HEAT-INDUCED STRUCTURAL CHANGES OF SMALL AND LARGE BARLEY STARCH GRANULES

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The small granules of barley starch were shown to differ from the large granules both in chemical composition and heat-induced functional properties. The small granules had a higher lipid:amylose ratio and also higher dissociation enthalpy of the amylose-lipid complex than the large granules. This may explain the better stability of amyllopectin in the gel of small starch granules as analysed by DSC. The small granules had a 4°C higher gelatinisation temperature than the large granules. During heating up to 90°C, the fraction obtained by aqueous leaching of small granules was mainly amylopectin, whereas the carbohydrate solubilised from large granules was mainly amylose.

Key Words: Barley, starch, small granules, large granules, gelatinisation, DSC, microstructure.

INTRODUCTION
Starch granules from mature barley kernels can be separated into two clearly defined populations: large A-granules, ca. 10–15 μm in mean diameter, and small B-granules, 2–5 μm in mean diameter.25 Generally, there appear to be only limited differences in the chemical composition and size of small and large granules. In many studies, higher amylose contents were observed in large granules15,22,30,35 whereas in some others the amylose content was not dependent on the granule size.1,12,26,29 In the recent results by Vasanthan and Bhatty,39 small differences were detected, but the differences in physico-chemical properties of barley starches were greater among the genotypes than between small and large granules from the same genotype.

The properties of small and large granules, particularly regarding gelatinisation and amylolysis, are of importance in the industrial applications of barley, especially in malting and brewing.23,31 Under normal mashing conditions an optimal starch extract is not achieved unless the gelatinisation temperature of the starch has been reached or exceeded.11 The gelatinisation temperature of small barley starch granules has been shown to be higher than that of large granules, as estimated from the loss of birefringence and by differential scanning calorimetry.12,21,39 The difference in gelatinisation temperature between small and large granules of ordinary, waxy, and high amylose barley starch varieties was 1.4–3.7°C as analysed by DSC.15,30,39 The gelatinisation enthalpy of the small granules was smaller than that of the large granules.24 Bathgate and Palmer2 studied the attack of malt alpha-amylase on barley and malt starch granules at 65°C. The small granules from barley were most resistant to malt alpha-amylase, also after complete gelatinisation. The small starch granules from malt were less susceptible than the large, but could be more easily hydrolysed than the small granules of unmalted barley. According to MacGregor and his coworkers,20,21,23 however, small ungelatinised barley and waxy barley starch granules were hydrolysed faster than the large granules by malt alpha-amylases at 18–35°C. A similar result was observed by Bertoft and Kulp.4 The different results obtained at 65°C and 20°C were explained by the faster gelatinisation and thus the better accessibility of large granules at elevated temperatures.21 Small granules, having a higher gelatinisation temperature, would require a longer time to gelatinise at 65°C, and would therefore be more slowly degraded.

Large granules from normal barley contain many pinholes after hydrolysis by malt alpha-amylase and appear to be hydrolysed from the inside out.21 No characteristic pinholes were observed in degraded small starch granules of either normal or waxy barley, both of which were hydrolysed by surface erosion. A similar observation was made by Bertoft and Henrikssön.3

The β-amylolysis limit value of small granules has been shown to be slightly higher (43–65%) than that of large granules (39–54%).12 Amylopectins of small and large starch granules of normal and waxy barley were, from enzymatic analyses, reported to have very similar structures,22 but large granules were shown to contain a larger amount of long amylopectin B chains.15,28

We have previously studied the heat induced changes and enzymatic accessibility of commercial barley starch (A-starch).11 In this study the differences in the heat-induced changes of small and large barley starch granules are further described.

MATERIALS AND METHODS

Materials
The 2-row malting barley variety Kustaa was kindly supplied by Risto Lampinen, Kesko, Finland.

Starch separation
Starch was isolated from the grains using the method of McDonald and Stark24 with minor modifications. Barley grains (6–20 g) were first husked with a special blocking machine, then milled with KT-mill (Koneteollisuus Ltd Finland) and suspended in 0.02 M sodium chloride (7 vols) once with toluene (1 vol), washed twice with water and twice with acetone, and finally air-dried (Fig. 1). Small and large granules were separated by the method of Decker and Hölker.7 Between 5-25 g starch as a 5% suspension in water was sedimented repeatedly (for 90 min at 5°C) in a vessel where the liquid height was 15 cm, and the suspension containing the small granules was carefully decanted. The sedimentation was repeated 15 times. The small granule fraction was purified by sedimenting it twice overnight (16 h) at 5°C. Both fractions were washed twice with acetone and air-dried.

Analytical methods
Starch content was determined enzymatically with small modifications by the method of Karkalas.13 Apparent
total amylose content was determined colorimetrically. The phospholipid content was determined by measuring the phosphorus content by the method of Morrison and using the conversion factor of 16.5. Protein content was determined as Dumas-protein using Carlo Erba Nitrogen analyzer 1500 (Milan, Italy).

