

Gelatinisation Characteristics and Enzyme Susceptibility of Different Types of Barley Starch in the Temperature Range 48–72°C¹

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ABSTRACT

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The gelatinisation temperatures, pasting characteristics and enzymic susceptibilities in the temperature range 48–72°C of normal, high amylose, low amylose and zero amylose barley starches were determined. Normal starches had the lowest gelatinisation temperatures, but low and zero amylose starches had the lowest pasting temperatures. Normal starches were the most readily soluble in water at 48–60°C in the presence of a mixture of α -amylase, β -amylase and limit dextrinase and were most readily broken down to reducing sugars by these enzymes. High amylose starch was the most resistant to enzymic hydrolysis in the temperature range 48–72°C and, hence, produced the lowest level of reducing sugars.

Key words: Barley, enzymic hydrolysis, gelatinisation, high amylose, pasting, starch, waxy.

INTRODUCTION

The rapid and efficient conversion of starch to fermentable carbohydrates during brewing is dependent on a number of parameters including the level of starch degrading enzymes in the malt, the gelatinisation temperature of the starch and the actual temperature program of the mash. Starch hydrolysis is carried out by a number of malt enzymes working together. These include α -amylase, β -amylase, limit dextrinase and α -glucosidase^{20,21}. Although α -amylase is able to hydrolyse intact starch granules, the rate of hydrolysis is very slow compared to that of solubilised starch^{27,29}. Effective hydrolysis by α -amylase, therefore, occurs only after the starch has been solubilised (or gelatinised). When this has occurred, the enzyme rapidly degrades the starch components through random hydrolysis with concomitant reduction in the viscosity of the starch solution and formation of degradation products²² that are substrates for β -amylase action. It is this action of

β -amylase that is largely responsible for the conversion of starch to the major fermentable carbohydrates, glucose, maltose and maltotriose²⁵. β -Amylase, however, is relatively heat labile and rapidly loses activity²⁸ when mashing temperatures rise over 62.5°C. It is essential, therefore, that malt starch is gelatinised at or below this temperature to permit β -amylase to complete its action before losing a significant amount of activity through heat inactivation.

The preferred substrates for limit dextrinase are the branched dextrans formed from starch by the combined action of α - and β -amylases²¹. Limit dextrinase appears to have only limited action during mashing, however¹⁰. This is caused, probably, through inhibition by low molecular weight inhibitors in the malt¹⁷ rather than by excessive lability at mashing temperatures²⁸. α -Glucosidase may play a role during mashing by hydrolysing small malto-dextrans to glucose but the importance of α -glucosidase action remains unclear³¹.

Several methods have been used to measure the gelatinisation (solubilisation) temperatures of starch granules but each tends to measure a different aspect of this complex phenomenon^{2,34} and so not all methods give the same result. One of the most common methods, differential scanning calorimetry (DSC) measures the temperature at which starch crystallites melt^{7,38}. Results using this technique indicate that waxy and high amylose barley starches have higher gelatinisation temperatures than does normal barley starch³³. Similar results have been reported when hot stage microscopy was used to determine the gelatinisation temperatures of barley starches containing different levels of amylose^{3,4}. The Brabender Amylograph^{15,23,36} and the increasingly popular Rapid ViscoAnalyzer (RVA)¹¹, measure the increase in viscosity that occurs as starch granules swell in water when the temperature is raised through the gelatinisation temperature of the granules. These latter techniques have been used to evaluate barley and malt quality parameters, including the pasting characteristics of barley and malt starches. Results have been used to provide information on the gelatinisation (solubilisation) temperature of barley starches²³.

Results generated by these techniques show that waxy starches appear to swell and generate viscous solutions at lower temperatures than do normal starches whereas high amylose starch exhibits limited swelling but does so at a higher temperature than normal starch^{12,30}. These results suggest that waxy starch, and especially, zero amylose

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starch, may become susceptible to enzyme hydrolysis at lower temperatures than does normal starch. If this were so then waxy barley starch would have an advantage over normal starch for brewing purposes. With this in mind, a project was developed to determine the solubility and enzyme susceptibility of normal, waxy and zero amylose barley starches in the temperature range of 48-72°C, which is typical of the starch conversion temperatures used in mashing. High amylose barley starch was included in the study for comparative purposes.

