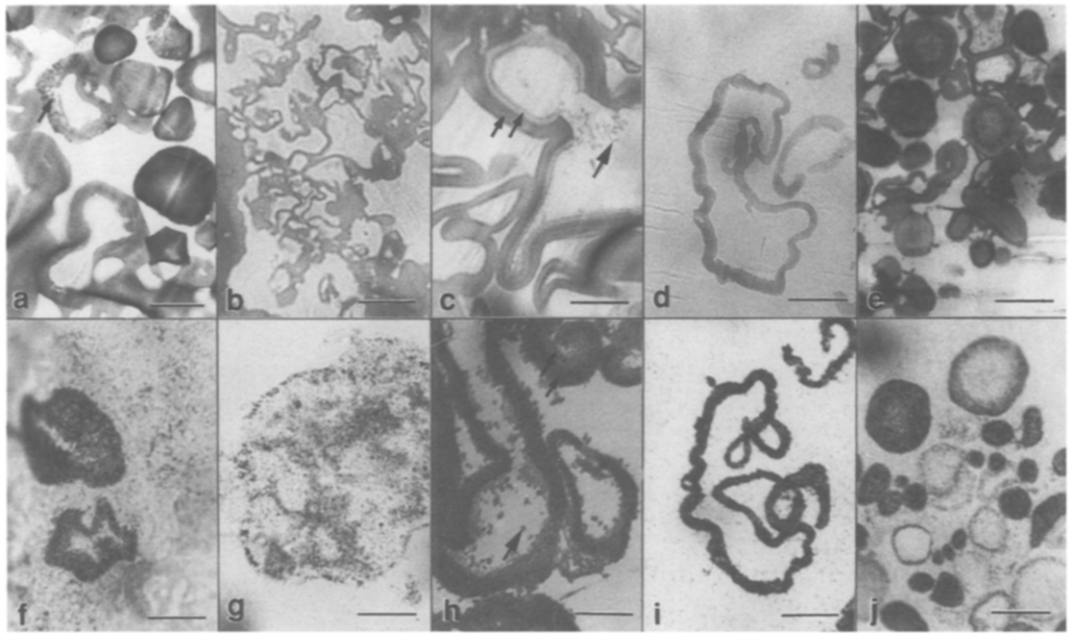

Fig. 2. The amylopectin content of isolated and washed starch granule ghosts as determined by PAS reaction and pullulanase-gold complex labelling on sectioned samples. (a) A section of waxy maize envelopes and ghosts at 70°C after staining with PAS reagent illustrating the carbohydrate content. An arrow indicates the granule surface structure with internal cavities, bar = 10 μm. (b) Waxy maize ghosts at 100°C stained with PAS reagent showing the web-like structure formed by several overlapping ghosts, bar = 10 μm. (c) Potato starch at 70°C stained with PAS illustrating layering of carbohydrate, small arrows, and the occurrence of particles associated with the ghosts, large arrow, bar = 10 μm. (d) Potato starch at 100°C illustrating thinning and the distribution of carbohydrate in the ghost structure, bar = 10 μm. (e) Hylon VII starch granules at 100°C illustrating a wide-range of granule and ghost structures, bar = 10 μm. (f) A section of waxy maize starch at 70°C labelled with pullulanase-gold complex to localise the amylopectin in granule surfaces, bar = 10 μm. (g) Waxy maize ghost at 100°C with pullulanase-gold labelling, bar = 10 μm. (h) Potato starch at 70°C labelled with the pullulanase-gold complex illustrating amylopectin content. The small arrows indicates layering of the ghosts and the large arrow points to amylopectin particles, bar = 10 μm. (i) Potato starch ghosts at 100°C illustrating thinning of the ghosts and general uniformity of the intensity of pullulanase label, bar = 10 μm. (j). Hylon VII starch granules at 100°C showing a range of granule architecture, illustrating dense pullulanase labelling of several granule outer layers and ghosts, which are composed of primarily amylopectin, bar = 10 μm.

determine the detailed structure of the starch envelopes and ghosts after initial hydration and heating to 40°C. By this technique, the surfaces of envelopes and ghosts were shown to be smooth, intact and to contain a high frequency of small (20 nm) pores [Fig. 5(a, b), small arrow].

The surface of the envelope appears to be highly flexible as indicated by the presence of numerous folds [Fig. 5(a), large arrow]. As the surface swells, when the temperature is increased to 70°C, the envelope generally remains intact, but many of the pores expand and merge forming large breaches up to 200 nm [Fig. 5(c), arrow]. At 100°C the envelope has ruptured becoming a ghost with a torn, web-like network connecting small areas of intact surface fragments [Fig. 5(d)].

