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# Heat-induced structural changes of acid-hydrolysed and hypochlorite-oxidized barley starches

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The effects of hydrolysis and oxidation on the heat-induced structural changes of barley starch dispersions were studied by dynamic viscoelastic methods and light microscopy. The effects of the modifications on the molecular weight of amylose and amylopectin were investigated by high-performance liquid chromatography (HPLC) with post-column iodine staining. Comparison of the molecular weights of oxidized and acid-hydrolysed barley starch dispersions with the same limiting viscosity values showed that the molecular weight of amylose was decreased more by oxidation than by hydrolysis, whereas the molecular weight of amylopectin was reduced to the same degree. Relative to acid hydrolysis, oxidation also reduced the temperature at which gel-formation occurred and weakened the gels. Irrespective of the type of modification, large amounts of granule residues were left after heating at 120 °C for 20 min. Microstructural studies of the gels showed that both hydrolysed and oxidized starches underwent phase separation into amylose- and amylopectin-rich phases. Copyright © 1996 Elsevier Science Ltd

## INTRODUCTION

Acid-modification and oxidation are widely applied to obtain starches with low cooking viscosity at relatively high starch concentration. Acid-hydrolysed starches are mainly used in the confectionary industry where a good gelling ability is required. Oxidized starches are used in the paper industry as surface sizes or coating binders. Oxidation introduces carbonyl and carboxyl groups which hinder gel formation. In most paper applications fast gel formation is an undesirable property.

Starch is composed of almost totally linear amylose and highly branched amylopectin. Amylose accounts for 18–33% of normal starch, while amylopectin is the major component. The amylose component, which forms a gel during cooling, is responsible for the gelling of starch pastes. Amylose forms a gel as a result of phase separation into polymer-rich and polymer-deficient regions (Miles *et al.*, 1985). On a longer time-scale, crystallization occurs, with the amylose chains adopting a double helical structure in the crystalline domains (Imberty & Perez, 1988). Although the extent of crystallization of the polymer-rich phase is still a subject of debate (Miles *et al.*, 1985; Gidley, 1989), it is now well established that amylose gels have a weak crystalline structure and consist largely of an amorphous fraction (Leloup *et al.*, 1992). Compared with amylose, cereal amylopectin is more easily solubilized in aqueous solution and requires much higher polymer concentration and longer times for gelation.

Mild acid-modification of barley granular starch promotes heat-induced solubilization of the starch molecules in water solution (Autio *et al.*, 1992; Pessa *et al.*, 1992). In addition to amylose, which will leach out of the granule, amylopectin will also be solubilized, at temperatures below 100°C where the solubility of native barley amylopectin is very limited (Autio *et al.*, 1992). In hydrolysed barley and maize starch gels heated to 90°C, the continuous phase is composed of both amylose and amylopectin (Autio *et al.*, 1992; Pessa *et al.*, 1992).

Mixing of pure soluble amylose and amylopectin has been shown to result in phase separation, one phase being enriched with amylose and the other with amylopectin (Kalichevsky & Ring, 1987). The polymer compositions of the continuous and dispersed phases are dependent on the ratio of amylose to amylopectin. By changing this ratio, it is possible to determine the point of phase-inversion from an amylopectin continuous network with amylose dispersed to a network in

which the amylose forms the continuous phase entrapping the amylopectin droplets. The inversion has been estimated to occur at an amylose:amylopectin ratio of about 30:70 on the basis of rigidity measurements in compression experiments, and at 15:85 on the basis of viscoelastic measurements (Leloup *et al.*, 1991; Doublier & Llamas, 1992). The results suggest that the amylose dominates in determining the properties of amylose plus amylopectin mixtures.

The aim of the present study was to compare the gel formation of oxidized and hydrolysed barley starch dispersions. To understand the basis for gel formation, we also studied solubility, the molecular weight distribution of amylose and amylopectin, and the heat-induced microstructure.

## MATERIALS AND METHODS

### Materials

Commercial barley starch (Raisio Group, Finland) was used as a raw material for acid-modification and oxidation.

