Problems Caused by Barley Beta-Glucans in the Brewing Industry

NEW DIAGNOSTIC KITS AID THE IDENTIFICATION AND RESOLUTION OF THESE PROBLEMS

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Brewing, the oldest application of biotechnology, is now a mix of trade art and modern science. This article describes new applications of enzyme chemistry to trouble-shooting in beer production.

What are Barley β-glucans?

Barley β-glucans are unbranched polymers of β-linked *D*-glucosyl residues. They are also referred to as mixed-linkage β-glucans, $(1\rightarrow 3)(1\rightarrow 4)$-$\beta$-*D*-glucans or barley gums. These β-glucans form a major part of the cell-walls in barley endosperm tissue, representing about 75% of total cell-wall carbohydrate.

Barley β-glucan extracted by water at 40°C is generally considered to be composed predominantly of cellotriosyl and cellotetraosyl residues separated by single $(1\rightarrow 3)$-β-linkages (refer to Scheme 1).

Small, but significant, proportions of longer blocks of up to 10 contiguous $(1\rightarrow 4)$-β-linkages are also present. Contiguous $(1\rightarrow 3)$-β-linkages would appear to be absent in the water-soluble glucan extracted from barley but have been reported to occur in β-glucan extracted from malt.

Problems caused by barley β-glucans

During the malting of barley grain, β-glucan and other major storage components are partially degraded by enzymes synthesised *de novo*. The major enzymes responsible for depolymerisation of the barley β-glucan, namely malt β-glucanases, rapidly increase in amount during the malting process and generally give effective removal of polymeric barley β-glucan.

If β-glucan is fully degraded to glucose during malting and mashing it contributes to fermentable sugars ("extract"). However, if this glucan is not fully degraded there is a loss of extract and high-molecular-weight, viscous material can be released into solution.

Loss of extract may be attributed both to incomplete degradation of β-glucan and also to the fact that sufficient cell-wall material may remain to provide a physical barrier to digestion of cell contents, particularly starch.

This problem is accentuated with modern, rapid, malting processes where the grain can be very unevenly modified by endogenous enzymes. Also, where unmodified barley adjunct is used as a less expensive source of carbohydrate it is essential to supply sufficient and suitable enzymes, from malt or otherwise, to ensure β-glucan breakdown.

Barley β-glucan is composed of both "soluble" and "insoluble" components and it is considered that it is the insoluble component which causes most problems in the brewing industry. The reasons for solubility or insolubility have not been clearly defined.

Some researchers consider that insolubility may be due to binding of the β-glucan through peptide linkages to protein, whereas others consider it is due to structural variation in the glucan itself, ie barley β-glucan subfractions with a high proportion of regions of sequential $(1\rightarrow 4)$-β-linked *D*-glucosyl residues could be expected to have a more cellulose-like nature and thus be less soluble.

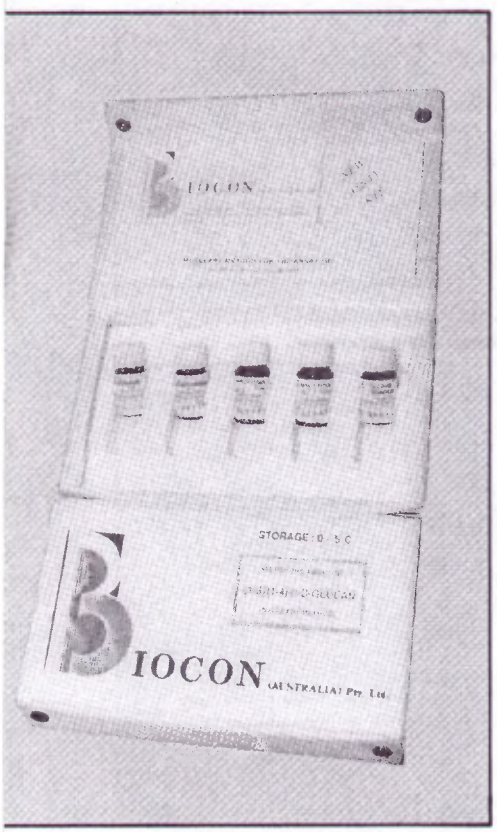
In contrast, our results indicate that there is no major difference between the soluble and insoluble fractions, although, with some barley flours we have found that the more readily extractible β-glucan has a lower molecular size.

