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Rheological and Microstructural Changes of Oat and Barley Starches During Heating and Cooling

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OAT AND BARLEY STARCHES DURING HEATING AND COOLING

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Abstract

Microstructural and rheological changes in barley and oat starch dispersions during heating and cooling were studied by light microscopy and dynamic viscoelastic measurements. The two starch pastes showed similar viscoelastic properties after gelatinization, but during cooling the 20% barley starch pastes heated at 95°C underwent a sharp transition in viscoelastic behaviour probably due to the gelation of amylose. This transition was shifted to lower temperatures at 10% starch concentration.

Microstructural studies of an 8% barley starch dispersion heated to 90°C using the smear technique showed amylose to form a network structure around the granules. The granules in starch paste heated to 95°C were poorly stained and amylopectin was fragmented. Microscopic examination of an embedded section of the cooled barley starch gel showed amylose to form a continuous phase in which starch granules were dispersed.

G' increased below 80°C during cooling of 10% oat starch dispersions preheated at 95°C. No rheological changes occurred when they were preheated at only 90°C.

Microstructural studies of an 8% oat starch dispersion heated to 90°C using the smear technique showed amylose to form a network structure around the granules. Part of the granule structure had already broken down. Heating to 95°C induced considerable changes in the granule structure of oat starch gels. Amylopectin formed a very fine network. Microscopic examination of embedded sections of the cooled, stored gel showed a much coarser structure compared with that of the smear.

Introduction

Starches have the useful property of thickening and gelling aqueous solutions. Before specific physical properties can be created at will, more information is needed on the relationship of properties to structure.

Starch-containing foods are usually cooked by heating in the presence of water. Above gelatinization temperature dramatic changes occur in the structure of starch. It has been proposed that gelatinization is "the collapse of molecular orders within the starch granule manifested in irreversible changes in properties such as granular swelling, native crystallite melting, loss of birefringence, and starch solubilization" (Atwell et al., 1987).

Dynamic viscoelastic methods provide an excellent tool for studying rheological changes during heating and cooling without breaking the structure. Eliasson (1986) studied the heat-induced changes in 10% wheat, maize, waxy barley and potato starches. For all these starches the storage modulus, G', and loss modulus, G", increased after gelatinization. Further heating caused a decrease of the moduli. At 90-95°C wheat and maize starches showed a second peak. The increase in G' was interpreted as starch granule swelling and the decrease in G' as a softening of the granules. Heat treatment of cereal starches gives rise to two stages of swelling and solubilization (Doublier, 1990). Amylose formed a gel during cooling of 10% maize and wheat starch pastes (Svegmark and Hermansson, 1990). Microstructural studies have shown that, in wheat starch paste, amylose forms the continuous phase in which the swollen starch granules are dispersed (Langton and Hermansson, 1989).

Little has been published on the changes in the structural properties of oat and barley during heating and cooling (Doublier et al., 1987; Gudmundsson et al., 1989). The few reports that do exist indicate, however, that oat starch possesses very different properties. Whereas in other cereal starches amylose is solubilized first, in oat starch pastes amylose and amylopectin are solubilized at the same time (Doublier et al., 1987). Oat starch differs rheologically from other cereal starches by exhibiting marked thixotropy at 70°C. Doublier et al. (1987) interpreted this behaviour as indicating the existence of a stronger structural network.

The aim of the present study was to monitor the

rheological changes of oat and barley starches during heating and cooling at varying starch concentrations and with different heat treatments. Light microscopy was used to evaluate the microstructural changes during heating and cooling.

Experimental

Materials

Barley starch was purchased from Raision Yhtymä Oy (Raisio, Finland), oat starch from Alko Ltd (Rajamäki, Finland). Concentrations of 8, 10 and 20% (w/w) were studied. The chemical analysis of the starches is presented in Table 1. The protein was determined by Kjeldahl nitrogen, and lipid content by a gravimetric method (AOAC, 1984). Moisture and ash were measured by AACC methods 44-15A and 08-01, respectively (AACC, 1986).

Table 1. Chemical analysis of the starches.

Starch	Moisture (%)	Lipid (%)	Protein (%)	Ash (%)
Oat	8.5	1.6	0.8	0.3
Barley	11.5	1.0	0.5	0.3

Sample preparation

20% starch dispersions were heated at 60°C for 5 minutes with minimal stirring. Some stirring is necessary before the onset of swelling in order to obtain a homogeneous dispersion. Samples were transferred immediately to the Bohlin VOR rheometer (temperature 60°C). In the rheometer the samples were heated from 60 to 90°C, either heated at 90°C for 30 minutes and cooled or immediately cooled.