### Modification of starch

Acid treatment of granular starch was carried out essentially as described by Cowie & Greenwood (1975). Barley starch was hydrolysed with 1 M HCl under argon as 2–10% suspensions in three-neck bottles with magnetic stirring at 40 °C for 2.5 (H1) and 7.0 h (H2).

The pH of 40% barley starch water suspension was adjusted to 10.0 with 2 M NaOH solution. Correct amount of 15% NaClO solution (activity 98.4 g Cl/kg) was added gradually during 30 min, at room temperature, to the stirred slurry. The amounts of NaClO reagent in two oxidations were 20 (O1) and 40 g Cl/kg starch (O2). The pH of the reaction mixture was maintained at 10.0 with NaOH solution during the addition of the reagent and for a further 50 min after the addition was stopped. The pH was then adjusted to 7.0 with 1 M $H_2SO_4$; the oxidized starch was filtered, washed twice with distilled water and airdried at 30°C.

### General methods

The carboxyl content of the oxidized starches was determined by a standard method, DIN 10386, and the carbonyl content by a titrimetric method (Smith, 1967). Starch sample (5 g) was slurried in 25 ml of 0.1 M hydrochloric acid and the solution was stirred occasionally over a period of 30 min. The slurry was then filtered through a medium-porosity fritted-glass funnel and the residue was washed with distilled water until it was chloride-free. The sample was transferred to a beaker and suspended in 300 ml of distilled water. The suspension was heated in a boiling water bath with continuous stirring for about 10 min to ensure complete gelatinization. The hot sample was titrated to pH 8.3 with 0.5 M sodium hydroxide solution.

For the carbonyl content determination, 2 g of starch sample was suspended in 100 ml of distilled water and the suspension was gelatinized by heating in a boiling water bath and cooled to 40°C. The pH was then adjusted to 3.2 and 15 ml of hydroxylamine reagent was added. The hydroxylamine reagent was prepared by dissolving 25 g hydroxylamine hydrochloride in distilled water, adding 100 ml of 0.5 M sodium hydroxide and diluting the mixture to 500 ml. The sample was covered with aluminium foil and placed in a water bath at 40°C. After 4 h excess hydroxylamine was determined by rapid titration of the reaction mixture to pH 3.2 with 0.1 M hydrochloric acid. Typical standard error of mean for carboxyl and carbonyl determinations was 7%.

Solubility was determined by the modified procedure of Leach *et al.* (1959). The starch samples (100±0.1 mg) were weighed into small, screw-capped test-tubes, 5 ml distilled water was added and the tubes were closed and mixed well with a Vortex mixer. The tubes were incubated in an 85°C water bath for 30 min, with occasional manual stirring, then cooled rapidly to room temperature and centrifuged for 15 min. The phases were separated immediately after the centrifugation, and the solubilized starch was determined as total carbohydrates in the supernatant by the method of Dubois *et al.* (1956).

### HPLC analysis

For the GPC analysis, a 200-mg starch sample was moistened for 1 h with 5 ml of water and 35 ml of 1 M sodium hydroxide solution was added. The sample was stirred overnight and diluted 2:5 with 1 M sodium hydroxide solution. The HPLC–GPC instrument consisted of a pump, M-715 automatic injector and μHydrogel 250, 500 and 2000 columns in series. Either a refractive index detector (M-411) or postcolumn addition of iodine with spectrophotometric detection was used. The eluent was 50 mM sodium hydroxide fed at a flow rate of 0.5 ml/min, and for the spectrophotometric detection 1.2 mM iodine containing 0.2% orthophosphoric acid was fed at a flow rate of 0.3 ml/min. The system was controlled and the data handled by a Maxima 820 workstation. All the equipment was from Millipore/Waters (Milliford, MA, USA).

Full range pullulan standards supplied by Showa Denko (Japan) were used as calibration standards. The time scale in all chromatograms was the same.