3.7. The effect of gelatinization on the macromolecular architecture of potato starch envelopes and ghosts

As with waxy maize starch, the potato granule surface at 40°C is initially smooth and intact with many visible pores of 20 nm [Fig. 6(a), small arrow]. Already, some of the granules are fully swollen and the envelopes have more breaches than waxy maize granule envelopes. Many regions of the envelope have become breached, forming web-like networks [Fig. 6(a), asterisk]. At 70°C, the ruptured starch envelopes have become ghosts and the majority of the pores in the surface have increased in size (> 200 nm) forming large breaches [Fig. 6(b)]. The majority of the ghosts at 100°C consist of a network with small, less frequent, areas of intact continuous surface which appear to be folded and entangled [Fig. 6(c)] losing the smooth appearance of the envelopes observed at 40°C.

3.8. The effects of gelatinization on the macromolecular architecture of Hylon VII starch granule surface

At 40°C, the Hylon VII starch granule surface appears smooth but is rippled and undulating [Fig. 6(d, e)] when compared to waxy maize and potato starch granule surfaces. The surface of Hylon VII granules has very few visible pores but still retains the flexibility indicated by the folds, also observed on the waxy maize and potato starch surfaces. As the temperature is increased to 70°C, with the development of envelopes, the size of the ripples increased and became more distinct [Fig. 6(f), arrow]. At 100°C, the envelopes form ghost structures to create the web-like

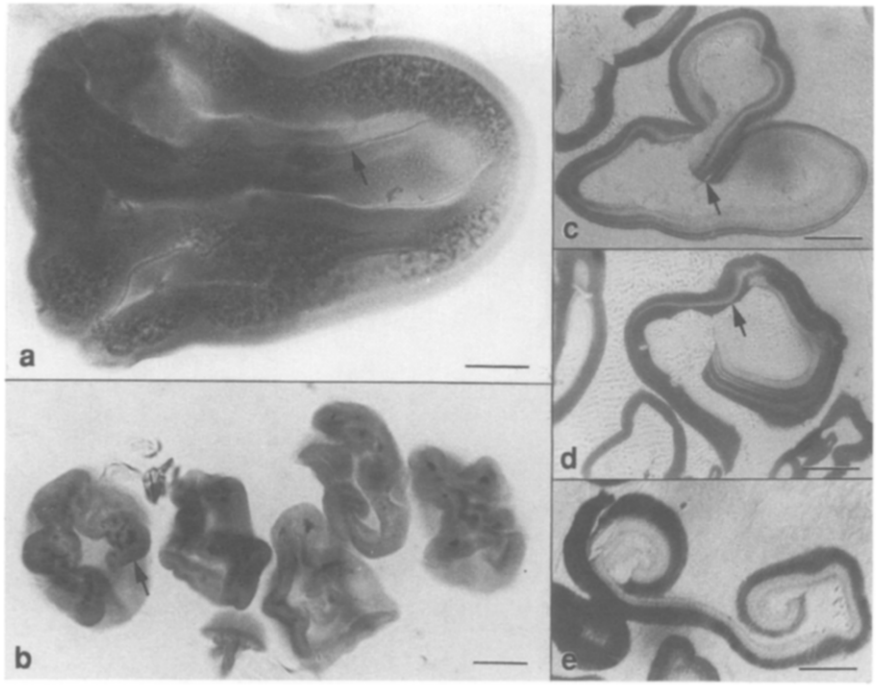

Fig. 3. The curling of starch granule ghosts illustrated by iodine staining and PAS reaction. (a) A potato starch ghost at 70°C stained with iodine vapour illustrating the folded, ruptured ghost. Arrow points to a fold, bar = 25 μm. (b) Waxy maize starch ghosts at 100°C stained with iodine vapour, illustrating the folds, indicated by arrow, bar = 5 μm. (c) Sections of potato ghosts at 70°C stained with PAS reagent to illustrate folds indicated by small arrow, bar = 10 μm. (d) Sections of potato ghosts at 70°C stained with PAS reagent to illustrate folds indicated by small arrow, bar = 10 μm. (e) Sections of potato ghosts at 70°C stained with PAS reagent to illustrate the coiling of the collapsed granule ghost, bar = 10 μm.

network [Fig. 6(g), small arrow] interconnecting small intact areas of surface [Fig. 6(g), large arrow].