The granule size distribution was measured by Coulter Multisizer II (Coulter Electronics Ltd, Luton, England). The counter was fitted with a 100 μm aperture tube and was calibrated with standard latex particles. Starch samples were first suspended into 2% NaCl-solution and pretreated with ultrasonication (30 s).

Swelling power and solubility at different temperatures were determined using the modified procedure of Leach et al. The starch sample (100 mg±0.1 mg) was 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 at different temperatures between 78 and 100°C for 30 min stirring manually occasionally, 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 using the method of Dubois et al. The soluble fractions were freeze-dried and amylose content were measured by the method of Morrison et al.

The microstructure of starch dispersions was studied by light microscopy using the smear technique and iodine staining as described previously. 8% starch suspensions were heated to the appropriate temperature and incubated 5 minutes prior to preparation of the samples. The DSC measurements were performed with a Mettler DSC-30S instrument equipped with a Mettler TC 11 analysis data station. Gelatinisation and retrogradation were studied in starch dispersions with a 50% (w/w) water content. The samples were prepared by weighing 3–5 mg of starch in DSC standard aluminum sample pans, 15 ml of distilled water was added, and the contents were mixed well with a needle. Excess water was removed by evaporation and the pans were sealed hermetically. The samples were heated from 10 to 100°C (heating rate 10°C/min). A pan of Al₂O₃ was used as reference. The temperatures reported correspond to the
maxima of the endotherm peaks. Transition enthalpies (H, J/g) were calculated from the area under the curve. The extent of recrystallisation was studied by reheating the pans after 1, 2, 4, 7, 11, and 15 d of storage at +4°C.

The dissociation of the amylose-lipid complex was studied at 70% moisture using medium pressure pans with water as the reference and by heating the samples from 10 to 130°C at 10°C/min.

RESULTS AND DISCUSSION

The purity of the isolated starch was 94%. Coulter counter analysis of the isolated starch showed that it contained 17% by volume small granules (Fig. 2a), which was more than the average 5–8% found in another common Finnish barley variety (Kymppi). The small and large starch granules of barley cv. Kustaa were separated by repeated decanting. The average size of the small granules was 3–4 μm and the size of the large granules was about 18 μm, as calculated from the volumetric data (Fig. 2b).

The lipid content of the small granules was higher than that of the large granules (Table I). A similar difference has been reported for small and large wheat starch granules.5 The apparent (free) amylose content of the small granules was lower, but the difference in the total amylose content of both granules was negligible. The lower (apparent) amylose content of the B-granules has also been reported earlier.12,15,16,21,13,39 It seems that the small granules differ from the large ones mainly in their lipid: amylose ratio, the small granules having a higher proportion of lipid-complexed amylose. Although the presence of amylose-lipid complexes in native starch granules was long debated, evidence by Morrison et al.29 suggests that lipid-complexed amylose exists already before gelatinisation.

The higher lipid content of the small granules was also reflected as a higher dissociation enthalpy of the amylose-lipid complex of the small granules (3.5 J/g) as compared to that (2.5 J/g) of the large (Table II). Eliasson and Karlsson16 also found the same difference between small and large wheat starch granules. The peak temperature for the amylose-lipid endotherm was 105°C, which is close to the results reported earlier for barley starch (94–109°C, depending upon the water content)6,13,14. The DSC analysis also showed that the gelatinisation peak temperature (62.0°C) of small barley starch was 4°C higher than that of large granules (57.8°C). A similar difference has also been reported earlier.15,30 The gelatinisation enthalpy was slightly lower for the small (10.2 J/g) than for the large (11.5 J/g) granules. The shape of the endotherm at 50% moisture was different (Figs. 3a and b). With the small granules the second peak area was larger indicating that a larger portion of the starch gelatinised at higher temperatures. The appearance of two peaks has been interpreted by the presence of two different phase changes in the starch granules: the hydration of the amorphous regions and the melting of the crystallites. In concentrated starch dispersions, parts of the crystallites melt at higher temperatures after redistribution of water from the amorphous phase.3

The difference in the gelatinisation behaviour of the two granule populations could also be observed in hydrothermal treatments at 50 and 55°C. After heating of a 7% starch solution for 3 hours, the large granules showed a higher degree of gelatinisation as measured by the reduction of gelatinisation enthalpy in DSC (Table III). At 55°C, the large granules were completely gelatinised, but the small granules still showed a gelatinisation enthalpy of 1.8 J/g. As the enzymatic accessibility of starch granules depends on their degree of gelatinisation,18 it is obvious that the difference in the rate of gelatinisation shown

### Table I. Chemical composition of the small and large starch granules in two rowed Kustaa barley cultivar

<table>
<thead>
<tr>
<th>Starch granules</th>
<th>Amylose content, %</th>
<th>Lipid content, %</th>
<th>Protein content, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>14.9</td>
<td>26.6</td>
<td>1.30</td>
</tr>
<tr>
<td>Large</td>
<td>21.4</td>
<td>28.5</td>
<td>0.90</td>
</tr>
</tbody>
</table>

