Heat-Induced Structural Changes in Acid-Modified Barley Starch Dispersions

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HEAT-INDUCED STRUCTURAL CHANGES IN ACID-MODIFIED BARLEY STARCH DISPERSIONS

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Abstract

The effect of heat treatment on the gel formation and microstructure of barley starch dispersions, hydrolyzed with 1 M HCl for 0.5, 1.0 and 4.0 hours, was studied by dynamic viscoelastic methods and by light microscopy. The effects of acid hydrolysis on the molecular weight of amylopectin and amylose were studied by high-performance liquid chromatography (HPLC) with post-column iodine staining.

Microstructural studies of 8% hydrolyzed barley starch dispersions heated to 90 °C showed that even a short acid treatment induced considerable changes in the granule structure. The molecular weight of amylopectin decreased substantially. As the hydrolysis progressed, the gel network composed of both amylose and amylopectin and separation into amylopectin-rich and amylose-rich domains took place. The gel formation of amylose weakened with increased hydrolysis time if heating was to 90 °C, mainly because the amount of amylopectin in the continuous phase increased.

Increasing the temperature from 90 to 98 °C induced dramatic changes in the structure of hydrolyzed starch pastes: most of the granules disintegrated. When the hydrolysis time was 0.5-1.0 hour, the starch paste assumed a phase-separated structure. The gels formed from the most extensively hydrolyzed barley starch sample had a dense network structure, with high storage modulus and low phase angle.

Key Words: Starch, barley, acid-modified, dispersions, gels, amylose, amylopectin, light microscopy, rheology, molecular weight.

Introduction

Starches are extensively utilized because of their ability to form viscous solutions, gels or films. A wide spectrum of starches with different properties can be prepared by varying the starch source and the type of modification. In most industrial applications, starches are heated in aqueous solutions. It is the changes that occur after heating that result in the development of interesting rheological properties.

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) have been used for studying the effects of acid or enzymatic hydrolysis on the ultrastructure of starch granules (Blanshard, 1987). The hydrolysis of starch begins in the amorphous parts of amylopectin, and in the first stage of hydrolysis, amylopectin is degraded (Blanshard, 1987; Pessa et al., 1992). Knowledge of the structural changes involved in the heating of hydrolyzed starch is important for developing hydrolysed starches with desired properties. Light microscopy combined with iodine staining provides information on the location of amylose and amylopectin in the starch pastes.

The gel formation of starches is an important property in the confectionery industry. The main events occurring during the heating process are swelling and solubilization of starch. After cooling of 10% native maize, wheat and barley starch dispersions, a gel is formed (Svegmark and Hermansson, 1991; Autio, 1990). Microstructural studies have shown that in the case of barley and wheat pastes, amylose forms a continuous phase in which swollen starch granules are dispersed (Autio, 1990; Langton and Hermansson, 1989). In the case of maize starch, both the viscosity of starch and gel formation of amylose decreased as the degree of hydrolysis increased (Pessa et al., 1992). The main reason for the poorer gelling properties was the fact that the ratio of amylose/amylopectin in the continuous phase decreased with increasing hydrolysis time.

Barley has traditionally been an important crop in Scandinavia. The main application has been in the malt and feed industry, but it could also have other uses in food industry.

The aim of the present work was to monitor the
gel formation of barley starch pastes subjected to different acid treatments. Emphasis was placed on the effect of heat treatment. Light microscopy was used to evaluate microstructural changes, and high-performance liquid chromatography (HPLC) was used for studying the molecular weight distribution of amylose and amyllopectin in the starch samples.

### Materials and Methods

#### Materials
Commercial barley starch (Raision Yhtymä, Finland) was used as a raw material for acid modification.

#### Acid hydrolysis
Acid treatment of granular starch was carried out essentially as described by Cowie and Greenwood (1957). Barley starch was hydrolyzed with 1 M HCl under argon as 2-10% suspensions in three-neck bottles with magnetic stirring at 40 °C for different periods of time (0.5, 1.0 and 4.0 hours).

After acid treatment the starch slurry was filtered rapidly with suction and washed with distilled water 7-8 times until no chloride reaction was observed with AgNO₃-solution. Filtered, washed starch material was suspended in acetone, filtered and washed once with acetone, and dried in ambient air.