### Rheological measurements

The limiting viscosity number was determined at 23°C by the method of Greenwood (1964). The viscoelastic

**Table 1. Limiting viscosity and solubility of starch samples**

| Sample | Limiting viscosity ml/g | Solubility % |
|---|---|---|
| O1 | 70±3 | 35±1 |
| O2 | 30±2 | 71±3 |
| H1 | 74±5 | 49±1 |
| H2 | 41±1 | 61±0 |

**Table 2. Carboxyl and carbonyl contents of the oxidized barley starches**

| Sample | COOH/100GU | CHO/100GU |
|---|---|---|
| O1 | 0.65 | 0.56 |
| O2 | 1.52 | 0.84 |

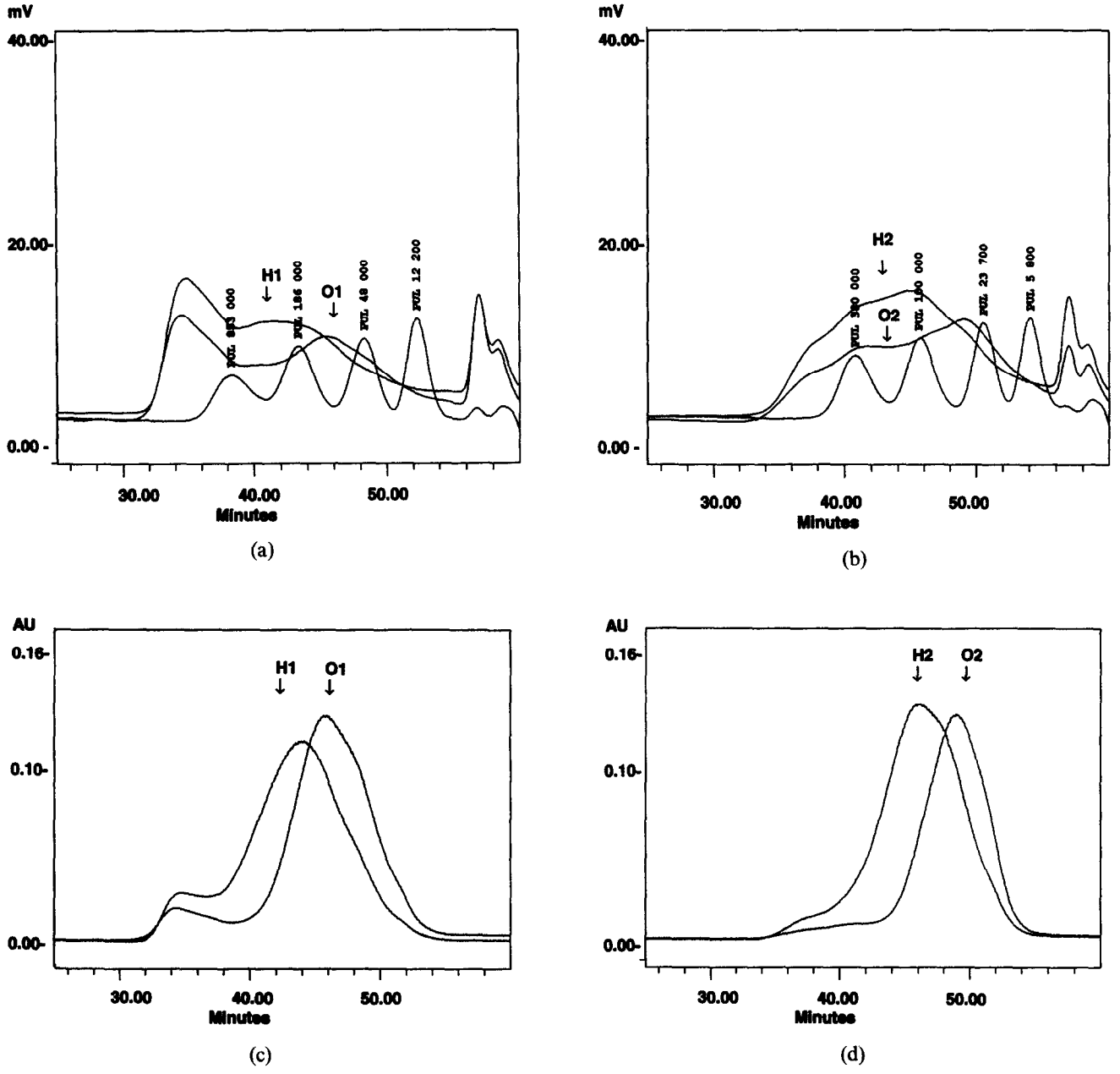


**Fig. 1.** (a) Comparison of molecular weight of H1 and O1. H1 is hydrolysed barley starch (hydrolysis time 2.5 h) and O1 is oxidized barley starch with low degree of oxidation. Detection by RI. Standards: Pullulans varying in molecular weight. (b) Comparison of molecular weight of H2 and O2. H2 is hydrolysed barley starch (hydrolysis time 7.0 h) and O2 is oxidized barley starch with high degree of oxidation. Detection by RI. Standards: Pullulans varying in molecular weight. (c) Comparison of molecular weight of H1 and O1. H1 is hydrolysed barley starch (hydrolysis time 2.5 h) and O1 is oxidized barley starch with low degree of oxidation. Post-column iodine dyeing, detection at 630 nm. (d) Comparison of molecular weight of H2 and O2. H2 is hydrolysed barley starch (hydrolysis time 7.0 h) and O2 is oxidized barley starch with high degree of oxidation. Post-column iodine dyeing, detection at 630 nm.

properties during cooling were measured with a small-amplitude oscillation test. Twenty-five per cent suspensions of acid-modified and oxidized starches in 50 mmol $NH_4Ac$ buffer, pH 5.0, were heated at 120°C for 20 min (mixing, pressure 1.6 bar) and then transferred to the Bohlin VOR rheometer (Bohlin Reologi Ab, Lund, Sweden). In the rheometer, the samples were cooled from 90 to 20°C at a rate of 1°C/min. The frequency was 1 Hz and the strain $1 \times 10^{-3}$.