4. Discussion

4.1. The structure of starch granule ghosts

The presence of macromolecular starch ghosts within fully dispersed starch polymers at the end stages of gelatinization (Atkin et al., 1998; Fannon & Bemiller, 1992) suggests that there is a structural difference between the surface and internal components of the starch granules. Several studies have examined the ghosts attempting to explain this difference. Derek et al. (1992) illustrated a lack of ordered structure within starch ghost remnants when compared to observations of intact granules using X-ray diffraction. Further evidence for a structural difference in granule composition is presented by Adler et al. (1995) suggesting that the surface acts as a shell for the granule. This study indicates a gradual change of amylopectin composition from the centre of the granule towards the surface. Such a change of the amylopectin architecture can explain the different properties of internal and surface amylopectin observed in our study.

We have demonstrated that starch granule ghosts are layered structures with increasing frequency of carbohydrate emanating from the inner surface. The layering was most prominent in potato starch ghosts and was also observed in waxy maize ghosts as well as in a minority of the Hylon VII ghosts. Study of sectioned ghosts stained with uranyl acetate/lead citrate, revealed thin bands of amorphous material separating wider layers of the increasing amylopectin networks. The size of the layers varied with up to 4–5 fine layers (450 nm/layer) on the inner side of the ghost and 2–3 larger layers (2 μm/layer) towards the outer surface in potato starch ghosts.

A pullulanase enzyme-gold complex was applied to provide direct visual evidence that starch ghosts are predominantly amylopectin. We cannot exclude the possible presence of amylose in the ghosts since pullulanase does not bind to linear starch polymer. However, the intense labelling by pullulanase provides strong evidence for the presence of large amounts of branched starch polymer in the ghosts. This predominance of amylopectin within the surface architecture was demonstrated for both high amylopectin starches, waxy maize and potato, as well as for the high amylose starch, Hylon VII. An enzymatic study of the amylopectin/amylose ratio in barley starch by Derek et al. (1992) also provided indirect evidence that the ghosts are primarily composed of amylopectin. Further evidence from Derek et al. (1992) suggested that the intermolecular interactions within the barley starch ghosts occur between amylopectin units, illustrating the importance of the amylopectin component to establish surface integrity.

Although the primary component of the granule ghosts has been shown to be amylopectin, there is evidence that

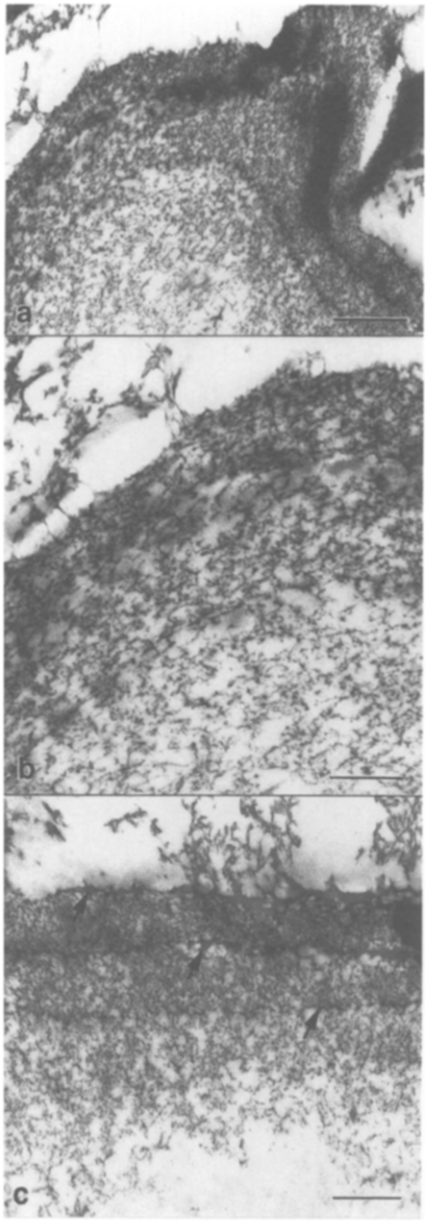

Fig. 4. The amylopectin network in the potato starch ghosts illustrated by staining with uranyl acetate and lead citrate visualised by transmission electron microscopy. (a) A cross-section through a potato starch ghost at 70°C revealing a layered web-like structure, bar = 1 μm. (b) The increasing carbohydrate frequency of the ghost layering is illustrated, bar = 500 nm. (c) An amorphous material appears to outline and define the layering of the ghost (arrows), bar = 500 nm.

