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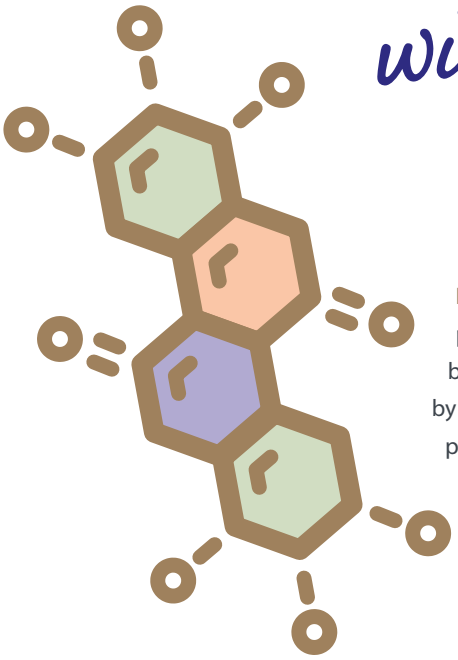
BETTER BREWING *with* ENZYMES



Enzymes are an integral part of life with millions of biochemical reactions catalysed by specific enzymes. The brewing process takes advantage of the natural biological processes of germination and fermentation, where enzymes play a critical role. During the malting process, plant hormones called gibberellins stimulate the aleurone layer of the barley seed to produce hydrolytic enzymes capable of breaking down starch. The α- and β-amylase enzymes present in the malt play a dominant role during mashing, breaking the starch molecules down into smaller fermentable sugars. During fermentation, multiple enzymes help facilitate cell division, import of sugar into the cell, production of alcohol, excretion of carbon dioxide and the formation and modification of many flavour compounds.

Historically, different beer styles were brewed using ingredients, equipment and processes adapted to each style. Brewers are now brewing more diverse styles than ever before including historical styles with a modern twist as well as completely novel styles. The use of unfamiliar ingredients and brewing processes to brew creative or novel beer styles can present challenges to the brewer. Processes such as high gravity brewing or mashing with non-traditional grains may be used to brew unique styles or reduce cost. However, high gravity brewing and the use of rye malt, wheat or other grains can result in a slow lauter or a stuck mash. Use of adjuncts such as maize and rice in dry or brut beer styles can lower nitrogen levels resulting in slow, sluggish fermentations in the nutrient depleted wort. Enzymes are the brewer's friend - The addition of exogenous enzymes can help the brewer in many of these instances by reducing production time, increasing yield and consistency, reducing off-flavors and lowering costs.

There are many exogenous enzymes available to the brewer to use throughout the brewing process. Several enzymes can be used at multiple points in the brewing process, such as in the mash tun, kettle, fermenter or maturation tank. A large proportion of exogenous enzymes are used upfront in the brewhouse during mash conversion. Brewhouse enzyme additions can be used to improve mash extract, wort fermentability, lauter efficiency, and free amino nitrogen levels. Enzymes added to the brewhouse are able to do their job, but they are then denatured by boiling in the kettle, so their activity doesn't carry through to fermentation or into the package. **This gives the brewer greater control – You decide exactly when enzyme activity starts and stops.**

Enzymes can also be added to the fermenter (FV) or maturation tank to improve attenuation and filterability, correct haze, promote biotransformation and improve product stability.

Some enzymes are very costly and it can be confusing which ones to use and where. In this document we will cover the options available to the brewer.

ENZYME SOLUTIONS

Mash conversion

The two main naturally occurring enzymes involved in the mashing process are β- and α-amylase. β-Amylase is a maltogenic enzyme, cleaving off two units of glucose (maltose) from the end of the amylose chain creating a highly fermentable wort. α-Amylase cleaves the 1,4 glucose linkages randomly, shortening the length of the chain, reducing viscosity, increasing extract but also increasing dextrins (Figure 1). The functions of these two enzymes generate the brewers window of optimal mash temperature between 62-70°C (Figure 2).

