Enzymes in brewing

The brewing process depends on enzymes. The enzymes from malt catalyse the hydrolysis of the endosperm cell walls, starch, protein, and lipid during malting and mashing to give wort which is then fermented by yeast into beer.

Yeast itself can be considered as a package of complex enzymes which catalyse the reactions necessary for converting fermentable sugars into alcohol.

Brewers often find themselves using malts of less than ideal quality and although they will attempt to make the best use of the enzymes from the malt, the variations in quality can lead to unpredictable brewhouse performance. The use of exogenous enzymes can supplement the malt enzymes and, in some cases, provide additional activities not inherently present in the malt to give consistent and efficient brewhouse performance.

Mashing

A number of factors may affect brewhouse performance including the presence of high molecular weight non-starch polysaccharides which can effect wort separation, small granular starch which may not be gelatinised at normal mashing saccharification temperatures and incomplete proteolysis may result in low α-amino nitrogen.

These various shortcomings can be tackled by the selection of appropriate enzymes.

To maximise extract and, in cases where high levels of adjunct are used, a blend of enzymes which should include one or more activities such as bacterial β-glucanase, protease, and α-amylase may be added directly into the mash vessel.

This type of enzyme blend should be used in a temperature controlled mashing profile which would include a proteolysis step at 45–55°C followed by a saccharification stage at 60–65°C and a finishing step at 74–76°C.

The times and temperatures are critical, particularly at the proteolysis and finishing steps. Too short a time, or too high a temperature, at the proteolysis step will lead to incomplete breakdown of the protein matrix surrounding the starch granules, which will be unavailable for conversion resulting in low extract.

Too low temperature at the finishing stage and any residual starch may not gelatinise and the starch may be carried through to the kettle unconverted. If the temperature is too high the α-amylase will be degraded and, although, the residual starch may be gelatinised it will not be converted.

The presence of unconverted starch carrying through from the mash will give low extracts and the potential for starch hazes in the finished beer. This can be avoided by the addition of a more heat stable α-amylase.

Additional proteolytic activity may be required when brewing with certain adjuncts or poor quality malts to improve extract and adjust the ratio of soluble nitrogen for yeast growth. A bacterial neutral protease is most suited for this purpose as its pH and temperature profile fits the conditions of a mash with a stand between 52–55°C.

Excessive proteolytic activity can have a detrimental effect on the beer foam stabilising proteins and increasing the soluble wort nitrogen too far can effect beer ester formation.

Using exogenous enzymes in the mash enables the brewer to substitute a substantial proportion of the malt with adjuncts such as maize, rice, unmalted barley or even sorghum.

Adjunct Cooking

Cereals such as maize and rice are commonly used as adjuncts in brewing. The gelatinisation temperatures of the starch in these adjuncts are higher than that used for saccharification in mashing. Therefore it is necessary to cook the adjunct prior to addition to the mash to ensure complete gelatinisation and liquefaction.

Adjunct cooking is traditionally carried out using the addition of some malt into the cereal cooker along with the adjunct. The α-amylase of the malt has sufficient activity at the higher temperatures of the cooker to liquefy the starch. However a more efficient method is the use of a heat stable α-amylase.

The cereal adjunct is often mashed in at a relatively low initial temperature of 45°C to 55°C with a small addition of malt or exogenous α-amylase to maintain the cooked adjunct in a liquid state. The cooker is then heated to 100°C for a 15 to 20 minute to complete the liquefaction. The cooked adjunct is then added to the mash mixing vessel after the proteolytic stand, to raise the combined temperature of the mash to between 62° and 65°C in the form of single decoction mashing.

Using a heat stable α-amylase allows for a shorter cooking cycle and, because of the relatively low amount of enzyme required, more adjunct may be accommodated in the cooker.

One of the major advantages of using a heat stable α-amylase over malt is that the brewer can ensure that there is no risk of residual starch carried over from the cereal cooker into the mash vessel. The presence of residual starch can have implications in beer filtration and haze formation.