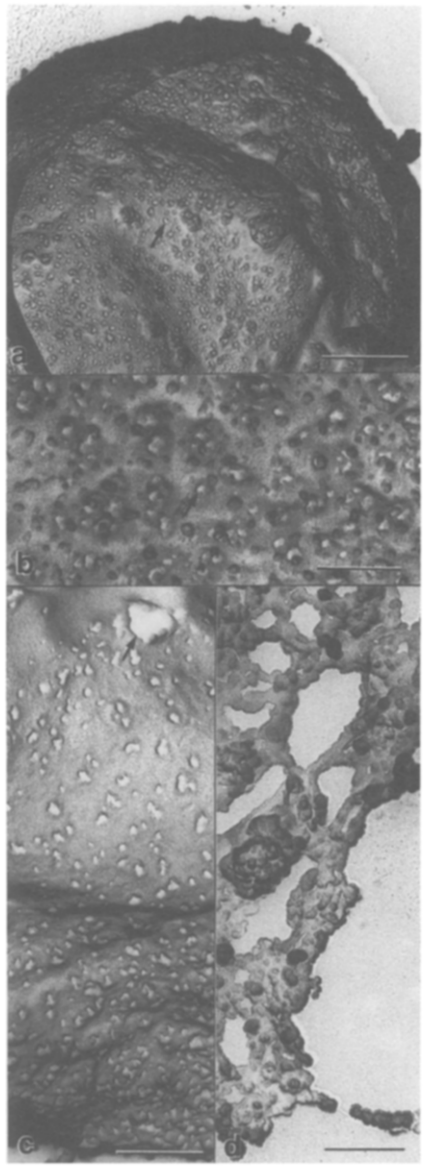

Fig. 5. The effects of hydration and heat on waxy maize starch ghosts as determined by the frozen replica technique. (a) Waxy maize starch granule surface at 40°C. The small arrow points to pores visible throughout the granule surface and the large arrow points to folds in the surface, bar = 500 nm. (b) Waxy maize starch granule surface at 40°C. The arrow points to a pore in surface, bar = 200 nm. (c) Waxy maize starch surface at 70°C. The arrows point to a pore of enlarged size, bar = 200 nm. (d) Waxy maize starch ghost at 100°C. A web-like network is formed through extensive heating, bar = 200 nm.

minor components also play important roles. Derek et al. (1992) illustrated that amylose, lysophospholipid and proteins are also present in the ghosts of barley starch granules. Although not fixed during the embedding procedure used, the lipid content of the ghosts is still thought to be present and trapped within the amylopectin architecture. The amorphous thin bands, separating the amylopectin networks, can account for the inclusion of a localised lipid component within the ghosts. These lipid complexes could play a role in maintaining structural integrity of the ghosts at higher temperatures, as observed in our study. The role of proteins within the outer surface of granules is undefined. These proteins are possibly involved with the arrangement of starch polymers in the granule surface. Overall, the major component of the granule surface, and subsequently the envelopes and ghost, is amylopectin. The presence of localised minor components and different orders of amylopectin packing may be responsible for the differences in physical properties between internal and surface amylopectin.