### Light microscopy

Small pieces of cooled starch gels, prepared as described above, were chemically fixed in 1% glutaraldehyde, then dehydrated and embedded in Historesin (Reichert-Jung, Germany) embedding medium, as recommended by the manufacturer. The samples were sectioned and stained with iodine and examined in an Olympus Vanox-T microscope.

## RESULTS AND DISCUSSION

The degree of acid-hydrolysis and oxidation was determined by measuring the limiting viscosity. Sample pairs (one oxidized and one hydrolysed), which had almost the same viscosity (Table 1), were selected for study.

The limiting viscosity values of the hydrolysed and oxidized starches are shown in Table 1. The solubilities of the hydrolysed and oxidized barley starch dispersion at 85°C were 35 and 49%, respectively, for lower degree of modification and 71 and 61%, respectively, for higher degree of modification. Amylose accounts for 29% of barley starch (Autio & Poutanen, 1993). Because amylopectin is the major component of starch, most of the increased solubility was attributed to solubilization of amylopectin. Since the solubility measurements were made at 1% starch concentration, the results cannot be compared with the rheological and microstructural tests where the starch concentration was 25%.

The carboxyl and carbonyl contents of oxidized barley starches are presented in Table 2.

Gel permeation chromatography (Fig. 1a and 1b) showed that most of the high molecular weight amylopectin of native starch, eluting at 28 min, had degraded to rather high molecular weight polymers (eluting at 35 min or more) (Autio *et al.*, 1992). Post-column iodine detection at 630 nm showed that the molecular weight of amylose, which is eluted after 40 min, is higher for the hydrolysed than the oxidized sample (Fig. 1c and 1d).

Comparison of samples H1, O1 and H2 reveals an interesting feature: the molecular weight of amylopectin is the same in samples H1 and O1 (Fig. 1a) and the molecular weight of amylose is the same in samples O1 and H2 (Fig. 1c and 1d). Moreover, the molecular weight of amylopectin and amylose is decreased in the more highly modified samples H2 and O2 (Fig. 1b) relative to the less modified samples H1 and O1 (Fig. 1a).