10 % starch dispersions were heated to 90 or 95°C, the 20% starch dispersions to 95°C (heating rate about 5°C/min) with minimal stirring. Upon reaching the desired temperature, the pastes were held at that temperature for a further 15 minutes. In the rheometer the samples were cooled from 90 to 25°C. At least three measurements were performed on each sample.

10% starch suspensions were heated to 95°C (heating rate 5°C/min). Upon reaching 95°C, the pastes were held at that temperature for 15 min. They were then cooled and stored in a refrigerator for one day, before embedding and microscopic examination.

8% starch dispersions were heated to 90 and 95°C (heating rate 5°C/min). On reaching the desired temperature and held at 95°C for 15 min, the starch dispersion was quickly smeared out onto an object glass and stained by iodine (0.33% iodine, 0.67% potassium iodide).

Embedded sections

The method developed by Langton and Hermansson (1989) was used. Samples were chemically fixed in 1 % glutaraldehyde with 0.1 % ruthenium red, dehydrated and embedded in historesin LKB. Sections (2-4 μm) were cut by a microtome (Reichert-Jung AG Heidelberg). The samples were sectioned and stained with iodine solution.

Light Microscopy

Reichert Polyvar and Olympus BH-2 microscopes were used for examining the samples.

Rheological measurements

Rheological measurements were performed with a Bohlin Rheometer VOR (Bohlin Reologi AB, Lund, Sweden) in the oscillatory mode. A concentric cylinder measuring system (C25) was used. Silicon oil was used to prevent drying. In the oscillation test, a small-amplitude oscillation is applied to a material and the resulting stress is compared with the strain. The amplitude ratio of the stress to strain gives the complex modulus, G*. The two sine waves have a phase shift, δ, which is used to give the storage and loss components: in-phase component being the storage or elastic component (G') and the quadrature component being the viscous or loss term (G"). The strain was 0.005, the frequency 1 Hz, and the heating and cooling rate 1°C/min.

Results and Discussion

Heat-induced microstructural changes in starch dispersions

Heating temperatures of 90 and 95°C were chosen to illustrate the dramatic changes in the microstructure of 8% oat starch dispersions (Figs. 1-3, color plate). Iodine stains amylose blue and amylopectin light to dark brown, allowing the demonstration of both starch components. At 90°C (Fig. 1) amylose formed a network structure around the granules; the microstructure resembled that of the other starches with the notable exception that part of the granule structure had broken down. The oat starch also consisted of a population of different kinds of granules. Raising the heating temperature from 90 to 95°C (Fig. 2) induced considerable changes in the granule structure of oat starch dispersion. On the basis of solubility studies, Doublier et al. (1987) reported that the solubility and swelling power of a 0.4% oat starch dispersion increased markedly when heating was to 95 rather than 90°C and in contrast to other cereal starches, amylose and amylopectin co-leached from the granule. On further heating at 95°C (Fig. 3) a still denser network structure was formed.

Figures 4-6 (color plate) demonstrate the heat-induced changes in barley starch dispersions. At 90°C amylose forms a network structure around the granules, which vary in the degree of swelling. The amylose still left inside the granule is concentrated in blue-stained zones. At 95°C, most of the granules are poorly stained and amylopectin is fragmented.