#### HPLC
Four hundred milligram samples of dried starch were suspended in 10 ml water with magnetic stirring and then dissolved by adding 10 ml 2 M NaOH solution followed by stirring with a magnetic stirrer for 6 hours. The samples were then diluted ten-fold with 1 M NaOH. The whole dissolution and dilution procedure was carried out under argon and strictly excluding oxygen. Other conditions were as described earlier (Suortti and Pessa, 1991). The resolution was improved by addition of third column (μ-Hydrogel 500) in series with the earlier column bank consisting of μ-Hydrogel 2000 and 250 columns. For detection with post-column dyeing a diode array detector was employed and detection at the wavelength of 630 nm was used.

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

#### Rheological properties
The viscoelastic properties during cooling were measured with a small-amplitude oscillation test. Fifteen percent starch dispersions were heated to 90 °C or at 98 °C for 10 minutes with minimal stirring and then transferred to the Bohlin VOR rheometer (temperature 90 °C). The viscosity was measured in the shear rate range between 18.6 and 147 s⁻¹ with a concentric cylinder system (DIN 53019).

#### Heat-induced microstructural changes in starch dispersions
Heating temperatures of 90 and 98 °C were chosen in order to illustrate the differences in stability between native and acid-modified barley starches. At 90 °C, the blue-stained amylose was evenly distributed between the swollen granules of the native barley starch paste (Fig. 1a). A small amount of amylose remains inside barley starch granules after heating to 95 °C (Autio, 1990). Even short acid treatment induced considerable changes in the granule structure at 90 °C (Fig. 1b); amyllopectin fragments can be seen in the granule and in

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**Table 1. Effect of hydrolysis time on the properties of acid-modified barley starch.**

<table>
<thead>
<tr>
<th>Hydrolysis time (hours)</th>
<th>Viscosity (mPas) at 46.0 s⁻¹, 80 °C</th>
<th>Swelling power (%) at 85 °C</th>
<th>Solubility (%) at 85 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1170±20</td>
<td>11.5</td>
<td>4.9</td>
</tr>
<tr>
<td>0.5</td>
<td>60.2±0.7</td>
<td>11.1</td>
<td>17.1</td>
</tr>
<tr>
<td>1.0</td>
<td>21.0±0.5</td>
<td>11.4</td>
<td>23.4</td>
</tr>
<tr>
<td>4.0</td>
<td>7.2±0.5</td>
<td>11.1</td>
<td>45.3</td>
</tr>
</tbody>
</table>
Heat-induced changes in acid-modified barley starches

the continuous amylose phase. As the hydrolysis progressed (1 hour) the network around the granules was formed from both amylose and amylopectin (Fig. 1c). It was not possible to judge whether the blue amylose-rich domains were dispersed or whether amylose and amylopectin formed two continuous interpenetrating networks.

The results in Table 1 demonstrate that acid treatment increased the solubility of starch. Mixing of soluble amylose and amylopectin has been shown to result in phase separation (Kalichevsky and Ring, 1987); amylose- and amylopectin-rich domains could be seen in the continuous phase of hydrolyzed (1 hour) starch dispersion (Fig. 1c). After 4 hours of hydrolysis, the structure of the pasic was composed of violet-stained granule residues with growth rings and soluble amylopectin droplets in the continuous phase of solubilized starch (Fig. 1d). A hydrolysis time of 4 hours resulted in the degradation of amylopectin to smaller molecules with higher solubility, resulting in a mixture in an increase in the miscibility between amylose and amylopectin. At 90 °C the violet-stained rings were still visible in the granule (Fig. 1d). In an earlier study (Pessa et al., 1992) from our laboratory, it was shown that acid-modified maize starch contained similar granule residues. It was concluded that these violet-stained granule residues were composed of amylose or mixtures of amylose and amylopectin since acid-modified waxy maize starch starch brown.

The microstructure of the continuous phase of the hydrolyzed barley starches resembled that of the hydrolyzed maize starch (Pessa et al., 1992). On the other hand, the disintegration of the granules occurred in a different way. In the case of maize starch, the whole granules were degraded to small pieces; whereas, in the case of barley starch, the hydrolysis occurred more evenly throughout the granule. Great differences, however, were observed between different granules. Amylose inside the granule seemed to be more resistant to acid hydrolysis and the growth rings of the walls could still be seen even in the most extensively hydrolyzed sample (Fig. 1d).

Increasing the temperature from 90 to 98 °C induced considerable changes in the structure of starch pastes. In the native barley starch dispersion the granules fragmented further than at 90 °C, and the continuous amylose phase was full of amylopectin fragments (Fig. 2a). In all acid-modified starch dispersions, most of the granules had broken down at 98 °C. When the hydrolysis time was 0.5 or 1.0 hour, the gel had a phase-separated structure (Fig. 2b). In the most acid-modified (4 hour) starches, a dense network structure was formed (Fig. 2c).

