Carbohydrate Research, 135 (1985) 271–281
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

THE ROLES OF AMYLOSE AND AMYLOPECTIN IN THE GELATION AND RETROGRADATION OF STARCH

MERVYN J. MILES, VICTOR J. MORRIS, PAUL D. ORFORD, AND STEPHEN G. RING
AFRC Food Research Institute, Colney Lane, Norwich, NR4 7UA (Great Britain)
(Received April 17th, 1984; accepted for publication, July 9th, 1984)

ABSTRACT

The retrogradation of starch gels has been studied by using X-ray diffraction, differential scanning calorimetry, and measurements of the shear modulus. Starch gels were considered as composites containing gelatinised granules embedded in an amylose matrix. The short-term development of gel structure and crystallinity in starch gels was found to be dominated by irreversible ($T < 100°$) gelation and crystallisation within the amylose matrix. Long-term increases in the modulus of starch gels were linked to a reversible crystallisation, involving amylopectin, within the granules on storage. It was considered that the crystallisation resulted in an increase in the rigidity of the granules and thus enhanced their reinforcement of the amylose matrix.

INTRODUCTION

Starch is a major component of many food plants where it occurs as water-insoluble granules. The granules have ordered structures which are semi-crystalline and birefringent. Two main polysaccharides, amylose and amylopectin, can be extracted from the granule. Amylose is an essentially linear (1→4)-linked α-D-glucan. Amylopectin is a highly branched macromolecule, which consists of short chains of (1→4)-linked α-D-glucose with (1→6)-α-linked branches. When starch granules are heated in water at a characteristic temperature (gelatinisation temperature, usually 60–70°), the granules swell irreversibly to many times their original size. At the same time, amylose is preferentially solubilised. During gelatinisation, granule birefringence and crystallinity disappear. At temperatures below 100° and in the absence of mechanical shear, granule integrity is maintained. As a consequence, concentrated suspensions of gelatinised starch are viscoelastic. The swollen granules, by their mutual interactions, form temporary networks. On cooling sufficiently concentrated starch dispersions ($\geq 6\%$*) to room temperature, an opaque starch-gel quickly develops, which becomes firmer on storage over several weeks[1,2]. These changes have a profound effect on the texture, and hence accepta-

---

*All percentages are w/w.

0008-6215/85/$ 03.30

bility, of many starch-containing foods, *e.g.*, the staling of bakery products[3]. It is an area of major commercial importance but, as yet, there is only a limited understanding of the molecular processes involved.

During gelation and ageing of starch gels, an X-ray diffraction pattern of the B-type develops slowly with time[4]. Amylose gels on storage, and amylose which has precipitated from aqueous solution, also give a weak X-ray diffraction pattern of the B-type[5]. This has been termed retrogradation, *i.e.*, return to the granular state. A line of evidence supporting the view that a crystallisation process is responsible for some of the observed changes in mechanical properties of the starch gel comes from kinetic studies[1].

In an appropriate system, the mode of nucleation, the growth-limiting process, and the geometry of the developing crystallite may be derived using the Avrami analysis[6–8]. Matured starch gels give an endothermic peak on differential thermal analysis[1], an expected result for the cooperative melting of a crystallite. From an application of the Avrami analysis to the development of this endotherm, the nature of the crystallisation was proposed[1] to be an instantaneous nucleation followed by a rod-like growth of the crystalline unit. The development of the endothermic peak was closely related to the changes in firmness of the gel.

Starch gels may be regarded as complex composites[9], in which swollen gelatinised granules are embedded in an interpenetrating amylose-gel matrix. It has been proposed[9] that the main factors which influence the mechanical behaviour of these materials at small deformations are the mechanical properties of the matrix gel and of the swollen granules (in particular their deformability), and the volume fraction and shape of the granules. It was the aim of this study to relate the molecular interactions observed during gelation and storage of starch gels to the development of the shear modulus. This was done by relating the molecular changes to the factors listed above.