MATERIALS AND METHODS

Starch preparation

Commercial barley starch was obtained from Primalco Grain Processing, Koskenkorva, Finland. The other starches used in this study were isolated from barley cultivars and experimental lines grown in Saskatchewan, Canada, in 1995. These were all 2-rowed, hull-less barleys. CDC Dawn is a feed barley with normal starch; SB92-55-08-31 is a high amylose line; CDC Alamo (formerly SB94794) is a zero-amylose line and SB94917 is a waxy line. Starches were isolated and purified from barley as described previously¹³.

DSC analysis

This was carried out as described previously¹³ using a 9900 DuPont thermal analyzer equipped with a 910 DSC high pressure cell base. Dispersions having a solids content of 17% (w/v) were used and the heating rate was 10°C/min. The results reported are means of at least duplicate analyses.

Pasting characteristics of starch

A viscometric method using the Rapid Visco-Analyser-3B (RVA) from Newport Scientific Pty Ltd., Australia as described in AACC method 76-21 (AACC 2000)¹ was used. The 30 min program was used with 3 g of starch and 25 mL of water in conjunction with the control software supplied with the instrument.

Materials

α -Amylase 2 and limit dextrinase enzymes were purified from green (unkilned) malt as described previously^{18,19}. Barley β -amylase was obtained from Megazyme International Ireland Ltd. All chemicals were ACS reagent grade or better. De-ionized, nanopure water (0.2- μ m final filter, 18.2 M Ohm) was used in all solutions and dilutions.

α -Amylase and β -amylase activities were determined using the Ceralpha and Betamyl assays as described by the manufacturer (Megazyme International Ireland Ltd.) except that 0.2M acetate buffer (pH 5.5) containing 1mM CaCl₂ was used for the α -amylase assay. Limit dextrinase activity was determined using the Limit Dextrzyme assay (Megazyme International Ireland Ltd.) using 0.1M acetate buffer (pH 5.5) containing 25mM dithiothreitol (DTT) and 0.5 mg/mL bovine serum albumin (BSA) as the assay buffer. Activity units for the enzymes were as described by the manufacturer of the assays.

Micro-scale mashing

Small scale mashing experiments were carried out as described previously¹⁶. Each mash consisted of 0.60 g starch, 0.05 g BSA, 20 U α -amylase, 7 U β -amylase, 250 mU limit dextrinase and 1mM CaCl₂ adjusted to pH 5.5 with dilute sulphuric acid to give a total volume of 4 mL. Each mash was held at 48°C for 30 min. For each temperature range the temperature was increased at 1°C/min and then held at the final temperature for 30 min.

At the completion of mashing, the mash liquors were cooled to ca. 25°C and poured into a centrifuge tube (50 mL). The mash vessel was rinsed with nanopure water (5 mL) and this was added to the centrifuge tube. The pH of the total mash liquor was lowered immediately to pH 2.5 with dilute sulphuric acid and the liquor was centrifuged (10 min at 10,000g). The pellet was rinsed with nanopure water (10 mL), re-centrifuged (10 min at 10,000g) and the two supernatants were combined. Dilute sodium hydroxide was added to bring the pH to 5.5 and the total volume was brought to 25 mL with nanopure water. Mashings were carried out at least in duplicate.

Mash analysis

Total starch solubilised during each mash was determined by digesting the mash liquor with thermostable α -amylase and amyloglucosidase and measuring the resulting glucose with a Gluco-quant assay kit (Boehringer, Mannheim) as described previously²⁶. The weight of solubilised starch was calculated as the weight of free glucose \times 0.90.

Reducing sugars were measured with a neocuproine reagent using maltose to standardize the assay⁸.