Rheological changes during heating and cooling

After gelatinization the storage modulus (G') of a 20% barley starch paste increased, with a maximum around 82°C (Fig. 7). Further heating caused a decrease in G'. After gelatinization, the phase angle was below one degree (Fig. 8), suggesting that the granules form a network structure. During the cooling stage, G' increased markedly below 60°C (Fig.7), probably due to the aggregation of amylose. Unexpectedly the phase angle increased at the same temperature (Fig. 8).
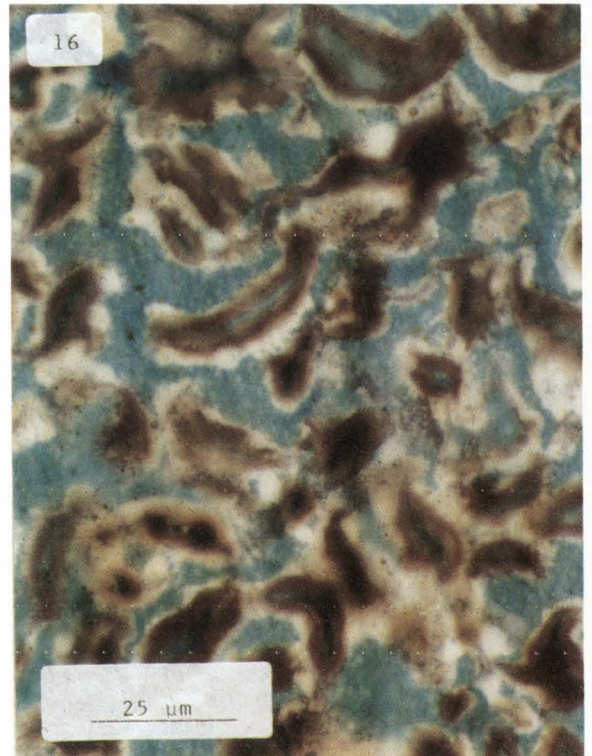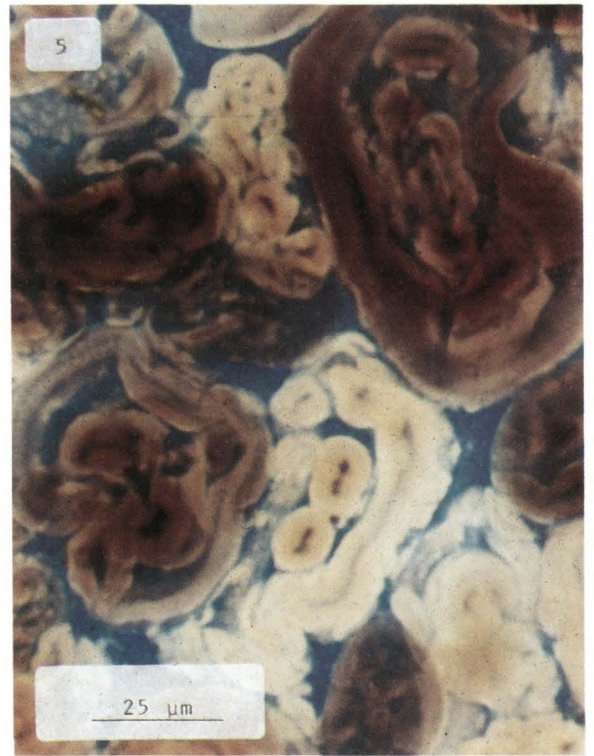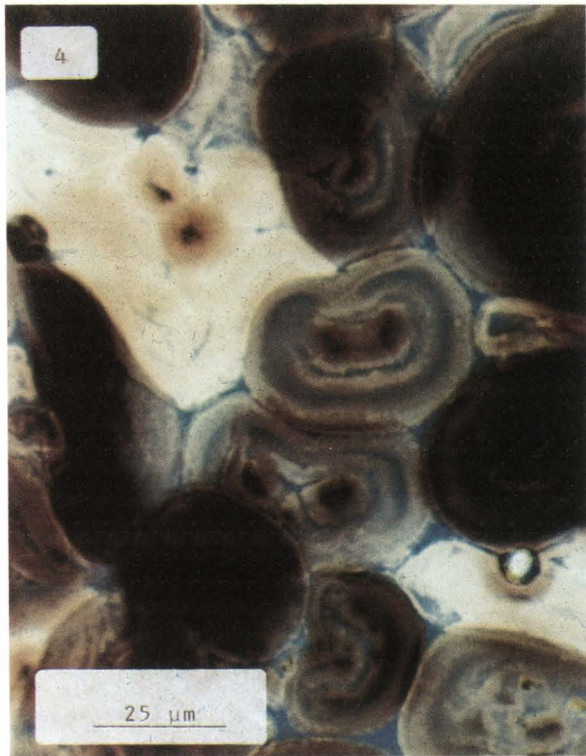
The heat-induced rheological changes in 20% oat
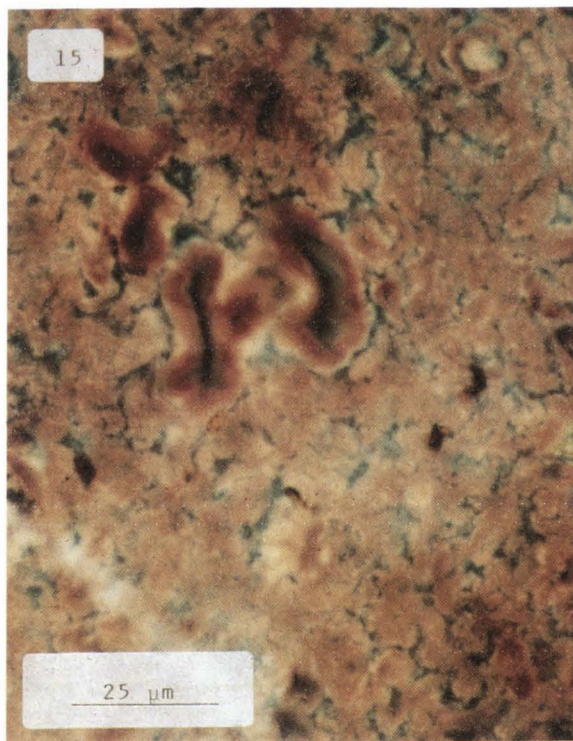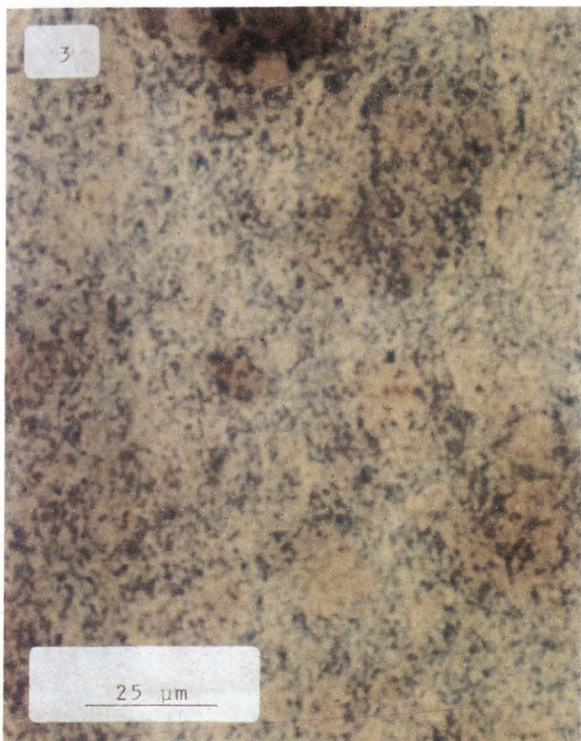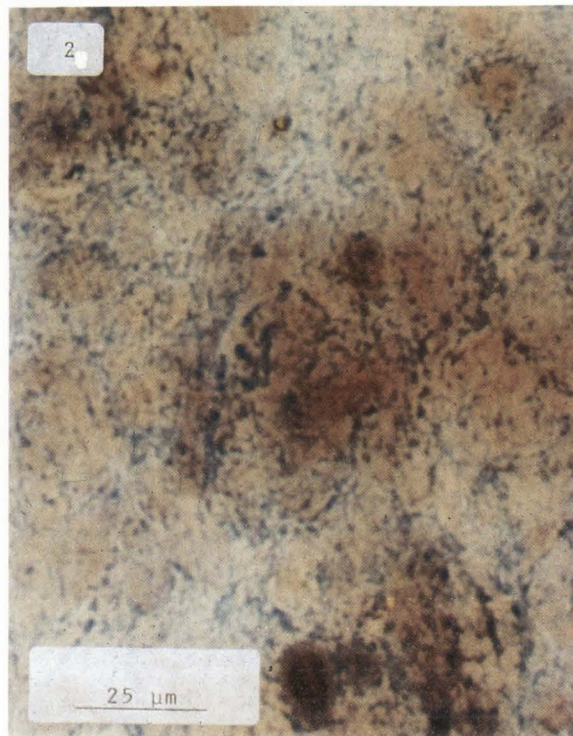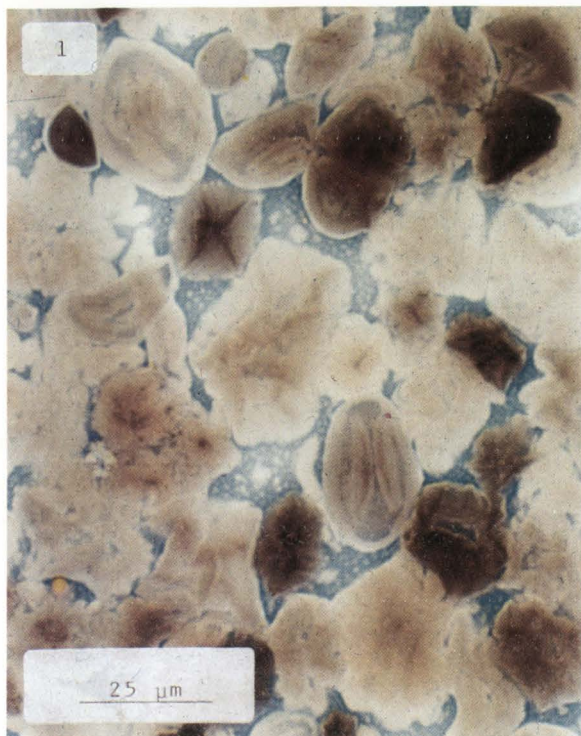
Figs. 1-3. Smears of 8% oat starch suspensions heated to 90 (**Fig. 1**), 95 (**Fig. 2**), and 95°C for 15 minutes (**Fig. 3**).
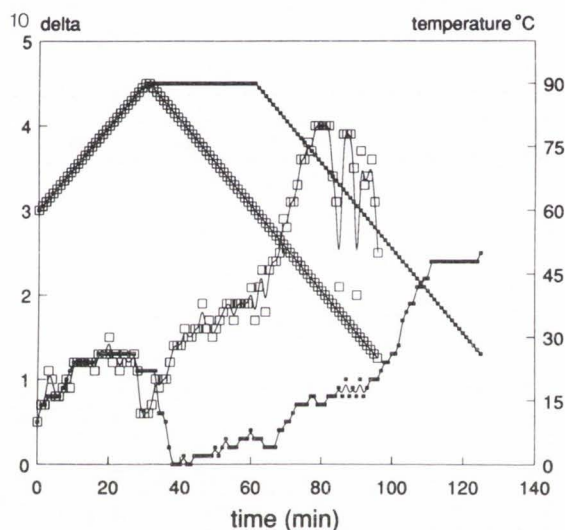
Fig. 15. Embedded section of 10% oat starch paste which was heated at 95°C for 15 minutes, cooled and stored in a refrigerator for one day.