Recently[10], and in the preceding paper[11], we reported studies of the gelation of amylose. Gelation occurs on cooling amylose solutions having concentrations greater than the overlap concentration[11] (C^*). Amylose gels are opaque, and it was considered that opacity arose from a phase separation of the amylose into polymer-rich and polymer-deficient regions on gelation. As the polymer chains were initially entangled, an interconnected network was produced. Crystallinity, as detected by X-ray diffraction, was a slower process that was proposed to occur in the polymer-rich regions of the gel[10,11]. A substantial part, >80% of the crystallisation of the amylose, occurred after the shear modulus had reached a constant value.

We now describe an extension of these studies to follow the gelation of starch.

EXPERIMENTAL

General. —Starch was isolated from smooth-seeded leafless peas, variety Filby, by an aqueous extraction procedure[12]. The iodine-binding behaviour of the purified starch and amylose fractions was determined by a semi-micro, differential potentiometric technique[13]. The purified starch had an iodine-binding capacity of 5% (*cf.* 19.5 ±0.5% for amylose[14]). On this basis, the amylose content of the pea starch is 25 ±1%. Pea starch was chosen for two reasons. Firstly, when the starch was gelatinised at 95°, ~21% of the starch was solubilised from a 1% suspension. The solubilised material bound 19.5 ±0.5% of iodine and was therefore an amylose of high purity. The solubilisation of amylopectin was not detected at this temperature and therefore pea starch was ideal for the study of the relative contributions of the solubilised amylose and of the amylopectin within the gelatinised granules to the rigidity of starch gels. Secondly, pea starch has[15] a lipid content of <0.03%. Some cereal starches have[16] a lipid content as high as 0.6%. Such concentrations of lipid would have the potential to complex significant quantities of amylose. As the lipid–amylose complex is crystalline, its absence is desirable for studies of the crystallisation of starch polysaccharides.

Preparation of amylose samples is described in detail elsewhere[11]. The purified amylose had an iodine-binding capacity of 19.5% and, in aqueous solution at 25°, had an intrinsic viscosity $[\eta]$ of 80 mL.g^{-1}. Since[14] $[\eta] = 0.113\ M_w^{0.5}$, M_w was 5×10^5.

Preparation of gels. — Aqueous suspensions of starch of the desired concentration were heated to 95° and constantly agitated to prevent sedimentation of the granules. Agitation did not lead to any disruption of the granular structure, as judged microscopically. When the viscosity of the suspension increased, indicating gelatinisation, agitation was stopped. The suspension was kept at 95° for 30 min before being cooled to 26°, the maturation temperature. No detectable loss of water occurred during storage.

Amylose gels were prepared by rapidly quenching amylose solutions to 26°. Amylose solutions of the desired concentration were regenerated from the amylose–1-butanol complex by heating to 95° followed by removal of the 1-butanol in a stream of hot nitrogen. The shear modulus of the amylose gels prepared from the fractions leached at 60–70° and at 60–95° differed by <10% at a fixed concentration[15].

The development of the shear modulus (G′) of the gels with time after quenching was monitored by using a modified pulse shearometer (Rank Brothers, Cambridge, U.K.) Details are discussed in the preceeding paper[11].

Differential scanning calorimetry. — D.s.c. was performed with a Perkin–Elmer DSC-2B instrument. The gel (~20 mg) was sealed in a Perkin–Elmer volatile-sample pan. An equal weight of water, sealed in another pan, was used as the reference material. Experiments were conducted at a heating rate of 10 K.min^{-1} over the temperature range 293–373 K. The enthalpies of the melting transition were determined from integration of the endothermic peak. The calorimeter was calibrated with indium, for which the heat of melting was taken to be 6.80 cal.g^{-1}. Gelatinisation behaviour was measured using a 10% suspension of pea starch.

After d.s.c., starch concentrations were determined by the phenol–sulphuric acid method[17].

X-Ray measurements. — Initial studies were made with a wide-angle flat-plate camera. Starch and amylose gels were prepared in thin-walled quartz capillaries, and, after storage, powder-type diffraction patterns were recorded with exposure times of ~1 h. In both cases, B-type patterns were obtained. In order to follow the development of crystallinity with time, changes in the 100 diffraction maximum were measured with an Anton Paar K.G. Kratky small-angle camera fitted with a proportional detector. The method is described in detail elsewhere[11]. Starch dispersions and amylose solutions were prepared in quartz capillaries and quenched from 90 to 26° within this camera.