Hydrolysed and oxidized starches are usually heated in a jet-cooker before use. The combination of shear, heat and pressure is assumed to have a marked effect on the rheological properties of starch dispersions. 25% suspensions were heated in an autoclave (120°C, 20 min, 1.6 bar, mixing), and rheological changes were measured during cooling. All starch samples formed a gel during cooling (Fig. 2). The rate of gelling decreased in the order H2 > H1 > O1 > O2, as did the rigidity. The gel formation occurred at higher temperature for the hydrolysed than oxidized samples. The higher molecular weight of amylose in the hydrolysed samples is probably one reason for the different gelling properties. However, the molecular weight of amylose is almost the same for samples H2 and O1, although the gelling properties differ greatly. Here the differences in gelling behaviour may be due to the presence of carboxyl and carbonyl groups in the O1 (Table 1) which is reported to hinder the gel formation of amylose (Boruch, 1985) or the amylopectin may influence the gelling of amylose in different ways depending on the molecular weight and branching of amylopectin.

Samples for microscopy were taken after cooling of the 25% starch gels. Iodine stains amylose blue and amylopectin brown allowing the demonstration of both starch components (Fig. 3a–d). Solubilized starch formed a continuous phase in which starch granules and amylose droplets were dispersed. Phase separation into

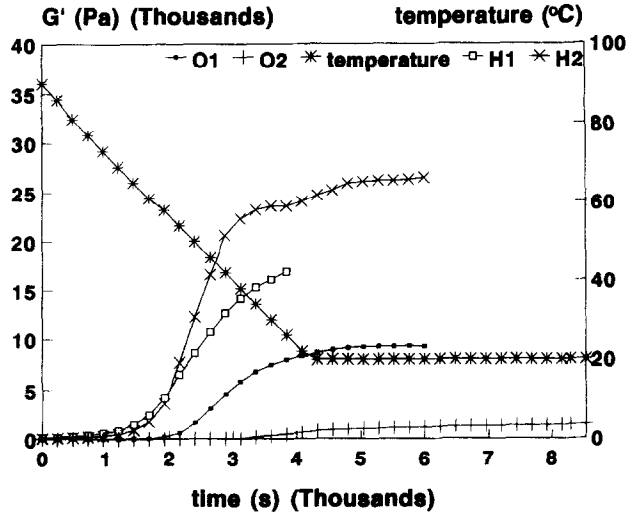


**Fig. 2.** Storage modulus (G') of 25% hydrolysed H1 (hydrolysis time 2.5 h) and H2 (hydrolysis time 7.0 h) and oxidized O1 (low degree of oxidation) and O2 (high degree of oxidation) barley starch dispersions preheated at 120°C for 20 min under shear, and cooled from 90 to 20°C in the rheometer.