RESULTS AND DISCUSSION

The gelatinisation properties of the starches, as measured by DSC, are given in Table I. A commercial sample of barley starch was included in the study as a reference because it has been used for other studies in the research program¹⁶. All other starches were purified from hull-less barley lines. There is little difference in the gelatinisation temperatures (T_m amylopectin) of barley and malt starches¹³. Therefore, the values of T_m amylopectin shown in Table I should mirror those that might be expected from starches isolated from malted samples of the corresponding barley lines but gelatinisation of malt starch may start at lower temperatures than that of barley starch⁵. The DSC runs were carried out at a solids content of 17% (w/w) to mimic the starch content in typical high gravity mashes. Starches from so-called normal barley

TABLE I. DSC thermal characteristics of barley starches.

Sample	T _m amylopectin ¹ (°C)	Δ H amylopectin ² (J/g)
Commercial barley starch	59.5 \pm 0.5	10.0 \pm 0.5
CDC Dawn	59.0 \pm 0.7	10.2 \pm 0.7
SB 94917	62.8 \pm 0.8	13.2 \pm 0.9
CDC Alamo	62.0 \pm 0.5	16.1 \pm 0.6
SB 92-55-08-31	63.5 \pm 0.9	9.8 \pm 0.9

¹ T_m amylopectin: melting (gelatinisation) peak temperature of amylopectin.

² Δ H amylopectin: enthalpy of gelatinisation of amylopectin.

lines (CDC Dawn and the commercial starch sample) had similar gelatinisation temperatures, that were lower than those of the other lines. This is in agreement with other results showing that normal barley starch has a lower gelatinisation temperature than either waxy or high amylose barley starches^{3,4,33}. Results for the starches from waxy and zero amylose lines were similar, as might be expected from other studies^{35,39}. The high amylose starch had the highest gelatinisation temperature but it was only slightly higher than that of the waxy starch. Waxy barley starches had higher gelatinisation enthalpies (ΔH amylopectin) than the other starches but this may just be a reflection of the increased amylopectin content of the

starch granules, and hence of the crystallinity of the waxy starch granules^{32,38}. It would be predicted from these results that normal barley starch (i.e. starch with an amylose content of ca. 25%) would be the most susceptible to enzymic hydrolysis at mashing temperatures of 60-70°C.

The pasting properties of the starches are shown in Fig. 1. For the purpose of this study, the most important parameter is the time to peak viscosity, shown as "a" in Fig 1. At this stage most of the granules are fully swollen and material starts to leach from the granules. Shortly afterwards the granular structure breaks down and the viscosity falls³⁴. At peak viscosity, or shortly thereafter, therefore, the granules might be expected to become