Molecular weight

Gel permeation chromatography with refractive index detection (Fig. 3) showed that already after 0.5 hours hydrolysis, most of the high molecular weight (Mw) amylopectin of native starch (eluting at 28 minutes) had degraded to rather high molecular weight polymers. These hydrolysis products still eluted earlier (about 35 minutes, Fig. 3, curve b) than native amylose (about 40 minutes, Fig. 4, curve a). As hydrolysis progressed, amylopectin hydrolysis products further reduced in size. Post-column iodine detection at 630 nm indicated that the molecular weight of amylose also decreased during hydrolysis (Fig. 4). It has been demonstrated with well-defined amylose solutions that the Mw has a great effect on the gelling of amylose (Gidley, 1989).

Rheological changes

Increasing the heating temperature from 90 to 98 °C changed the native barley starch pastes from an elastic to more viscous type. It is probable that the amylopectin fragments interfered with the gel formation of amylose. The gel formation of amylose weakened with the increasing degree of hydrolysis if heating was to 90 °C, but not if heating was to 98 °C. At 90 °C, hydrolysis caused a decrease of the storage modulus (G') and an increase of 6 (Table 2), which means that the gels became less elastic and less rigid (Fig. 5). In the native barley starch paste the amylose alone forms an elastic network structure, and the granules are fillers in the gel. Amylose gel formation in native maize starch was hindered by the addition of a small amount of hydrolyzed waxy maize starch (Pessa et al., 1992). Microstructural studies suggested that soluble amylopectin and amylopectin fragments interfere with the gel formation of amylose. It is not, however, possible to judge whether the decreased Mw of amylose is responsible for the weakened gelling ability of hydrolyzed barley starches at 98 °C, since many parameters, like concentration and chemical structure of amylose in the continuous phase may also vary between the samples.

Starch gels composed of mixtures of amylose and amylopectin can be regarded as two-phase mixed gels. In these mixed gels, the ratio of amylose to amylopectin is important, and the higher the amylose/amylopectin ratio the better are the mechanical properties of the gels (Leloup et al., 1991). The increased amount of amylopectin in the continuous phase of the hydrolyzed barley starch pastes seems also to be the explanation for the poorer gelling properties of hydrolyzed barley starch gels preheated at 90 °C.

Increasing the temperature from 90 to 98 °C induced considerable changes in the viscoelastic properties of hydrolyzed barley starch gels. Heating to 98 °C instead of 90 °C increased the elasticity and rigidity of the gel made of the most extensively hydrolyzed barley starch (Fig. 6). The low phase angle of this gel indicated the existence of a strong network structure (Table 2). The micrographs suggest that heating to 98 °C liberated the residual amylose from the granule residue to the continuous phase. Instead of a phase-separated structure, a more elastic gel structure was formed. The gel made of the least hydrolyzed barley starch (0.5 hours) was less elastic (Table 2) and had a phase-separated structure (Figs. 2b).

Acid modification decreased substantially the hot paste viscosity of starch dispersions (Table 1). The flow
Figures 1a-d. Smears of 8% native and hydrolyzed barley starch dispersions heated to 90 °C: a) native barley starch; b) hydrolysis time 0.5 hours; c) hydrolysis time 1.0 hour; d) hydrolysis time 4.0 hours. Bar = 20 μm.

Figures 2a-c. Smears of 15% hydrolyzed barley starch dispersions heated at 98 °C for 10 minutes: a) native barley starch; b) hydrolysis time 0.5 hours; c) hydrolysis time 4 hours. Bar = 20 μm.
Heat-induced changes in acid-modified barley starches

Figure 3 (at top). Effect of hydrolysis time on the molecular weight distribution of starch. Detection by refraction index: a) native; b) hydrolysis time 0.5 hours; c) hydrolysis time 4.0 hours.

Figure 4 (at bottom). Effect of hydrolysis time on the molecular weight distribution of starch. Post-column iodine dyeing, detection at 630 nm, samples the same as in Fig. 3.
Figure 5. Storage modulus ($G'$) of 15% native starch and of hydrolyzed barley starch dispersions heated to 90 °C: a) native starch; b) hydrolysis time 1.0 hour; and c) hydrolysis time 4.0 hours. Cooling rate 1 °C/min.