RESULTS

Fig. 1 shows the development of the shear modulus with time for 10% and 20% suspensions of gelatinised starch after quenching to 26°. Initially, the sus-

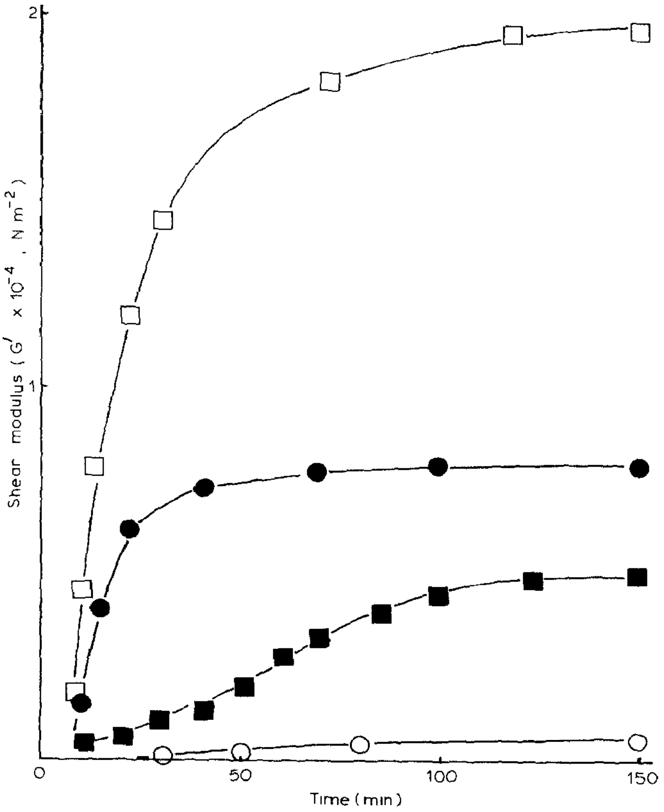

Fig. 1. Development of the shear modulus (G′) with time for 10% (■) and 20% (□) starch gels, and 2.4% (○) and 3.2% (●) amylose gels.

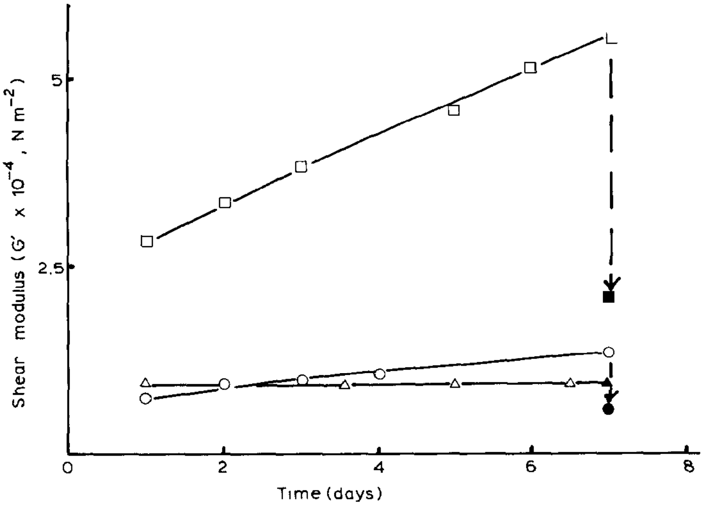

Fig. 2. Long-time development of the shear modulus (G') for 10% (○) and 20% (□) starch gels, and a 3.2% (△) amylose gel at 26°. The dotted line indicates change after heating to 90° and cooling.

pension was viscoelastic. The gel behaved as a Hookean solid at strains below 0.2. The shear modulus of the gel reached a constant value after only 100–150 min. The maximum amount of amylose which could be solubilised at a gelatinisation temperature of 95° from 10% and 20% suspensions of starch would be 2.1% and 4.2%, respectively. Therefore, gelation was monitored within this concentration range. Fig. 1 shows the development of the shear modulus with time for 2.4% and 3.2% solutions of amylose after quenching to 26°. The solutions became turbid and started to gel after 15 and 8 min, respectively. The development of modulus was rapid and approached a constant value after 100 min. Thus, the development of opacity and the gel network was similar for the starch and amylose gels. The long-term behaviour of the starch and amylose gels is dramatically different (Fig. 2). The shear modulus of the starch gels continued to increase slowly with time, whereas the shear modulus of the amylose gel remained constant. When the matured starch gels were heated to 95°, and then cooled to 26°, the shear modulus fell to the value held after 24 h, *i.e.*, the long-term change was thermally reversible. In comparable experiments, the shear modulus of the amylose gel did not change on heating and cooling the gel.