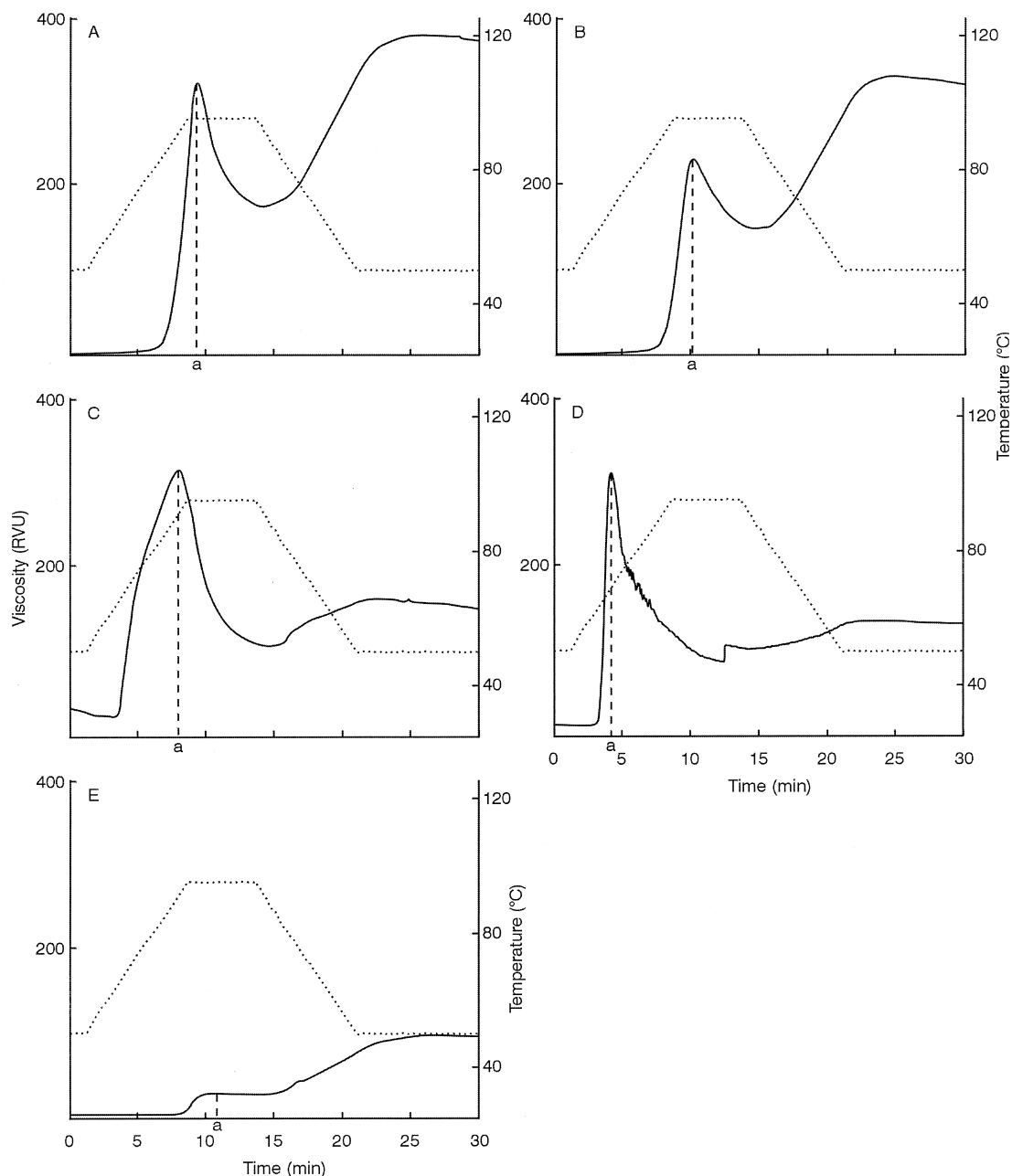


FIG. 1. Pasting profiles of barley starches measured by the Rapid Visco Analyzer. (....., temperature profile; — pasting curve; a, time required to reach maximum viscosity). Graph A - Commercial starch; Graph B - CDC Dawn starch; Graph C - SB 94917 starch; Graph D - CDC Alamo starch; Graph E - SB 92-55-08-31 starch.

susceptible to enzymic hydrolysis. Time to peak viscosity was similar for the two normal starches (Fig. 1A and B) but it was lower for the waxy starch and much lower for the zero amylose starch (Fig. 1C and D). This difference in the pasting characteristics between waxy and zero amylose barley starches is similar to that reported previously⁶. Very little swelling was detected with the high amylose starch (Fig. 1E) in agreement with previous findings^{12,37}. It might be expected from these results that the waxy and zero amylose starches would become susceptible to enzymic degradation at a lower temperature than normal starch.

The relative solubilities of the starches at different temperatures and in the presence of starch degrading enzymes are shown in Fig. 2. These results represent the formation of soluble products from the combined effects of temperature solubilisation and enzymic hydrolysis of the starches. They do not simply reflect differences in starch solubility but, rather, differences in susceptibility of the starches to enzymic hydrolysis. This is what is important from a mashing perspective. High percentages (ca. 80%) of the normal starches were solubilised when the temperature of their suspensions was raised to 60°C. Little further solubilisation occurred in suspensions raised to 63 or 65°C. A small reduction in solubility appeared to occur in the suspensions taken up to 72°C. In contrast, the waxy and high amylose starches were much less soluble (ca. 40%) during the 48-60°C treatment but became more soluble at higher temperatures. The high amylose starch was markedly less soluble than the other starches during all temperature treatments. Clearly, higher temperatures are required to render the waxy and zero amylose starches susceptible to enzymic breakdown than are required for the normal starches as would be predicted from the DSC results. Inferring, from the RVA results, that the zero amylose starch might be the most readily solubilised would be misleading.