Figure 6. Storage modulus ($G'$) of 15% native starch and of hydrolyzed barley starch dispersions heated at 98 °C for 10 minutes: a) native starch; b) hydrolysis time 0.5 hours; and c) hydrolysis time 4.0 hours. Cooling rate 1 °C/min.

Table 2. Effect of hydrolysis time on the phase angle of the gels at 25 °C.

<table>
<thead>
<tr>
<th>Hydrolysis time</th>
<th>$\delta$ (gels heated at 90 °C)</th>
<th>$\delta$ (gels heated at 98 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.4±0.1</td>
<td>5.7±0.2</td>
</tr>
<tr>
<td>0.5</td>
<td>5.6±0.2</td>
<td>5.6±0.3</td>
</tr>
<tr>
<td>4.0</td>
<td>5.3±0.3</td>
<td>1.5±0.1</td>
</tr>
</tbody>
</table>

behavior of native barley starch dispersion was pseudo-plastic, whereas that of acid-modified starches was more Newtonian. Doublier (1990) reported that the viscosity of highly concentrated (> 10%) native wheat and corn starch pastes heated below 85 °C, was governed primarily by the volume fraction of swollen particles. When heated to near 100 °C, the contribution of continuous phase cannot be neglected. It is assumed that the rheology of the continuous phase governs the viscosity of acid-modified starches.

Conclusions

1. Acid treatment increased the solubility of starch molecules, decreased the molecular weight of both amylose and amylopectin, and decreased the hot paste viscosity of starch dispersions.

2. A 15% native barley starch dispersion formed an elastic and rigid gel on cooling if preheating was to 90 °C. The microstructural studies showed that the amylose formed the continuous phase of the gel, in which starch granules were fillers. Heating to 98 °C induced fragmentation of amylopectin, and the gel formed on cooling had higher $\delta$ and lower $G'$.

3. When the hydrolysis time was 0.5-1.0 hour the starch pastes assumed a phase-separated structure at both heating temperatures. Increasing the temperature from 90 to 98 °C caused substantial changes in the structure of the most hydrolyzed barley starch paste; residual amylose was liberated from the granule and formed a very dense network with good rheological properties.

Acknowledgement

The authors thank Liisa Anäkainen for expert technical assistance.

References


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Discussion with Reviewers

A-M. Hermansson: It is stated that the molecular weight of both amylose and amylopectin decrease on acid treatment, but the discussion is focused on the increase in solubility of amylopectin. Could you comment on this?

Authors: The microstructural studies (Figs. 1a-e) indicate that the amount of amylopectin-like material increases with the increased hydrolysis time in the continuous phase of hydrolyzed barley starch dispersions heated to 90°C. Instead of amylose network, a network composed of both amylose and amylopectin is formed. It has been well documented that amylopectin fragments or solubilized amylopectin weakens the gelling of amylose. In order to relate observed molecular differences in amylose with the gelling behavior molecularly well-defined model systems are needed. Many parameters, e.g., concentration, branching frequency and branch lengths of amylose in the continuous phase may also vary between the samples and it is not possible to discuss on the role of amylose on the gelling behavior of such a composite system.

A-M. Hermansson: The differences in the rheological behavior between 90 and 98°C is very interesting. In wheat starch, there is a dramatic increase in the leakage of amylose from the granules as well as the swelling behavior in this temperature range when the granules change from the first to the second stage of swelling. Does the same occur for barley starch?

Authors: The same occurs for barley starch.

A.-C. Eliasson: How is it that the swelling power (Table 1) is more or less unaffected, whereas the solubility changes to such an extent with hydrolysis time?

Authors: Dilute acid hydrolysis at 40°C combined with heating at 85°C increased solubility of starch. Swelling power at 85°C was more or less unaffected since it was calculated as the amount of water bound by the sediment, and the amount of sediment decreased with increased hydrolysis. The microstructural studies showed that the pastes were composed of differently gelatinized starch granules. Some of the granules were less degraded and were still capable of retaining water.

A.-C. Eliasson: The viscosity seems to be very much related to the solubility, i.e., a high viscosity is obtained for the starch with low solubility, and vice versa. Could these results reflect the deformability of the starch?