D.s.c. studies of 10% and 20% starch gels, which had been stored at 26° for 7 days, showed an endothermic transition at 63°. Similar experiments on the 3.2% amylose gel showed no such transition. The rate of development of the endothermic peak with time for the 20% gel is shown in Fig. 3. After 2 h at 26°, no peak was detectable, but one developed during the next 7 days. The enthalpy change measured after storage for 7 days was 0.745 mcal/mg of starch. The endothermic change at 62° observed during the gelatinisation of pea starch was 3.02 mcal/mg of starch.

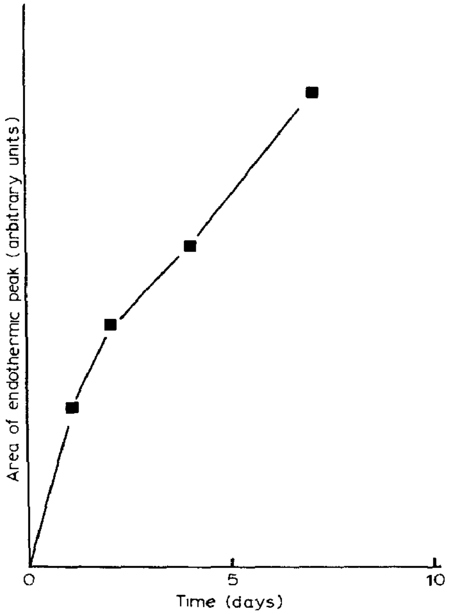

Fig. 3. Development of the endothermic peak obtained by d.s.c. for a 20% starch gel.

The use of X-ray diffraction to study the development of crystallinity in amylose gels has been discussed elsewhere[10,11]. We shall discuss here the differences in the appearance of crystallinity within both amylose and starch gels. Starch and amylose gels which had been stored at 26° for 14 days gave X-ray diffraction patterns of the B-type. The development of crystallinity was monitored by following the development of the 100 reflection (d spacing = 1.6 nm), which is an indicator of the side-by-side packing of amylose helices. Fig. 4 shows a graph of the angular dependence of the X-ray intensity for a 3.2% amylose gel after storage for 1 h at 26° (Fig. 4*a*) and after 8 days (Fig. 4*b*). The presence of the diffraction peak on the X-ray scattering curve is clearly indicated. Fig. 5 shows the development of crystallinity with time during the maturation at 26° of a 3.2% amylose gel and a 10% starch gel. For both the amylose and the starch gel, the initial development of crystallinity occurred at a similar rate. The crystallisation of amylose effectively reached a limit after 48 h, whereas the crystallinity of the starch gel continued to increase. This slow increase in crystallinity, unique to the starch gel, appears to be related to the slow increase in the modulus and also to the development of the endothermic transition at 63°, as observed by d.s.c.

On heating the matured starch gels to 90°, ~70% of the crystallinity of the starch gel was abolished, whereas that of the amylose gel was reduced by only 25%. In an attempt to isolate the contribution of crystallisation within the swollen granule to the crystallinity of the starch gel, experiments were performed on swollen starch granules.