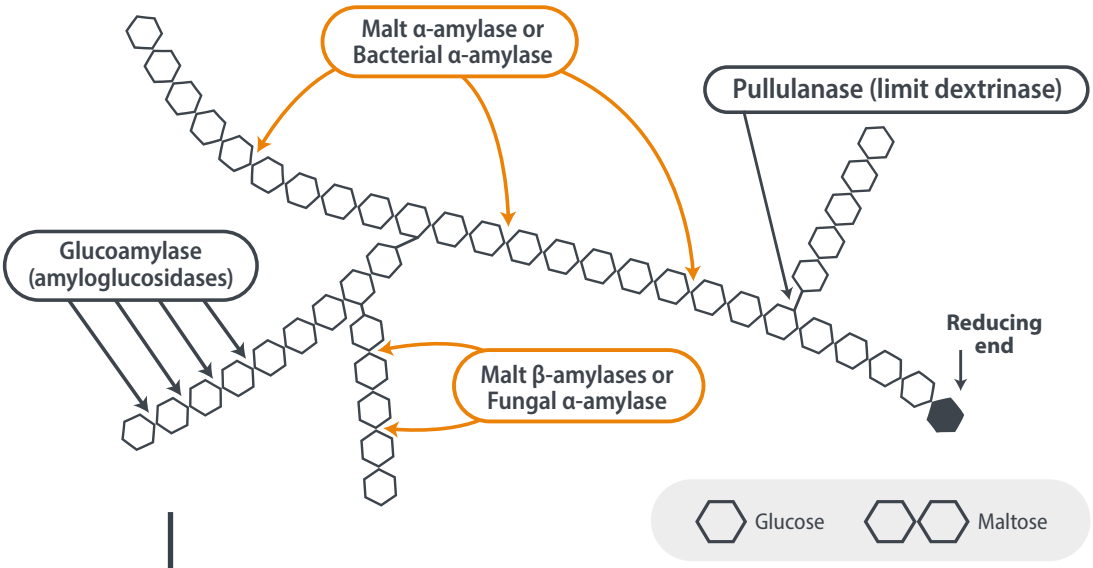


FIGURE 1: Specific enzyme activities of different starch degrading enzymes.

The malt-derived enzymes are limited for the high mash temperatures required when using adjuncts with higher gelatinisation temperatures, such as rice and maize. β-Amylase denatures above about 65°C and α-amylase above 75°C, so these enzymes are denatured at the high temperatures found in a cereal cooker. **Bacterial α-amylases** are thermally stable, some at ultra-high temperatures (UHT), and can be used to reduce viscosity in the cereal cooker when using cereals with high gelatinisation temperatures. Due to the thermal stability of bacterial α-amylase, the active enzyme activity carries over from the cereal cooker to mash conversion for further breakdown of starch to dextrins. **Fungal α-amylase** is a maltogenic enzyme with similar activity to malt β-amylase and can be used to increase wort fermentability at normal mash temperatures, especially at the higher end of the brewer's window where malt β-amylase activity is reduced.

Traditionally, light beers were produced by using a long mash at low temperature to increase starch conversion levels and attenuation. Brewers now brewing these styles can opt for using an **glucoamylase** enzyme (amyloglucosidase). Glucoamylase works by cleaving the α-1,4 linkage at the non-reducing end of a starch molecule to liberate single glucose units up to a branch point (Figure 1). This produces a high level of fermentable sugars for the yeast to utilise. Glucoamylase is used for many applications including "light" or "brut" styles, low carb / low calorie beers, and for decreasing body and improving drinkability in high gravity beers. Using glucoamylase is economical as it increases brewhouse throughput by keeping mash times short and allows you to reduce your grain bill due to the increased extract efficiency.

Glucoamylase can cleave 1,6 branch points as well, but not very efficiently. Combining glucoamylase with a pullulanase enzyme (limit dextrinase) to cleave the 1,6 branch points will facilitate maximum starch conversion (Figure 1). Pullulanase enzymes are very expensive, however, and similarly high mash conversion can be achieved using a blend of fungal α-amylase and glucoamylase as long as mash temperatures are not above 65°C.