### Table II. The transition temperature and enthalpy of the amylose-lipid-complex of small and large barley starch granules

<table>
<thead>
<tr>
<th>Starch granules</th>
<th>Tp (°C)</th>
<th>H (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>105</td>
<td>3.5</td>
</tr>
<tr>
<td>Large</td>
<td>106</td>
<td>2.5</td>
</tr>
</tbody>
</table>

### Table III. Effect of hydrothermal treatments (3 h) at 50 and 55°C on the gelatinisation properties of small and large starch granules

<table>
<thead>
<tr>
<th>Preheating (°C)</th>
<th>Starch granules</th>
<th>Tp (°C)</th>
<th>Tp (°C)</th>
<th>Tp (°C)</th>
<th>H (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>Small</td>
<td>64.4</td>
<td>68.1</td>
<td>73.3</td>
<td>6.1</td>
</tr>
<tr>
<td>50</td>
<td>Large</td>
<td>60.8</td>
<td>67.8</td>
<td>73.4</td>
<td>2.4</td>
</tr>
<tr>
<td>55</td>
<td>Small</td>
<td>63.7</td>
<td>71.5</td>
<td>76.8</td>
<td>1.8</td>
</tr>
<tr>
<td>55</td>
<td>Large</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

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Fig. 3. The gelatinisation endotherms of large and small starch granules at 50% moisture.

Fig. 4. Retrogradation enthalpy of small and large starch granules during storage at 4°C.
in Table II is one reason for the reported differences in enzymatic accessibility of small and large granules.\textsuperscript{2,21,23}

The recrystallisation rate of the small granules was slower than that of the large granules during the first 4 days of storage (Fig. 4). The final retrogradation enthalpy was 6–7 J/g for both small and large granules. The slow rate of recrystallisation was probably due to the presence of a higher amount of lipid in the small granules. The role of lipids in retarding starch recrystallisation in oat starch has been reported previously.\textsuperscript{13,14}

The changes in swelling power and apparent solubility due to heating are presented in Figures 5a and b. Up to 90°C, the swelling power of small granules was slightly lower. This is probably due to the higher lipid content of the small granules, as the lipids or lipid complexed amylose are thought to inhibit swelling of barley starch granules.\textsuperscript{26,29,37,38} Alternatively, the organisation or molecular weight of amylose and amyllopectin may differ between the large and small granules. Above 95°C, it was impossible to separate the insoluble and soluble phases of small granules. Most of the carbohydrate solubilized from the large granules was amylose (Fig. 6). In contrast, solubilisation of the small granules began with release of higher amounts of amyllopectin than from the large granules (Fig. 6).

The heat-induced changes in the microstructure of the starch pastes were studied using iodine staining and light microscopy. The amylose-rich phase stains blue and the amyllopectin-rich phase stains brown. At 90°C, blue-stained amylose formed a network structure between the large granules (Fig. 7a). Only a little solubilized amylose could be seen between the small granules (Fig. 7b and c).

Raising the heating temperature from 90 to 95°C increased the relative amount of amylose leaching out from the small granules (Fig. 6) and induced considerable changes in the structure of the small granules (Fig. 8a). The pasting behaviour of the small granules resembles that of oat starch granules: in oat starch the apparent solubilisation of amylose and amyllopectin has been reported to occur simultaneously.\textsuperscript{1,8} It is not possible to judge whether amyllopectin formed a continuous phase or if amylose and amyllopectin formed two separate, interpenetrating networks. Under the same heating conditions, the large granules degraded less (Fig. 8b). Amylose seemed to

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{Swelling power (a) and solubility (b) of small and large starch granules at different temperatures.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{The amylose content of the solubilized carbohydrates of small and large starch granules at different temperatures}
\end{figure}
Fig. 7. Micrographs of 8% dispersions of barley starch granules heated to 90°C. (a) Large granules, bar is 50 μm. (b) Small granules, bar is 20 μm. (c) Small granules, bar is 20 μm.
form a continuous phase in which granule residues and amylopectin fragments were dispersed.

**CONCLUSION**

The average size of starch granules in the Finnish barley variety Kustaa was about 4 μm for small granules and 18 μm for large granules. The lipid content of small granules (1.30%) was higher than that of large ones (0.90%). The small granules contained more lipid-complexed amylose than the large granules, which may explain the differences between the two granule classes with respect to swelling, solubility, and gelatinisation properties. At temperatures lower than 90°C, amylose preferentially leached out from the large granules whereas more amylopectin was released from the small granules under these conditions. The higher amount of lipid-complexed amylose is said to inhibit swelling and gelatinisation. At 95°C, small granules solubilized completely, but some large granules still remained as particles, indicating a more stable structure.

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