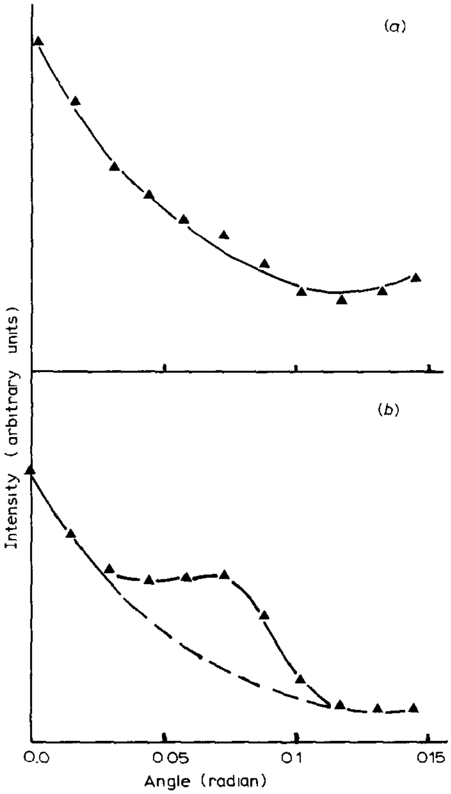

Fig. 4. Graph of scattered X-ray intensity *versus* angle for a 3.2% amylose gel (*a*) after 1 h at 26°, and (*b*) after 8 days.

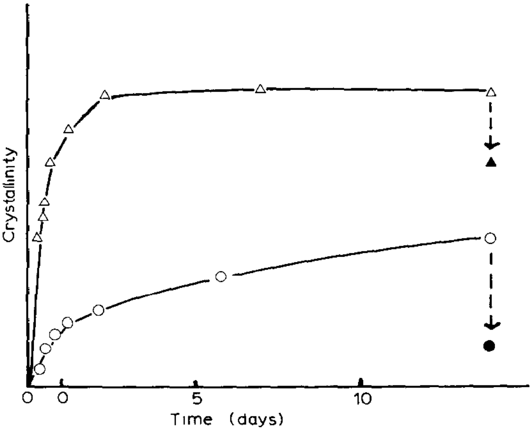

Fig. 5. Development of crystallinity (measured as the integrated intensity of the 100 diffraction peak) as a function of time for the 3.2% amylose gel (△) and a 10% starch gel (○), and after heating to 90°.

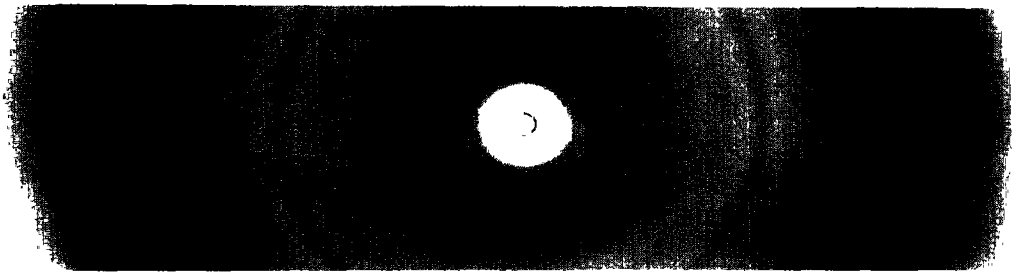

Fig. 6. Wide-angle diffraction pattern of gelatinised starch granules after storage for 14 days at 26°.

The granules were chromatographically washed at 95° to remove soluble amylose. This reduced the iodine-binding capacity of the gelatinised granule to 1%, indicating an amylose content of <5%. X-Ray diffraction studies of the granules immediately after cooling to 26° showed no evidence of a detectable X-ray diffraction pattern. After storage for 14 days at 26°, the B-type pattern shown in Fig. 6 was obtained. Fig. 7 shows a scan of the 100 diffraction peak before (*a*) and after (*b*) heating these granules to 70°. The crystallinity completely disappears on heating.

DISCUSSION

The present study suggests that the retrogradation of starch consists of two separable processes, one involving the amylose solubilised during gelatinisation, and the other involving amylopectin within the gelatinised granule. The initial development of shear modulus and crystallinity occurs at a similar rate in both the amylose and starch gels. The main difference between the development of the shear modulus within amylose and starch gels was that, whereas the shear modulus of an amylose gel effectively reached a constant value after 24 h, the shear modulus of a starch gel continued to increase during several weeks. A major difference between the two types of gels was that the increase in modulus of the starch gel after 24 h could be reversed by heating to 100°. The shear modulus of an amylose gel showed no such decrease. Therefore, the changes in modulus of the starch gel after 24 h most likely involve changes in the mechanical properties of the gelatinised starch granules. The present results suggest that the crystallisation of polymer chains within the gelatinised granule was responsible for these changes. The development of crystallinity, as detected by X-ray diffraction and as indicated by the development of an endothermic transition in d.s.c., was closely related to the slow development of the shear modulus of the starch gel on storage.