Figs. 4-6. Smears of 8% barley starch suspensions heated to 90 (Fig. 4), 95 (Fig. 5), and 95°C for 15 minutes (Fig. 6).
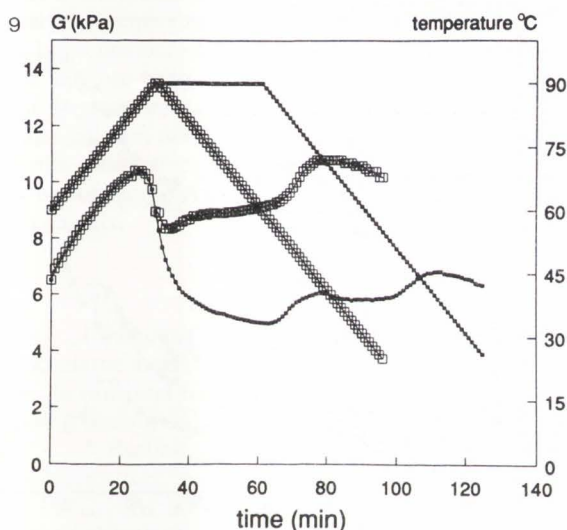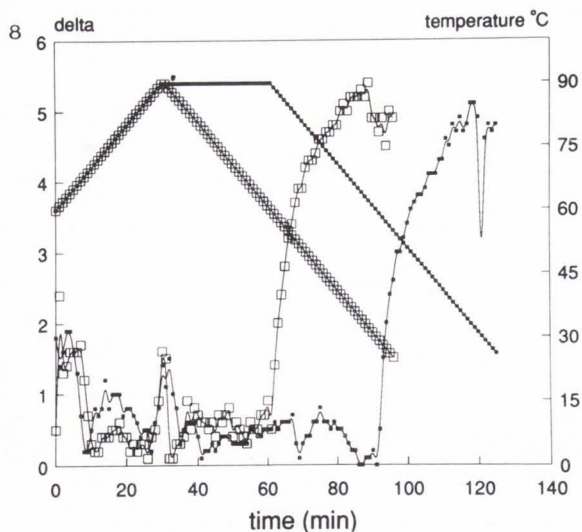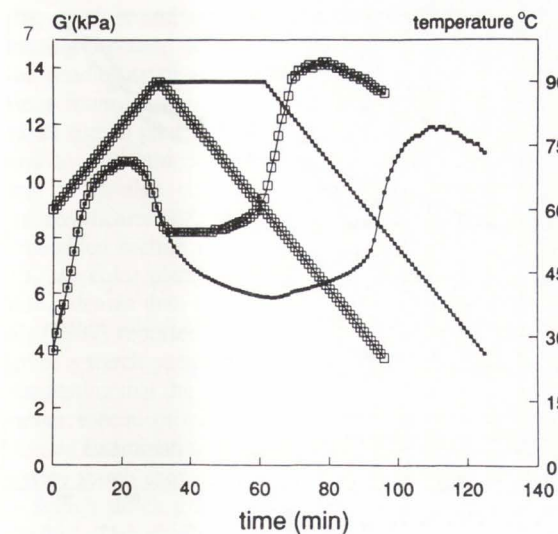
Fig. 16. Embedded section of 10% barley starch paste which was heated at 95°C for 15 minutes, cooled and stored in a refrigerator for one day.