Authors: The rheology of starch dispersions depends on many parameters, including the volume fraction occupied by the dispersed phase, the deformability and rheological characteristics of swollen particles, the rheology of the continuous phase, and the interactions between the dispersed phase and the continuous phase. The starch pastes were heated at 98°C for 10 minutes and the viscosity was measured at 80°C. Microstructural studies indicate that heating under these conditions induced considerable changes in the granule structure of acid-modified starches. It is assumed that the deformability of starch granules increases in hydrolyzed starches, but that the rheology of the continuous phase has more contribution to the viscosity of acid-modified starches.

A.-C. Eliasson: Are there not risks for degradation of starch molecules during the treatment with 2 M NaOH for 6 hours?

Authors: The degradation of starch because of treatment in 2 N NaOH has been shown to be minimal in experiments with waxy maize and amylo maize. Starch is degraded more seriously by the shear forces in column inlet and outlet frits.

A.-C. Eliasson: For Rheological measurements, and for smears, how was the heating to 90 or 98°C carried out? The sedimentation of starch granules should be avoided, but also too much damage due to shearing?

Authors: As stated in the text, the samples were prepared with minimal stirring. This is necessary for obtaining non-sedimenting dispersions.

A.-C. Eliasson: Under Viscoelastic properties you state "The frequency was 1 Hz and the strain 3 x 10^-3". Are these conditions within the linear viscoelastic region for the starch samples?

Authors: The strain applied was within the linear region.

A.-C. Eliasson: Regarding Figures 3 and 4, is it possible to tell from these figures which part is due to amylose and which is due to amylopectin? Would that be possible if the absorption of iodine-starch complex as a function of wavelength was shown?

Authors: The amylose and amylopectin part can very easily be detected in native starch by their characteristic spectra, but the fraction resulting from the chemical degradation of amylopectin have very similar spectra to those of amylose.

J.N. BeMiller: A concern of mine is that barley starch has a bi- or trimodal distribution of granules and nothing is said about the different behaviors of different granule types.

Authors: The commercial starch sample used as raw material in this study contains mainly large (A-type) granules.

J.N. BeMiller: The starch samples used were gelatinized (not dissolved) by adding NaOH (see HPLC, under Materials and Methods). HPLC was done on what I
hope was a molecular dispersion. It most likely was not a solution.

Authors: It is very difficult to understand that anything that is not dissolved might be chromatographed through the very efficient relative filter consisting of 90 cm column packed with 6 μm particles and six 2 μm column inlet and outlet frits.

A-C. Eliasson: Why was the temperature of 85 °C chosen for Swelling power and solubility, especially as the rheological measurements were carried out at 80, 90 or 98 °C?

J.N. BeMiller: For swelling and solubility, why 85 °C?

Authors: It is not possible to compare the swelling power and rheological properties at the same conditions. In rheological and microstructural studies the starch concentration is clearly higher. It is obvious that in addition to temperature, also starch concentration has a great effect on solubility and swelling.

J.N. BeMiller: Normal size-exclusion chromatography gives two peaks -- a large broad one for amylopectin and a smaller one for amylose. Where are they in Figure 3 for example?

J.N. BeMiller: Linear chains released from amylopectin could stain blue like amylose, and short chains produced by depolymerization could stain reddish like amylopectin!

Authors: In the chromatogram of native starch, three peaks were obtained. The first peak, with a retention time of about 28 minutes, is amylopectin; the second peak, with a retention time of 35 minutes, is the so-called pseudoamylopectin (Fig. 3, curve a). Amylose will eluate at the retention time of 41 minutes. The premier breakdown product of amylopectin, which can be seen as the major peak in Figure 3 curve b, stains like amylopectin. The secondary breakdown product, which can be seen at the retention time of 46 minutes (Fig. 3, curve c), stains like amylose. Thus, in the chemical degradation, the amylopectin fragments are linearized. In the enzymatic degradation with α-amylase, the spectra of stained fragments do not change.

J.N. BeMiller: Each of the photographs in Figures 1 and 2 are of samples that were heated at different temperatures for different times. How can comparisons be made? And they were cooled before viewing?

Authors: Heating was continued at 98 °C for 10 minutes in order to detect the extent of granule degradation that can be achieved at normal vapor pressure. Hot starch dispersion was smeared out onto an object glass and stained directly.

J.N. BeMiller: Do the authors know that there was no residual acid in the starch? Starch binds acid very strongly. Granule-bound acid can be determined by titration after pasting. What is the pH of the cooks?

Authors: The starch samples were carefully washed with distilled water (7-8 times), until no chloride reaction was observed with AgNO₃. The pH of the samples was measured after heat treatment, it was about 4.8. The pH variations between the samples were so small that they were not considered important.