The molecular species involved in this crystallisation must be amylopectin. In the native state, amylopectin is considered to be the crystalline component of starch[14]. Although it is unusual for a highly branched molecule such as amylopectin to occur in crystalline form, the evidence is compelling. For instance, waxy-maize

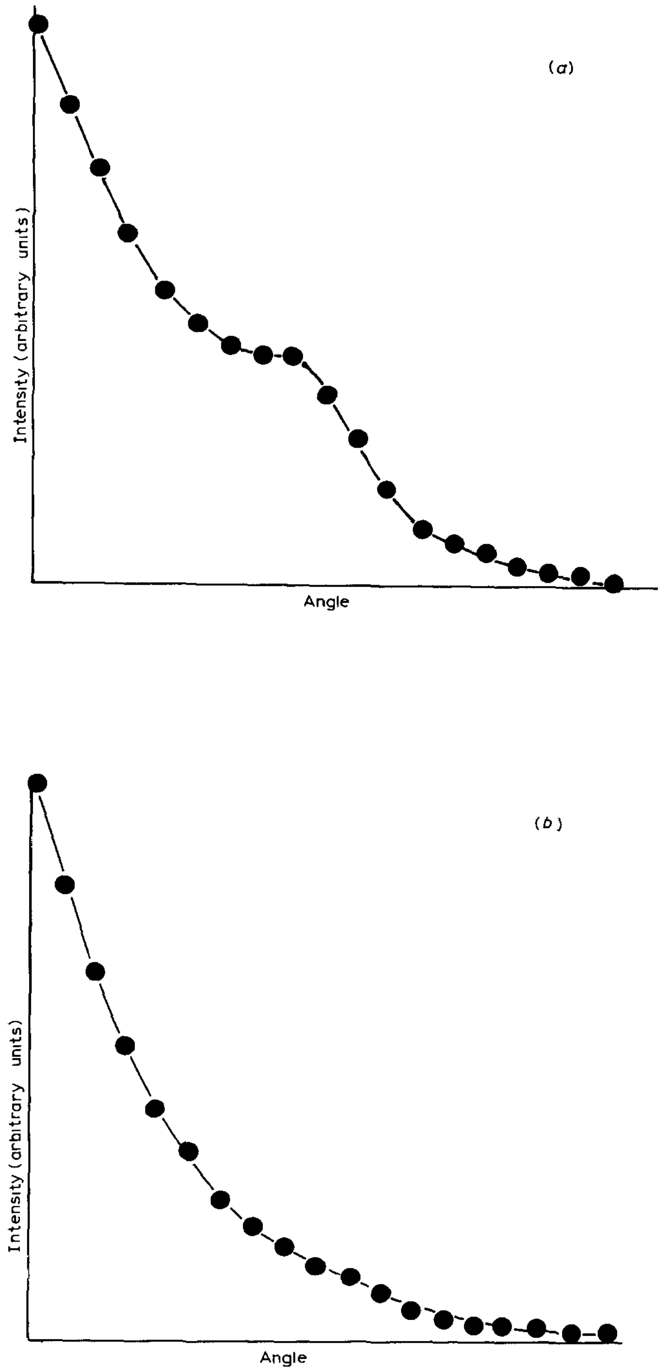

Fig. 7. Scan of 100 diffraction peak of stored, gelatinised starch granules before (*a*) and after (*b*) heating to 70°.

starches, which contain only amylopectin, are crystalline[14]. In the starch gel, two types of molecular interaction leading to crystallinity can be envisaged, one between amylose and amylopectin, and the other involving only amylopectin.

The experiments reported here demonstrate that crystallisation, involving amylopectin only, may occur within the gelatinised starch granule. This supports the suggestion made by Schoch[18] that crystallisation of amylopectin alone is responsible for the long-term changes in the firmness of starch gels on storage.

An interesting feature of the crystallisation involving amylopectin, as compared to that of amylose, is its reversibility on heating to 100°. Additionally, the temperature for the midpoint of the transition, 63°, is close to the gelatinisation temperature of the starch granule. Amylopectin has a bimodal distribution of chain lengths[14], with modal values of d.p. of ~15 and 50. The smaller chains are believed to be attached as clusters on the longer chains; therefore, inter-chain association can only extend over 15 or so residues before it is interrupted by a branch point.