Wort Separation

In barley and malt there are non-starch polysaccharides, which, when solubilised, contribute to the viscosity of wort. The most significant is β-glucan which is solubilised into the wort by the enzymes of malted barley. Whilst β-glucan accounts for a substantial part of the troublesome polysaccharides in barley and malt, there are other polysaccharides such as arabinoxylans, often referred to as pentosans, which can also contribute to wort viscosity. Arabinoxylans are present in higher concentrations in other cereals such as wheat.

The degradation of mash β-glucans by the addition of exogenous fungal β-glucanases has been demonstrated to decrease wort and beer viscosity and to improve wort separation. These enzymes often contain a secondary enzyme activity of xylanase or arabinoxylanase, which is sometimes referred to as pentosanase.

Fungal β-glucanases differ from bacterial β-glucanases in their ability to breakdown β-glucan and reduce wort viscosity, this may be due to the presence of secondary enzyme activities.

The correct choice of enzyme will depend on a number of factors such as the type of adjunct, if any, used, and the temperature profile of the mash. Using an adjunct such as wheat will introduce the possibility of some high molecular weight arabinoxylan being present in the wort, then there may be a requirement for a β-glucanase with a substantial secondary arabinoxylanase activity.

Some high molecular weight β-glucans may be solubilised at higher temperatures which may be outside the temperature range of some β-glucanase preparations. In such a case a β-glucanase with a higher heat stability may be the preferred option.

In all cases the use of a β-glucanase should result in wort which contains the lowest possible level of high molecular weight β-glucans and a viscosity sufficiently low to

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**Optimum temperature and pH ranges of Enzymes used in Mashing**

<table>
<thead>
<tr>
<th>Activity</th>
<th>pH Range</th>
<th>Temperature Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-amylase</td>
<td>5.0–7.5</td>
<td>60–80°C</td>
</tr>
<tr>
<td>β-glucanase</td>
<td>5.0–7.5</td>
<td>50–55°C</td>
</tr>
<tr>
<td>Neutral Protease</td>
<td>5.0–8.5</td>
<td>50–57°C</td>
</tr>
</tbody>
</table>

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Increasing Fermentability

Controlling the fermentability of the wort enables the brewer to produce a new range of products. Highly attenuated or low carbohydrate beers may be produced by the addition of enzymes to the wort during fermentation.

Although malt is able to produce all the enzymes needed to break starch down into fermentable sugars, the debranching enzyme, malt limit dextrinase, is very heat labile and is inactivated at the temperatures required for gelatinising the starch in malt. Therefore since the α-1,6 bonds in amylpectin are not hydrolysed by α- and β-amylase, the maximum fermentability in normal beers is around 70% due to the presence of limit dextrins.

Pullulanase can hydrolyse the α-1,6 links and enable the production of more fermentable sugars from the wort. Using pullulanase on its own, in the fermenter, will not give an increase in fermentability and is usually accompanied by either fungal α-amylase or β-amylase.

Used in the fermenter, fungal α-amylase alone will give a small increase in fermentability of 1 to 2 degrees, and may be useful in the cases of stuck or incomplete fermentations. By far the largest increase in fermentability can be achieved by using glucoamylase which can hydrolyse both the α-1,4 and α-1,6 linkages to give glucose. The rate of hydrolysis of α-1,6 linkages is only slow and may be enhanced by supplementation with pullulanase.

Glucoamylase is relatively heat stable and, when used in the fermenter, may survive pasteurisation, and may carry through into the beer. The consequence is that, if any substrate is still available, the sweetness of the beer may increase throughout its packaged life. For this reason some brewers add amyloglucosidase to the mash or to the wort after separation to ensure that the enzyme is inactivated during the boil.

Beer Stabilisation

As well as hazes due to polysaccharides, non-biological haze in beer can result from the cross linkage of soluble proteins and polyphenols which are naturally present in beer. The polyphenols combine slowly with protein to form chill haze when cooled, but which re-dissolve when warmed up. Eventually as the polyphenols polymerise and increase in size they become insoluble at room temperature to form irreversible or permanent haze.

The traditional method of stabilising a beer was to promote the aggregation of these soluble proteins and polyphenols by reducing the temperature of the beer in the maturation tank and holding for an extended time. The aggregates become so large that they settle and can be removed by cold filtration.