Differences in the levels of starch conversion into reducing sugars (Fig. 3) can be explained on the basis of the solubility results. At the lowest mashing temperature used (48-60°C), there was a large difference in starch conversion between the normal starches and the other starches, as would be expected from the starch solubilisa-

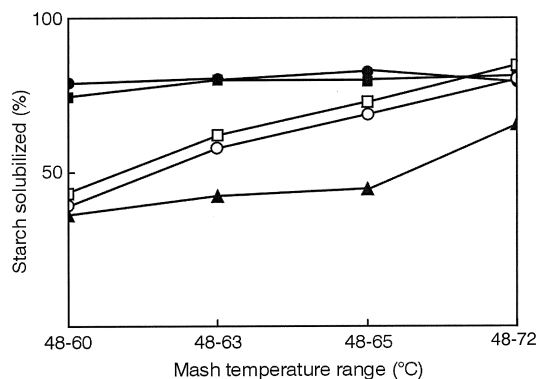


FIG. 2. Starch solubilised at different temperatures in the presence of α -amylase, β -amylase and limit dextrinase. (● - Commercial; ■ - CDC Dawn; □ - CDC Alamo; ○ - SB 94917; ▲ - SB 92-55-08-31).

tion results. Conversion of the waxy starches increased with mashing temperature but that of normal starches remained constant and even declined at the highest mashing temperature. Low conversion of the high amylose starch was observed at all mashing temperatures, indicating that this type of starch is quite unsuited for brewing and distilling purposes in agreement with previous findings on high amylose starch⁹.

There is a high correlation between the levels of reducing sugars and those of fermentable carbohydrates in a mash¹⁶. Therefore, the results shown in Fig. 3 would be expected to reflect the conversion of starch to fermentable sugars in these simulated mashing experiments.

Enzyme levels used in this study were chosen to be less than optimum levels required for the complete conversion of normal starch to fermentable sugars¹⁶. This was done to differentiate more clearly amongst the starches in terms of their enzyme susceptibility at temperatures normally used for mashing.

It is clear from Fig. 3 that normal barley starches are more susceptible than waxy starches to enzymic breakdown during mashing. These findings would be predicted clearly from the DSC results but would not be so obvious from the RVA results.

Swelling of starch granules, as measured by the RVA technique, does not necessarily indicate the extent of solubilisation, or of the enzymic susceptibility of starch granules.

Clearly, normal barley starch would be preferred over waxy, and especially, over high amylose starch, for malting, brewing and distilling purposes.

Barleys containing waxy or high amylose starches have two further disadvantages for potential use in fermentation industries. They tend to have a lower starch content and increased levels of β -glucan^{14,24}. Both of these characteristics are undesirable in barleys intended for the malting, brewing and distilling industries.

CONCLUSIONS

Waxy barleys have lower susceptibilities to enzymic degradation at mashing temperatures than do normal barleys despite having lower pasting temperatures. Starch

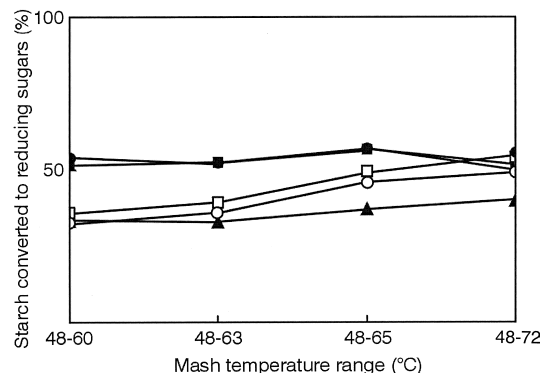


FIG. 3. Extent of starch conversion to reducing sugars (maltose equivalents) at different temperatures in the presence of α -amylase, β -amylase and limit dextrinase. (● - Commercial; ■ - CDC Dawn; □ - CDC Alamo; ○ - SB 94917; ▲ - SB 92-55-08-31).

gelatinisation temperatures, as determined by DSC analysis, are a better predictor than starch pasting characteristics of starch susceptibility to breakdown by enzymes during early stages of mashing. Barley starches having either lower or higher amylose contents than normal starch (ca. 25% amylose) are not desirable for fermentation purposes.

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