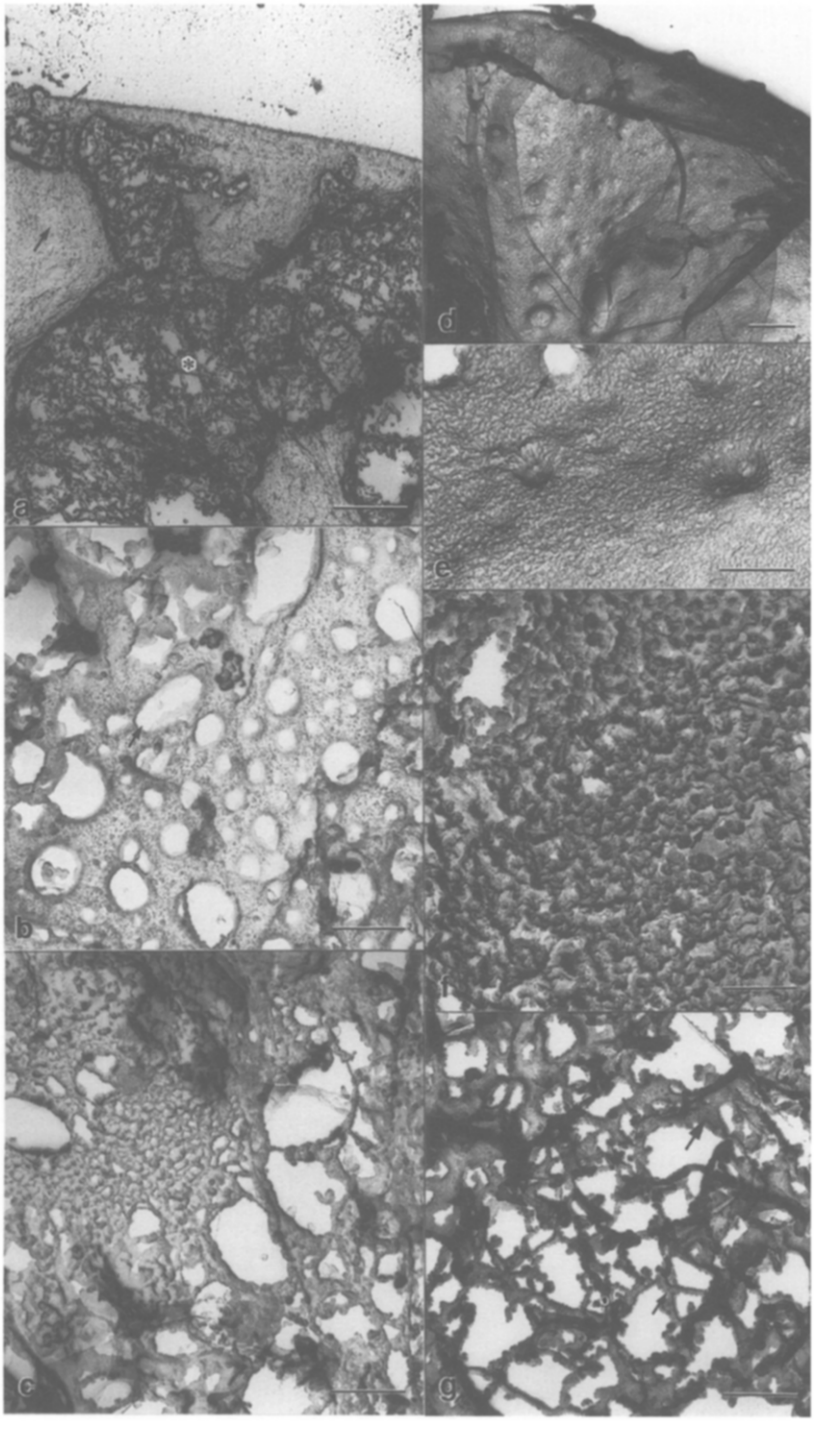

Fig. 6. The effect of hydration and heat on the ghost structure of potato and Hylon VII starch granules as determined by the frozen replica technique. (a) Part of a potato starch granule surface at 40°C. The arrow indicates pores visible throughout the granule surface. The asterisk indicates a large breach in the surface with web-like network formed, bar = 1 μm. (b) Potato starch granule surface at 70°C, arrow points to a large pore formed through the swelling of the granule, bar = 200 nm. (c) Potato starch ghost structure at 100°C showing a fibrous network, bar = 200 nm. (d) Starch granule surface illustrating large folds in Hylon VII granules at 40°C, bar = 250 nm. (e) Hylon VII starch granule surface at 40°C. The arrow points to a visible large pore, bar = 200 nm. (f) Hylon VII starch granule surface at 70°C. Arrow points to the undulating surface formed through granule swelling, bar = 200 nm. (g) The Hylon VII ghost surface at 100°C illustrating merging pores forming a web-like network (small arrow) linking intact areas of granule surface (large arrow) as seen in all the examined starches, bar = 200 nm.

4.2. The properties of starch granule ghosts

The swollen envelopes exhibit regions of folds and ripples, which indicate localised areas of varying elastic/plastic properties. This localised effect is most prominent in the waxy maize envelopes but is also observed in both potato and Hylon VII starches. When the envelope is ruptured, the newly formed ghost partially contracts upon itself creating taut regions and folds. This contraction is further reflected in acute curls visible in cross-sections of ruptured granules. These curls are distinct from the natural curvature of the surface caused by the internal granule pressure during gelatinization. The direction of the curl is always towards the granule interior. This may indicate that the outermost surface of the ghost has a greater elastic limit becoming plastically deformed or is structurally weaker, than the inner surface of the ghost. The curling of the ghost remnants is most prominent in potato starch. Recently, Fisher et al. (1997) using pressure measurements at the surface of potato starch granules have also clearly demonstrated starch granules to be 'elastic bodies'.