In hydrated endosperm cell-walls the concentration of β-glucan is very high (approximately 20% w/v) and a high degree of molecular entanglement and self-association is likely to occur. β-glucan subfraction of higher molecular weight are likely to be more highly entangled and thus more difficult to extract, particularly under the relatively mild conditions of agitation employed in mashing.

The ratio of soluble to insoluble β-glucan is also likely to be affected by the physical state of the barley grist or malt being extracted and the conditions of agitation employed during the extraction process.

During the early stages of malting, large amounts of a highly viscous β-glucan are released from the endosperm cell-walls by an enzyme or enzymes. This enzyme is heat stable and thus cannot be malt β-glucanases {(1→3)(1→4)-β-glucanase} as this enzyme is very temperature labile.

It has been suggested that the enzyme involved may act by cleaving peptide bridges between β-glucan chains, and thus induce solubility by destroying a three-dimensional polymer network. However, highly purified proteases do not catalyse this reaction and, although a carboxypeptidase has been implicated, the known action pattern of this group of enzymes (*exo*-peptidases) is inconsistent with this proposal. It is most probable that the enzyme involved is a temperature-stable β-glucanase (cellulase) possibly originating from fungi growing on the barley husk and seed coat.



High levels of partially depolymerised soluble β-glucan in the mash-tun increase wort viscosity, which reduces the rate of leaching of extract from the grist and thus increases the time required to obtain maximum extract.

Due to the instability of endogenous β-glucan degrading enzymes at temperatures above 45 °C, there is only limited further depolymerisation of barley β-glucan during an infusion mash of a mixture of malt and barley at commonly employed mashing temperatures (ie 65 °C). This undegraded β-glucan remains largely unchanged during the fermentation process and, due to the removal of maltose and other low molecular weight components (which apparently retard polysaccharide interaction) it may precipitate as a jelly-like material during storage, resulting in filtration problems during beer processing.

Heavy precipitates of barley β-glucan in final beers have been reported to occur, particularly in high gravity beers. Inadvertant freezing of beer has also led to β-glucan precipitates on rethawing.

Enzymic degradation of barley β-glucans

Barley β-glucans are susceptible to hydrolysis by three types of *endo*-glucanase, namely:

endo (1→4)-β-glucanase (cellulase)
endo (1→3)(1→4)-β-*D*-glucanase (lichenase and malt β-glucanase), and
(1→3)(4)-β-*D*-glucanase {non-specific *endo*-(1→3)-β-*D*-glucanase}.

Another *endo*-β-*D*-glucanase which has been implicated in the depolymerisation of high molecular weight β-glucans from malt is specific *endo*-(1→3)-β-*D*-glucanase, but the importance of this enzyme has not been conclusively demonstrated.

Endo-(1→4)-β-glucanase is mainly confined to the husk and most of this activity has been shown to originate from micro-organisms which contaminate the grain.

Various studies have indicated that the major, if not the only β-glucanase which plays a key physiological role in the *endo*-depolymerisation of barley β-glucan is malt β-glucanase. This enzyme is essentially absent in raw barley and activity increases rapidly during germination or malting (germination under controlled conditions of moisture, temperature and aeration). This enzyme is very heat labile and without particular care it can be substantially inactivated during kilning. It is essential that most of the moisture is removed from the malt before high kilning temperatures are attained.

In aqueous solution, malt β-glucanase is rapidly inactivated at temperatures above 55 °C. For this reason, the enzyme is unlikely to play a major role in the depolymerization of barley β-glucan during infusion mashing which is usually performed at 65 °C.

Measurement of barley β-glucan

Because of the problems caused by barley β-glucan in the brewing and animal-feed industries, a major aim of most barley breeding programs is the identification and establishment of varieties with reduced levels of this glucan. There is also a desire to measure this component in malt, wort and beer.

Over the past decade, a range of analytical procedures for barley β-glucan have been described. Most of these procedures employ crude cellulase preparations to depolymerise the glucan to glucose, and this glucose is subsequently analysed.