Different techniques may be employed to prevent the formation of cross linkages between the protein and the polyphenols such as the removal of the haze forming protein with silica hydrogel or tannic acid, or the removal of some of the polyphenols using polyvinylpyrrolidone (PVPP).

Enzymes such as papain have been used to hydrolyse the protein fraction and thereby prevent haze formation. Effective use of papain is best achieved by addition into cold storage so as to obtain maximum contact time. Papain is also readily absorbed onto the surface of yeast and will be removed during filtration.

Papain is a non-specific protease and enzymes preparations based on papain may have an adverse effect upon beer head retention, particularly at high dose rates and long contact time.

Recently a new enzyme product has been introduced – a proline specific endo-protease that could cleave on one side of a proline residue only in a protein. This prevents the polyphenol bridging chains of peptides and therefore significantly reduces the large aggregated network from forming.

The action of the proline specific endo-protease is based on the idea that the particular proteins involved in such reactions are rich in the amino acid proline. It is the cross-linking of these, so-called haze active (HA), proteins with the polyphenols which eventually produce a colloidal haze.

The enzyme is added to the cooled wort prior to fermentation and, since there is little, if any, proline in the proteins responsible for foam beer foam is not affected.

Maturation

One of the rate limiting steps in beer maturation is the time needed for the yeast to reduce diacetyl levels to below a certain limit.

Diacetyl has a very low flavour threshold and gives a flavour often described as “toffee” or “butterscotch”. This is one of the most common flavour defects in lager.

A by-product of amino acid metabolism, diacetyl is formed by the oxidative decarboxylation of α-acetolactate during the exponential growth phase of yeast in primary fermentation.

Towards the end of the fermentation and
during maturation, yeast is capable of metabolising the diacetyl to a compound with a much lower flavour threshold, acetoin which is almost tasteless.

By adding an \(\alpha\)-acetolactate decarboxylase at the beginning of the primary fermentation process, it is possible to convert the precursor, \(\alpha\)-acetolactate, directly into acetoin. Since most of the \(\alpha\)-acetolactate is degraded before it has a chance to oxidise, less diacetyl is formed.

This reduces the need for a long maturation or diacetyl stand after fermentation, enabling the beer to be ready quicker thus improving vessel utilisation.

**Inactivation of Enzymes**

Exogenous enzymes used in the adjunct cooker or mash before wort boiling in the kettle will have exceeded their thermal limit and have no residual activity after boiling. The combination of high temperature, relatively low pH, time, and the absence of substrate will reduce enzyme stability so that even heat stable \(\alpha\)-amylases will be inactivated.

Enzymes used in fermentation or maturation rely on pasteurisation for inactivation, there is a risk of enzyme activity being carried over into the beer.

### A word on GMOs

The vast majority of exogenous enzymes used in brewing are produced from the fermentation of bacterial or fungal micro-organisms. There are occasions when the desired enzyme is not produced in economical amounts in the micro-organism. In such a case the enzyme producer having identified the gene responsible for expressing the enzyme protein, may choose to insert multiple copies of the gene into the production strain. Such a technique is referred to as homologous recombination or self-cloning.

In other occasions the desired enzyme may be produced by a micro-organism that is not a good or well known production organism. In this case the gene encoding the enzyme may be placed in a more efficient or better known production micro-organism. Such a technique is known as heterologous recombination.

In both of these cases the enzyme itself is not genetically modified, it is the producing micro-organism that is genetically modified. The enzyme protein is the same whether the producing micro-organism is genetically modified or not.

It is also a subject of debate whether a self-cloned micro-organism containing no foreign DNA can be considered as a GMO.

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**The author**

Ian Bentley has 22 years of experience in the application of enzymes into brewing and potable alcohol. He joined ABM Chemicals, Brewing and Food in May 1984 as a scientist in the Technical Services Laboratory giving technical support to customers. Since the acquisition by firstly Genencor in 2002 and subsequently Danisco in 2005 he has taken more responsibility on the business side of supplying enzymes to the brewing and potable alcohol market. He still retains a technical role supporting customers in the application of enzymes.