4.3. The structural difference between the granule surface amylopectin and the internal granule amylopectin

The differences in the surface amylopectin and internal amylopectin could be due to a number of reasons. Firstly, the localised activity of branching/debranching enzymes in the granule can be expected to cause a differentiation in amylopectin architecture resulting in surface amylopectin and internal amylopectin. The more probable reason is the initial exposure of the granule's surface to heat, which creates differences in amylopectin from the internal granule regions compared to the amylopectin from the granule surface. Heat can cause rapid alteration of the granule surface, restructuring the molecular architecture. This rearrangement possibly causes separation of the lipid component from the carbohydrate, leading to concentration of lipid within the outer layers. The impact of subsequent heat transfer and water exchange results in the disruption of internal granule structure (Atkin et al., 1998) due to enveloping outer layers enforcing steric restrictions as osmotic pressures increase.

The increasing polymer frequency could also account for the differences between the amylopectin of the surface and the internal granule. The conformation of surface amylopectin can influence heat resistance and structural integrity of the granule outer layers. The imbalance of polymers from the inner surface of the ghost to the outer surface is probably responsible for curling of the ghosts. The amylopectin of the outer most layer of the ghost, although tightly packed, may have less intermolecular interactions and therefore have greater flexibility than the inner layers of the ghost causing inward curling when disrupted. Alternatively the polymer imbalance may generate a difference between the hydrophilic natures of each side of the ghost, causing the ghost to curl as both sides react with the surrounding water molecules.

Internal granule amylopectin has been shown to form 400 nm particles during gelatinization (Atkin et al., 1998). No particles were formed from the granule envelopes during gelatinization or from the ghost at the end of gelatinization. Transmission electron microscopy of ghosts illustrated a fibrillar network. The particles present inside ghosts, observed in the light microscopy studies, are the trapped internal amylopectin units, which were not expelled as the envelope collapsed after rupture. The structural distinction between surface and internal amylopectin is illustrated in Fig. 3(a). This suggests that the amylopectin of the surface is of a different conformation to the internal amylopectin found as single units.

4.4. The effects of hydration on starch granule surface and the formation of the granule ghosts

The effect of hydration and heating on the granule surface, with subsequent ghost development, has been clearly illustrated in this study. The intact hydrated granule surface at 40°C contains many small pores. Increasing the temperature led to these pores becoming enlarged, forming breaches in the outer surface of the granules. The swelling of the granule surface forms a translucent envelope which contains the disrupted internal starch. Further increases in temperature cause greater swelling, stretching the envelope as water fully permeates the granule. A maximum degree of swelling occurs, specific for different starch origins, increasing the stress on weakened breaches causing surface ruptures and eventually a major structural collapse at a critical stress point. This critical stress point is defined as the point of equilibrium between the elastic limit and the plastic tension of the envelope, where the swelling pressures is exerted by the free starch particles and water pressure within the granule surface. As the envelope ruptures, it collapses creating a ghost and becoming a web-like network of thick fibres. Studies were carried out by Fannon & Bemiller (1992) on maize starch and Jing-Ming & Sen-Lin (1990) on potato and maize starch using cryo-scanning electron microscopy on granule remnants after gelatinization. These studies agree with our observations where it was illustrated that the ghosts are composed of a network of fibres with small intact areas of granule surface. We have used an ultra-rapid freezing technique to reduce the possibility of freezing artefacts associated with liquid nitrogen freezing for cryo-scanning electron microscopy. The possibility of freezing artefacts has to be considered, as these may cause some exaggeration of the pore size and general decomposition of the granule surface. The use of ultra-rapid freezing (cooling rate $> 2.2 \times 10^3\,^{\circ}C\,s^{-1}$) (Robards & Abeysekera, 1992) has reduced ice crystal damage to the granule surfaces and ghosts in our studies. The nodules (25–50 nm and 100–300 nm) observed by Baldwin et al. (1997) on the surface of intact potato starch

granules, were not visualised in our study on the surfaces of hydrated potato starch granules at 40°C, 70°C and 100°C.