Figs. 7 and 8. Storage modulus, G', and temperature profile (**Fig. 7**), and phase angle, δ (delta, **Fig. 8**) for 20% barley starch suspensions which were heated from 60 to 90°C, either heated at 90°C for 30 minutes and cooled (▪▪▪▪) or immediately cooled (□□□□). Heating and cooling rate was 1°C/min.

Figs. 9 and 10. Storage modulus, G', and temperature profile (**Fig. 9**), and phase angle, δ (delta, **Fig. 10**) for 20% oat starch suspensions which were heated from 60 to 90°C, either heated at 90°C for 30 minutes and cooled (▪▪▪▪) or immediately cooled (□□□□). Heating and cooling rate was 1°C/min.

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starch paste were similar to those of barley, but the cooling curves were very different (Fig. 9). Two minor changes in G' were observed during cooling, one below 80°C and the other below 50°C (Fig. 9). As with 20% barley starch dispersions, the increase in G' was accompanied by the increase of phase angle (Fig. 10). The microstructural studies suggest that the transition below 50°C is related to the aggregation of amylose. No such transition was seen in the oat starch pastes heated at 95°C (Figs. 11 and 12). The amylopectin could be expected to interfere with the gel-formation of amylose.

Heating at 95°C induced changes in the rheological properties of 20% barley starch dispersions. G' was substantially lower than in dispersions heated at 90°C, and began to increase as the temperature dropped below 85°C (Figs. 11 and 12). A major structural change was again observed below 60°C. The fragmentation of amylopectin is expected to influence the rheological properties of starch gels. Langton and Hermansson (1989) have reported phase separation of small amylopectin fragments during storage of wheat starch gels.
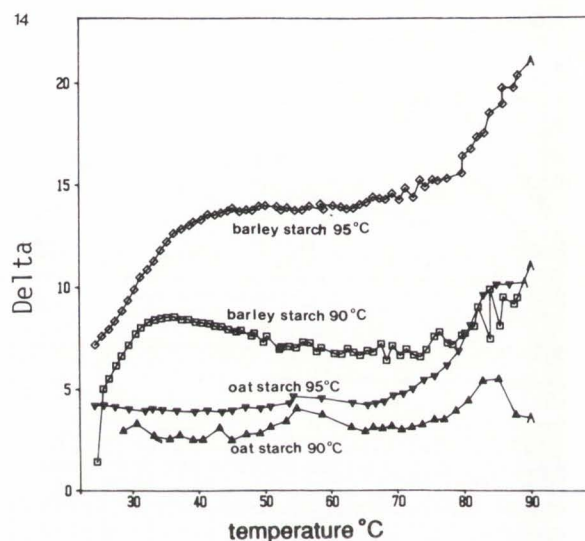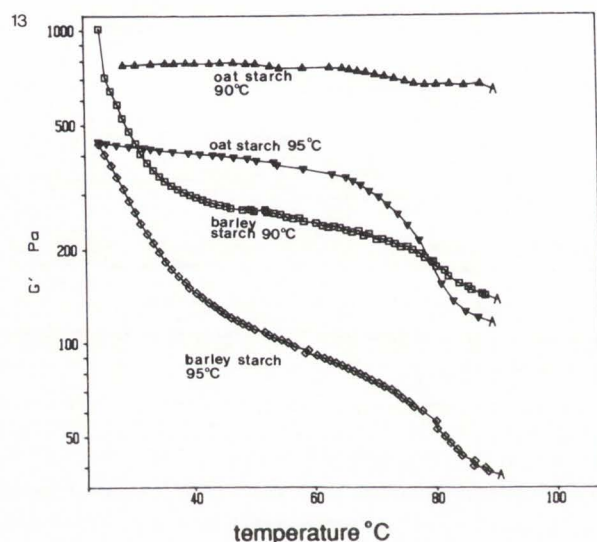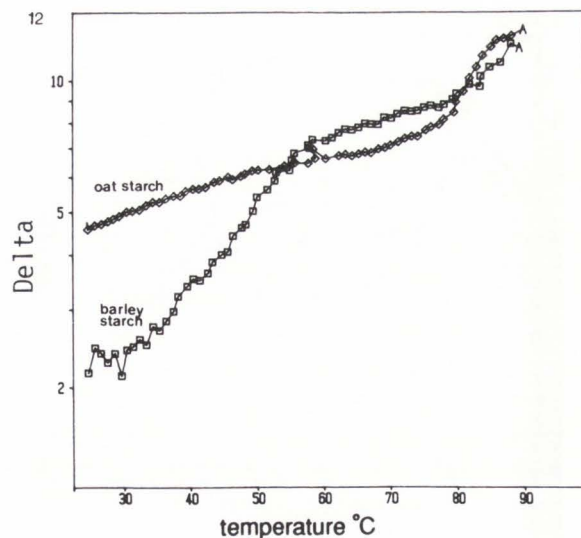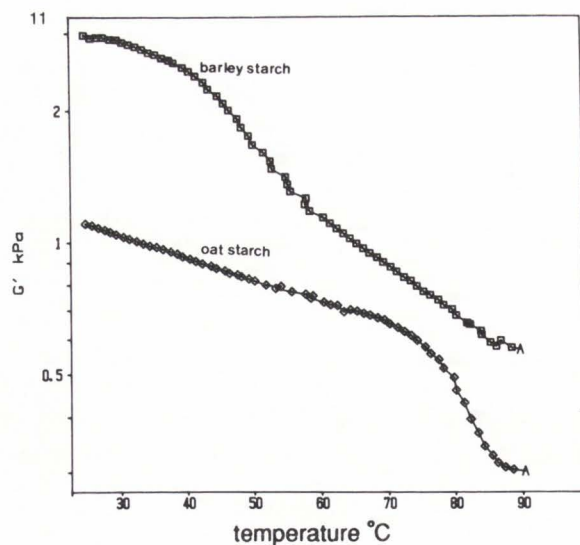
Figs. 11 and 12. G' (**Fig. 11**) and phase angle, δ (delta, **Fig. 12**) for 20% barley and oat starch suspensions which were preheated at 95°C for 15 minutes. Cooling rate was 1°C/min.