The basic problem with these procedures is that quite often the cellulase preparations employed contained side activities which released glucose from other carbohydrates (particularly starch) which led to inflated β-glucan values.

Other procedures involved the use of a highly purified (1→3)(1→4)-β-glucanase (lichenase) to depolymerise the β-glucan to oligosaccharides which were then either recovered and acid hydrolysed and the released glucose measured, or alternatively, the oligosaccharides were measured by a reducing sugar procedure.

Both of these methods are significantly better than those employing crude cellulase preparations but each has its own limitations. The former method is very accurate, but also very tedious, while the latter employs a non-specific and non-stoichoimetric reducing sugar assay to measure released oligosaccharides, and thus has limited accuracy.

In the author's laboratory an alternative, totally enzymic procedure was developed. This procedure involves the depolymerisation of barley β-glucan with lichenase followed by hydrolysis of oligosaccharides to glucose with another enzyme, β-glucosidase (refer to Scheme 1). The released glucose is specifically measured with glucose oxidase/peroxidase reagent. This procedure is both rapid and accurate, allowing the analysis of at least 30 samples per day.

Evaluation of the technique on different days has indicated a mean standard error of 0.1 for barley flour samples containing 3.8 and 4.6% (w/w) β-glucan content. The use of highly purified enzymes allows the analysis of β-glucan in whole grain homogenates. There is no requirement to extract or purify the β-glucan before analysis.

Because highly purified enzymes are required in this assay procedure it was unlikely that the method could be generally adopted unless either the enzyme or the assay procedure became commercially available. This problem was resolved when Biocon (Australia) Pty Ltd, took up the option to develop and market commercial kits based on the described basic research.

Kits were developed and are marketed worldwide through the 26 Biocon subsidiaries. The procedure has been succesfully evaluated by a sub-committee of the Royal Australian Chemical Institute,

Cereal Chemistry Division and now finds worldwide application for the assay of β-glucan in barley, malt, wort and beer. The procedure is currently being evaluated by the European Brewing Convention.

Measurement of malt β-glucanase

With the confirmed central role played by malt β-glucanase in the depolymerisation of barley β-glucan during malting and during mashing (if mashing-in temperatures below 50°C are employed), there has been considerable interest in the availability of a simple and reliable method for the estimation of the β-glucanase content of malt.

The method previously employed is based on the measurement of the decrease in viscosity of solutions of barley β-glucan. Although the limited accuracy and reliability of this method has been known for some time, the method persisted due to the unavailability of a more reliable alternative procedure.

An alternative approach to the analysis of polysaccharide *endo*-hydrolases is through the use of dye-labelled substrates, ie carbohydrate polymers to which a dye is covalently attached. Such substrates have found use in the analysis of alpha-amylase, cellulase, xylanase and β-mannanase.

In our laboratory we have used this technology to develop a substrate for the assay of malt β-glucanase. In the development of this substrate we found that when barley β-glucan was dyed with a reactive dye such as Remazolbrilliant Blue R an insoluble product is formed which is quite difficult to employ as a standard substrate.

This problem has been overcome by increasing the solubility of barley β-glucan, through carboxymethylation, before dyeing with Remazolbrilliant Blue R. The product is a soluble, dyed β-glucan substrate which is readily attacked by malt β-glucanase. This substrate has been incorporated into an assay procedure (outlined in Scheme 2) which has also been evaluated by a sub-committee of the Royal Australian Chemical Institute, Cereal Chemistry Division.

Evaluation of the procedure in 18 laboratories (14 in Australia and four in the UK and Europe) on five malt samples yielded interlaboratory coefficient of variation values of less than 6% for each of the samples. These results were so favourable that there was an immediate demand for the assay procedure.

The substrate for the assay of malt β-glucanase, ie Azo-Barley Glucan, is currently being prepared by Biocon and will be marketed through Biocon (Australia) Pty Ltd, in a ready to use kit form. The assay procedure can be used to follow changes in β-glucanase levels in developing malt and during the mashing process.

It can also be used to assay the activity of other enzyme active on barley β-glucan such as those present in commercial fungal and bacterial preparations.