4.5. The contribution of granule surface to gelatinization of starch in excess water

Discovering the role of the starch granule surface during the gelatinization process is vital to gaining a complete understanding of the entire gelatinization process of starch granules described in our previous study (Atkin et al., 1998). Through a series of impressive micromanipulation experiments, Fisher et al. (1997) provided evidence for the presence of an encapsulating membrane capable of supporting an internal granule pressure of approximately 10^5 Pa. We have illustrated in the present study the effects of hydration and temperature on the formation of the granule ghosts. By comparing the pathway of ghost formation with that described for the gelatinization of the complete granule (Atkin et al., 1998) several comparisons have been made.

Initially, with hydration at ambient temperature, the entire granule expands by approximately 80% causing the granule surface to increase correspondingly. This would 'open' the surface, creating small surface pores and increasing water exchange from the external solution to the internal components of the granule. Amylose molecules near the surface, can leach out of the granule into the external solution as a result of this water exchange, as predicted in current models (Blanshard, 1987). The size of the surface pores, which are approximately 20 nm, would prevent any large molecules such as the 400 nm amylopectin units escaping from the granule (Atkin et al., 1998). The intact amylopectin surface itself, which does not form the 400 nm units, would also reduce the rate of molecular release controlling the leaching of the internal starch polymers and, therefore, the gelatinization endotherm.

As the temperature is increased, the granule swells further up to 200% of the original diameter. The central structure of the granule is broken down becoming disordered and molecular leaching is increased as the pores of the surface increase in size. The intact amylopectin surface forms an envelope surrounding the disordered internal starch granule components. This 'boil in the bag' scenario (Atkin et al., 1998) continues as the temperature is raised until the critical point of swelling is reached. At this point the envelope ruptures at the weakest region, releasing a large quantity of the 400 nm internal amylopectin units. The envelope having undergone a massive structural collapse, trapping small amounts of remaining starch molecules, degrades into ghost remnants as gelatinization is completed for the entire system. The starch solution now contains a mixture of dispersed gelatinized amylose and amylopectin in a continuous phase with fragmented ghost structures in an excluded phase. The extent of the fragmentation of the ghosts is dependent on the origin of the starch and temperature of granule processing.

Blanshard (1987) discusses DSC thermograms of potato starch demonstrating the energy requirements of starch gelatinization under various conditions. Our study illustrates that physical effect of the granule surface structure is most acute as the gelatinization peak is reached (Fig. 7). The envelope retains the internal starch molecules until the critical stress point of granule swelling is reached, which would coincide with the peak of gelatinization shown by the DSC thermograms. Damaged surfaces have been shown to allow internal components to be released early (Adler et al., 1995) lowering the gelatinization point of the granule components. This observation can be explained by our results as the damaged outer surface forms an inferior envelope which cannot retain the internal dispersed starch polymers during heating and releases its contents prematurely resulting in a lower gelatinization point.

4.6. The contribution of granule surface to gelatinization in limited water

The review by Blanshard (1987) and the study by Cameron & Donald (1993) examine the effect of limited water systems on starch gelatinization. The swelling process does not occur in most granules in a limited water system and the contribution of the surface to the gelatinization process in such conditions is severely reduced. The control of granule swelling and disruption of internal granule components is not the limiting factor of the gelatinization endotherm peak in limited water conditions. The important factors involved in control of the gelatinization endotherm in limited water systems are concerned with internal architecture and granule composition and not the formation of the controlling envelope.

4.7. The effect of ghost fragments on the gelatinized starch gel

Once gelatinized, the ghosts play an important role within a gel system. Studies by Svegmark & Hermansson (1991) and Fannon & Bemiller (1992) have illustrated by light microscopy and scanning electron microscopy, the presence of ghost structures within the gelatinized starch paste. These studies consider the distribution of amylose and amylopectin in starch pastes and show a varying range of physical properties with regard to different starch origins. Our study illustrates the existence of the starch ghosts in the gelatinized system and reveals three components in a phase separating system, amylose polymers, amylopectin polymer units and amylopectin ghost remnants.

4.8. The role of the starch granule ghosts in plants

Within the plant cell the starch granules are unlikely to undergo any radical changes of environment causing the