Figs. 13 and 14. G' (**Fig. 13**) and phase angle, δ (delta, **Fig. 14**) for 10% barley and oat starch suspensions which were preheated at 95 or 90°C for 15 minutes. Cooling rate was 1°C/min.

Heating temperature greatly affected the rheological properties of 10% oat and barley starch dispersions. Microscopic examination suggest that amylopectin hydration and fragmentation are responsible for the low G' of barley starch dispersions heated at 95°C. On cooling 10% barley starch dispersions heated at 90 and 95°C, a shift in the G' and δ respectively, was observed below 35°C (Figs. 13 and 14). Svegmark and Hermansson (1990) have reported that in maize and wheat starch dispersions the shift in the complex modulus is delayed at lower concentrations. The same holds true for pure amylose water systems (Clark et al., 1988).

No transition was observed on cooling the oat starch paste. The fact that only a minor change occurred in 20% oat starch dispersions heated to 90°C might indicate that at 10% starch concentration the amylose concentration is too low for gel·formation. Heating at 95°C decreased the rigidity of both the barley and oat starch gels (Fig. 13). The marked solubilization of amylopectin in oat starch dispersions subjected to heating at 95°C (Doublier et al., 1987) explains the substantial decrease of G'. On cooling the oat starch paste major shifts in G' and phase angle were detected below 80°C (Fig. 13 and 14). Doublier et al. (1987) have reported

that amylose and amylopectin are co-leached throughout the pasting process. Kalichevsky and Ring (1987) in a study of the miscibility of amylose and amylopectin in aqueous solutions found that the interaction of the polymers at 70-90°C gives rise to phase separation with the formation of amylose and amylopectin rich phases. The occurrence of phase separation is also supported by the difference between the microstructure of the smear (Fig. 3) and that of the embedded section of the cooled, stored oat starch gel (Fig. 15, see color plate). The network structure of the smear is much denser than that of the embedded section. Doublier et al. (1987) reported that removal of internal lipids from oats gives a starch paste similar to native wheat and corn starches suggesting that the starch-bound lipids are responsible for the rather special properties of oat starch.