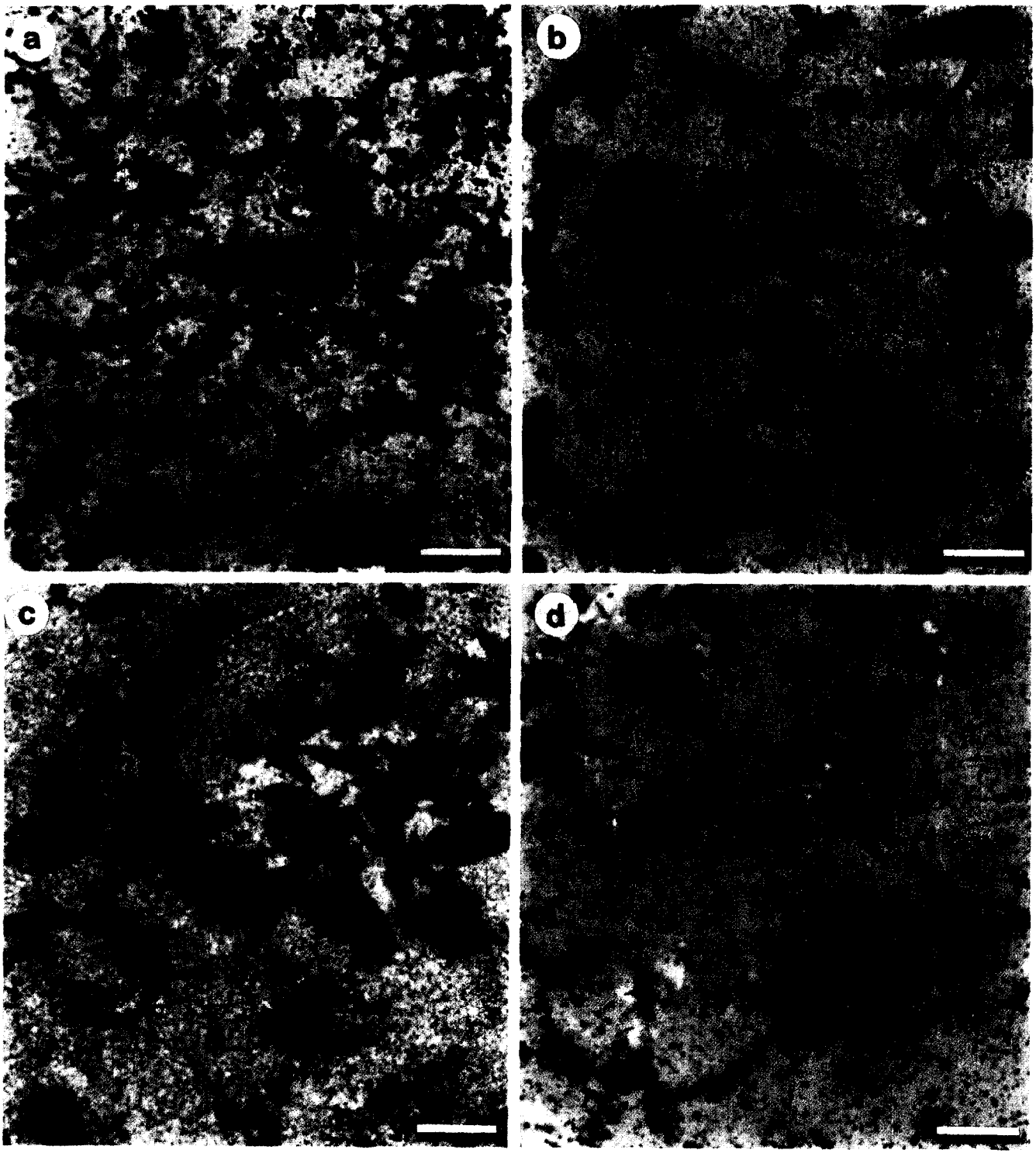


**Fig. 3.** Embedded sections of 25% starch dispersions after cooling. (a) Hydrolysed H1 (hydrolysis time 2.5 h). (b) Oxidized O1 (lower degree of oxidation). (c) Hydrolysed H2 (hydrolysis time 7.0 h) and (d) oxidized O2 (high degree of oxidation).

amylose- and amylopectin-rich phases occurred for both the hydrolysed and oxidized starches. All the samples contained starch granules, but the gel prepared from O2 contained the lowest amount. In the hydrolysed starch gels, no amylose could be observed inside the granules. By contrast, in the oxidized starch gels, weak violet-stained zones were visible. Blue-stained, large amylose-rich domains were visible in all gels. The size distribution of these domains varied widely. The largest droplets visible in Fig. 3a, b and c are discontinuous. It has been shown that only part of the amylose in native starch is effective in forming a gel (Doublier & Llamas, 1992). Our results suggest that in starch systems, amylose may be located in both continuous and dispersed phase. Since native starch systems also contain amylose–lipid complex, their structure is far more complex than that of molecular mixtures of amylose and amylopectin. On the basis of the micrographs it is not possible to say which component forms the continuous phase. The very different gelling rate of the samples, however, suggests that, at least in the hydrolysed samples, amylose dominates in the structure and probably forms the continuous phase.

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