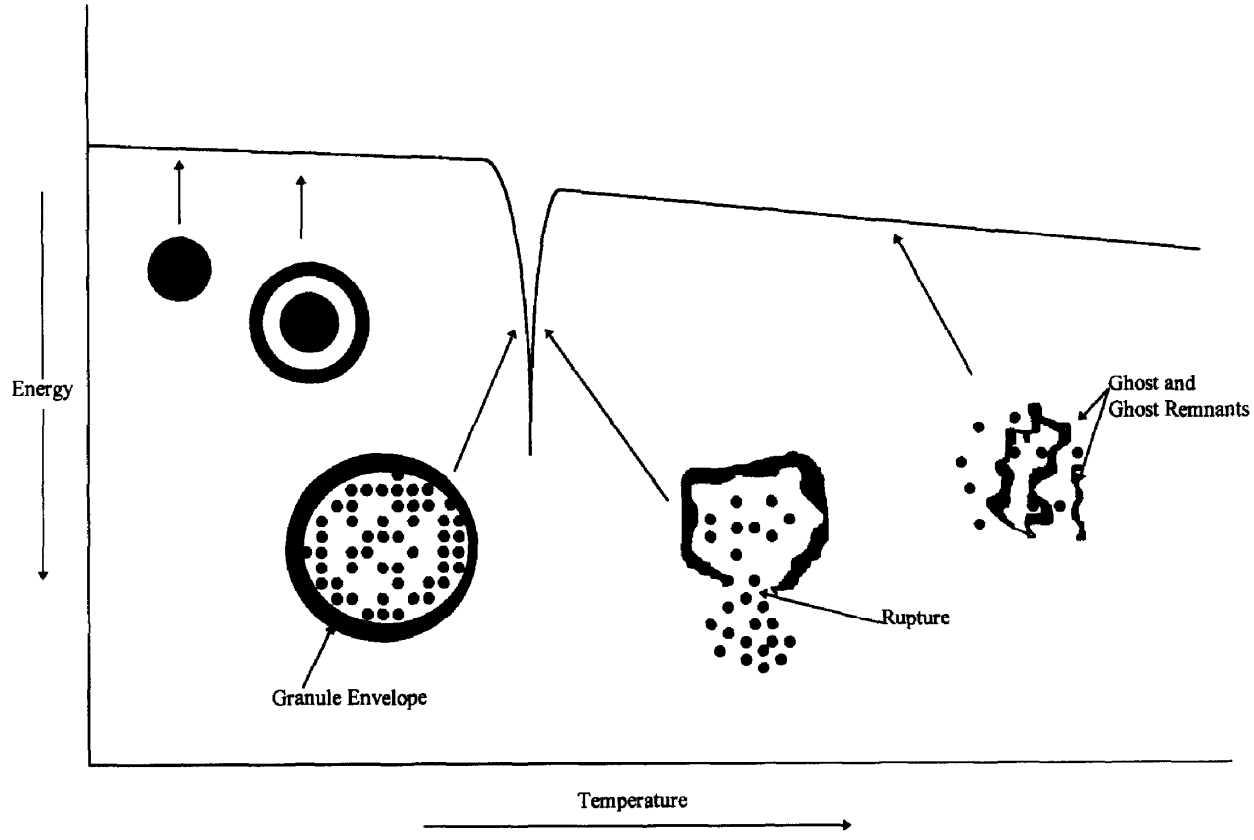

Fig. 7. The model of starch gelatinization. A model of the molecular events of gelatinization, the formation, control and degradation of granule ghosts coincident with a hypothetically fitted DSC curve.

ghosts to develop. If the structural difference between surface and internal amylopectin is due to biosynthesis then this must be for some other function of the starch granules such as the formation of an enzyme resistant layer or due to the mechanism of granule synthesis.

4.9. The industrial importance of the starch granule surface and ghosts

The real value of understanding the granule surface structures lies with controlling the effects which the surface has on gelatinization. It should be possible by adjusting the factors that influence the pathway of gelatinization to create a more favourable result, e.g. lower the gelatinization peak or increase the peak preventing gelatinization. By altering the structure of the granule surface, either genetically or chemically, the process of gelatinization can be influenced and controlled.

5. Conclusions

We have characterised the remnants of starch granules after gelatinization as starch ghosts illustrating the development of these remnants from the outer layers of the granules. We have directly shown that the starch granule ghosts are primarily composed of amylopectin and that they exhibit elastic/plastic properties. The amylopectin, which forms the granule surface, was shown to possess distinct structure and properties when compared to the internal amylopectin.

We illustrate how the granule swells and the surface develops to create a translucent envelope during the early stages of gelatinization controlling the endotherm. We demonstrate that the envelopes reach a critical stress point at which they rupture and collapse, releasing the trapped starch molecules into the external solution completing gelatinization. The remaining surface structure is then shown to degrade to a web-like network, the ghost. By controlling the granule surface, the energetics of the gelatinization can be favourably influenced and controlled, improving product quality and processing efficiency.

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