Examination of the embedded section of cooled stored barley starch showed the amylose to form a continuous phase in which starch granules were dispersed (Fig. 16, see color plate). The starch granules also contained some amylose. There is a difference in the microstructure of the smear and that of the embedded section of the cooled, stored starch gel. A large number of amylopectin fragments are dispersed in the amylose phase in the gel shown in Figure 6. Langton and Hermansson (1989) reported that amylose phase became more uniform during storage because the amylopectin fragments clustered together. Further studies are needed on the effect of storage on the microstructure of barley and oat starch gels.

Conclusions

Combining light microscopy technique with dynamic viscoelastic measurements allowed the following observations on the structural changes of oat and barley starch dispersions during heating and cooling:

* Heating to 95°C induced considerable changes in the microstructure of oat starch dispersion relative to heating to 90°C. At 90°C amylose formed a network structure around the granules and part of the granule structure was broken down. At 95°C amylopectin formed a very dense network.

* G' increased markedly below 80°C on cooling of oat starch dispersions preheated at 95°C. No such transition has been observed for other native cereal starches.

* During cooling, the barley starch dispersions underwent a transition in viscoelastic behaviour. At lower starch concentration the transition shifted to lower temperatures. Microscopic examination of starch dispersions heated to 90°C showed amylose to form a continuous phase in which starch granules were dispersed. The granules heated to 95°C were poorly stained and amylopectin was fragmented.

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Discussion with Reviewers

J.L. Doublier: On cooling barley starch paste, the G' increase at 60°C is accompanied by a sharp δ increase. The G' evolution is to be ascribed to amylose gelation which should result in the rigidity increase of the system. The δ increase suggests on the contrary that there are reduction of the elastic character of the system. Both evolutions thus appear to provide contradictory information. Can you comment on this?

Reviewer I: How do you explain the major differences in the δ evolutions on cooling 10% and 20% barley starch pastes when they were heated, both at 90°C?

Author: The increase of G' was accompanied by the increase of phase angle on cooling concentrated (> 10%) barley starch pastes which were heated to 90°C. Under these conditions it is expected that the solubility of amylose is restricted and the volume fraction of the dispersed phase is high. The rheological measurements suggest that under these conditions we are not monitoring a gelation phenomena but aggregation of amylose. On cooling 10% barley starch paste, G' increased and phase angle decreased, suggesting that amylose forms a network structure.

D.J. Gallant: Could you explain why samples were fixed in 1% glutaraldehyde with 0.1% ruthenium red and what kind

of reaction may occur between fixative, stain and starch before iodine staining? Do you think that ruthenium interacts with iodine? Does this technique explain differences between colorations shown in Figures 4 and 8?

Author: Ruthenium red was used to visualize the small sample piece in sectioning. Parallel experiments were done without and with ruthenium. No visual changes could be seen in the micrographs.

Reviewer II: Do you plan to study starch dispersions using TEM and/or SEM techniques? Do you think that could improve our knowledge on viscoelastic behaviour of starch dispersions during heating and cooling ?

Author: In order to understand fully the behaviour of starches, all levels of structures should be studied. The electron microscopic structure of materials deals with the aggregation of molecules. TEM would give interesting information on the aggregation and gelation of amylose, and on the aggregation state of amylose inside and outside the granules. Unfortunately we do not have the experience needed to prepare samples with high water content for examination by TEM.

A.C. Eliasson: The micrographs show the pastes after one day in the refrigerator. How does the storage affect the appearance of the gels?

Author: The microstructure of the embedded section of the cooled, stored starch pastes is clearly different from that of the smear, indicating that the structure of the pastes has changed during cooling and/or cold storage. The effect of storage needs to be studied further.

Reviewer III: The decrease in phase angle and increase in G' in the case of barley starch is interpreted as being due to decreased granule interaction, to amylose leaching, and to increased amylose aggregation. Is there any evidence for amylose inhibiting granule-granule contact? What is the mechanism, and why does barley behave different from oat in this respect?

Author: In the case of oat starch paste most of the granule structure was broken down at 95°C, and the amylopectin formed a dense network structure, which would be expected to interfere with the gel-formation of amylose. In the case of barley starch the granule structure still existed although a large number of amylopectin fragments were observed. The granule-granule contacts are dependent on the concentration. At 10% concentration the granules were found to act as fillers in the amylose network. The microstructural studies suggest that during cooling and/or storage the amylopectin fragments will cluster together.