The future

The methods developed in our laboratory should help resolve the problems caused by barley β-glucans in the brewing process by:

(1) allowing identification and culling of barley varieties high in β-glucan;
(2) allowing the selection of varieties which synthesise high levels of β-glucanase during malting;
(3) aiding the malster and the brewer in monitoring changes in malt β-glucanase during malting, kilning and mashing processes;
(4) allowing the judicious use of commercial enzymes in relieving β-glucan related problems.

Acknowledgement

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Further reading

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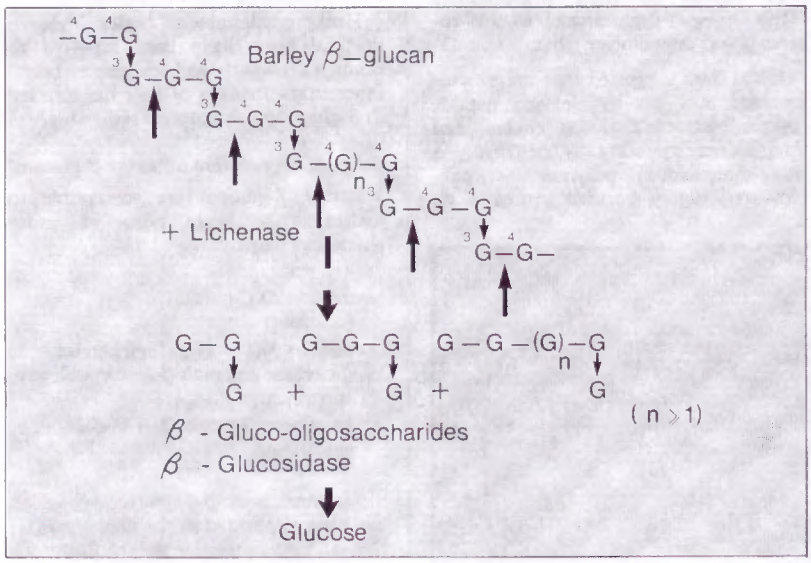
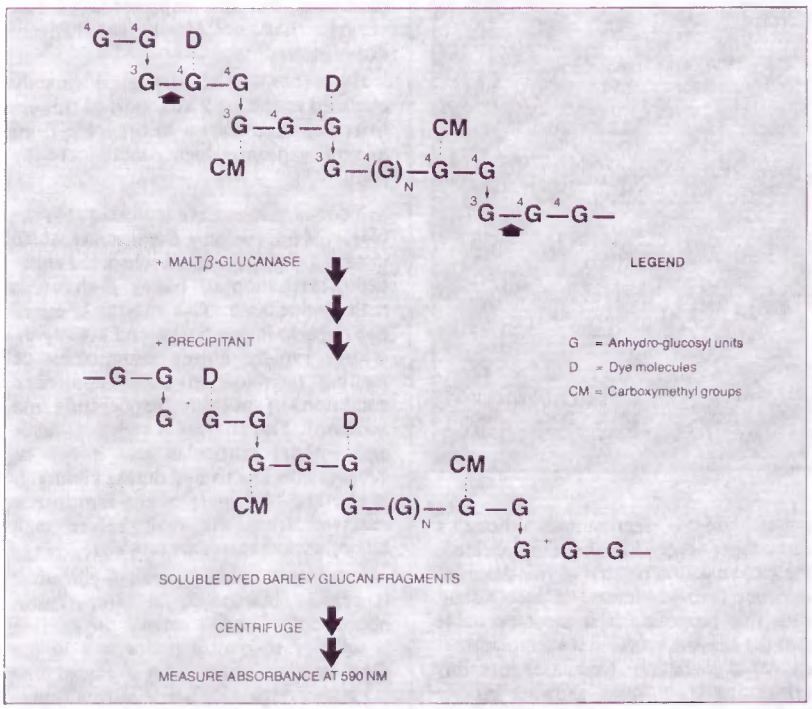


Scheme 1: Theoretical basis of the barley β-glucan assay procedure.



Scheme 2: Theoretical basis of the malt β-glucanase assay procedure.