Amylose, on the other hand, because it is essentially linear, can form inter-chain associations with other amylose molecules over many more residues, which presumably accounts for the greater stability of amylose crystallites. Whilst the crystallinity of "aged" swollen starch granules can be abolished by heating to 100°, some crystallinity still remains in the starch gel. This residual crystallinity is attributed to the amylose.

As the crystallisation process in the starch gel involves two separate polysaccharides, it is not possible to apply the Avrami analysis to the X-ray data. D.s.c. selectively examines the crystallisation of amylopectin, and, in the past, the analysis has been successfully applied to these data[1]. There are objections, however, in applying it to systems, like the starch gel, which contain a polymer and a diluent[8].

CONCLUSIONS

The initial stages of gelation of starch appear to be dominated by the gelation of the solubilised amylose resulting from a phase separation into polymer-rich and polymer-deficient phases. This is followed by a slower crystallisation, presumed to occur in the polymer-rich phase. The amylose-gel modulus and crystallinity could not be reversed by heating to 100°. Starch gels also showed this behaviour, but, in addition, exhibited a thermal reversibility in the component of modulus and crystallinity, which developed more slowly and did not occur in amylose gels alone.

In the starch gels, the granules act as fillers in the amylose matrix. Isolated amylopectin granules show the same slow increase in crystallinity, which, without the amylose component, is completely thermally reversible. This demonstrates the possibility that amylopectin alone could be responsible for the thermally reversible component of crystallinity in the starch gel, although it does not exclude co-precipitation and co-crystallisation of the amylose with the amylopectin within the granule as an alternative process. The fact that the long-term, thermally reversible changes in modulus of the starch gel parallel the changes in crystallinity suggests that partial crystallisation within the granules results in an increase in rigidity of the granules, which enhances their reinforcement of the amylose matrix.

ACKNOWLEDGMENTS

The authors thank J. R. Bacon and D. J. Wright for advice and assistance with the d.s.c. measurements.

REFERENCES

1 R. G. McIver, D. W. E. Axford, K. H. Colwell. and G. A. H. Elton, *J. Sci. Food Agric.*, 19 (1968) 560–563.
2 J. E. Cluskey, N. W. Taylor, and F. R. Senti, *Cereal Chem.*, 36 (1959) 236–242.
3 E. M. A. Willhoft, in J. A. Radley (Ed.), *Industrial Uses of Starch and its Derivatives,* 4th edn., Chapman and Hall, London, 1968.
4 J. R. Katz, *Z. Phys. Chem.*, 150 (1930) 37–59.
5 R. E. Rundle, L. Daasch, and D. French, *J. Am. Chem. Soc.*, 66 (1944) 130–134.
6 M. Avrami, *J. Chem. Phys.*, 7 (1939) 1103–1112.
7 M. Avrami, *J. Chem. Phys.*, 8 (1940) 212–224.
8 L. Mandelkern, *Crystallisation of Polymers,* McGraw-Hill, New York, 1964.
9 S. G. Ring and G. Stainsby, *Prog. Food Nutr. Sci.*, 6 (1982) 323–329.
10 M. J. Miles, V. J. Morris, and S. G. Ring, *Carbohydr. Polym.*, 4 (1984) 73–78.
11 M. J. Miles, V. J. Morris, and S. G. Ring, *Carbohydr. Res.*, 135 (1985) 257–269.
12 G. K. Adkins and C. T. Greenwood, *Staerke,* 18 (1966) 213–218.
13 W. Banks, C. T. Greenwood, and D. D. Muir, *Staerke,* 23 (1971) 118–124.
14 W. Banks and C. T. Greenwood, *Starch and its Components,* Edinburgh University Press, Edinburgh, 1975.
15 S. G. Ring, Ph.D. Thesis, University of Leeds, 1983.
16 W. R. Morrison, *J. Sci. Food Agric.*, 26 (1975) 507–521.
17 M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
18 T. J. Schoch, *Bakers Digest,* 39 (1965) 48–54.