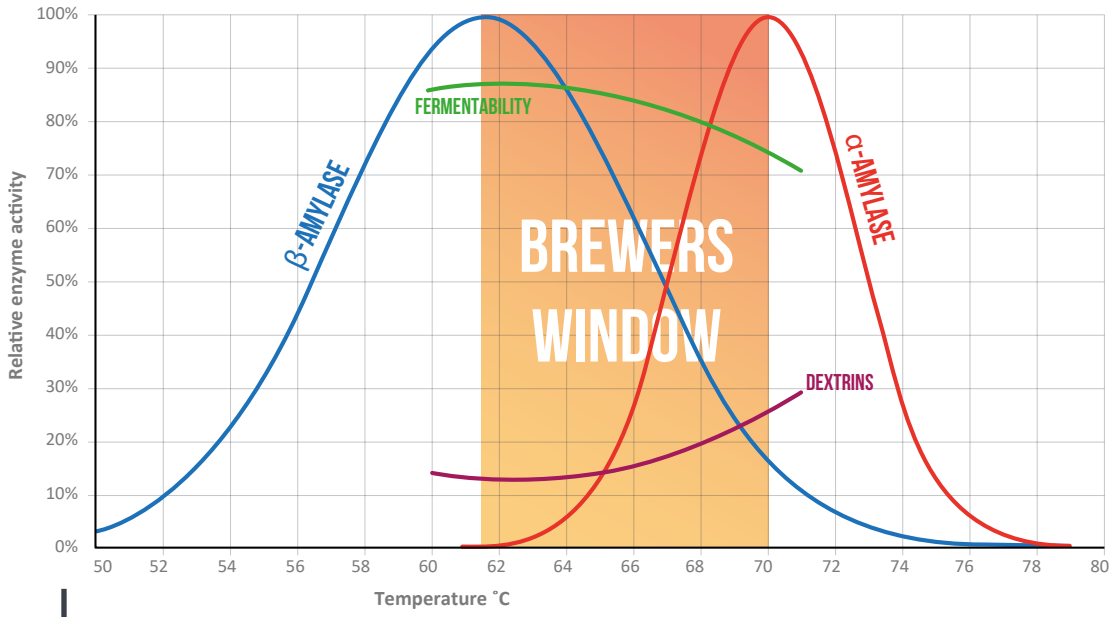


FIGURE 2: Different types of enzymes have different specific activities during mash conversion that influence the composition of fermentable and non-fermentable sugars in the wort.

Glucoamylase can be used either in the mash or the FV. Though it is more expensive to use in the mash (a much higher addition rate is required), this method provides more control compared to adding to the FV. Denaturation of the glucoamylase enzyme only occurs at high temperatures (>85°C for 10 mins) or very low or high pH. If added to the FV, the active enzyme may transfer to the packaged product or pass into further brews with the cropped yeast. Some glucoamylase activity may also survive pasteurization. Dextrins in the packaged beer will be slowly broken down into fermentable sugars by the glucoamylase enzyme. If active yeast is present, this sugar will be fermented resulting in increased CO_2 and alcohol. If no yeast is present, the beer will gradually sweeten over time due to the production of glucose.

FAN – Proteases

Using high levels of adjunct in your grist can be desirable from a cost point of view, but many such as corn, rice, maize, raw barley and sugar will dilute the level of nitrogen in the wort produced. Yeast requires nitrogen in the form of free amino nitrogen (FAN) to support its metabolism.

Introducing a protein rest can help to increase the level of FAN, but only up to ~10%. The addition of a **neutral protease** enzyme to the mash will result in more protein broken down into usable peptides and increased levels of FAN. This enzyme has a low temperature tolerance and a mash step of 45-48°C for a minimum of 20 minutes will need to be included.

The addition of a neutral protease is also required for brewing with unmalted barley. When using high levels of raw barley, a cocktail of enzymes is required including α-amylase, β-glucanase, neutral protease and a fungal α-amylase. By using a blend of these products it is possible to brew with upwards of 50% raw barley in the grist.

Wort separation

Seasonal variation in the levels of non-starch polysaccharides (NSPs) such as β-glucan can cause the brewer a serious headache. High levels of NSP (over 140ppm) can cause wort viscosities to increase. This in turn causes lautering issues resulting in lower extract, reduced brewhouse throughput and poor beer filtration. Using speciality grains such as rye and high levels of wheat will also affect lautering speeds and brewhouse performance due to high levels of other NSPs such as xylanases or arabinoxylans. NSPs can be broken down by adding a **β-glucanase** enzyme, which can be derived from either fungal or bacterial origins, each of which has its advantages. **Fungal β-glucanase** tends to have a lower temperature tolerance but has a broader range of side activities such as cellulase and xylanase, which is important when using adjuncts such as rye and wheat. **Bacterial β-glucanase** has a higher temperature tolerance, but acts more specifically on β-glucans. The seasonal variation in malt β-glucan levels can be ironed out by using a relatively inexpensive bacterial β-glucanase. Companies offer enzymes blends to take advantage of the benefits of both types. You will need to decide which product is best for your application.

Fermentation

To a non-brewer, the process of making beer should be a simple one. You use the same malt, yeast, hops and water every time, therefore surely the outcome should always be the same. Anyone working in the industry knows this isn't the case. Perfect, consistent fermentations are the holy grail for most brewers. Fermentation is a hive of activity and the biochemical performance of the yeast is fundamental to the beer quality, as is the medium it ferments. However, common issues the brewer faces such as temperature control, time constraints and capacity limitations can result in poor fermentations leading to off-flavors and stalled or incomplete fermentations.

Diacetyl is a common fermentation biproduct that is perceived by most people as an off-flavor. It is produced from a side reaction by yeast metabolising amino acids into valine (Figure 3). The yeast produces α-acetolactate, which is then excreted out of the cell. The α-acetolactate is then decarboxylated into diacetyl and reabsorbed back into the yeast at the end of fermentation where it is metabolised into acetoin, a flavourless compound. Diacetyl reabsorption by the yeast takes time and is faster at warmer ale temperatures compared to cooler lager temperatures. Diacetyl may be present in packaged beer when fermentations are incomplete and the yeast is unable to completely reabsorb the diacetyl. This may be due to premature transfer from the fermenter, nutrient deficiency, yeast stress or infection.

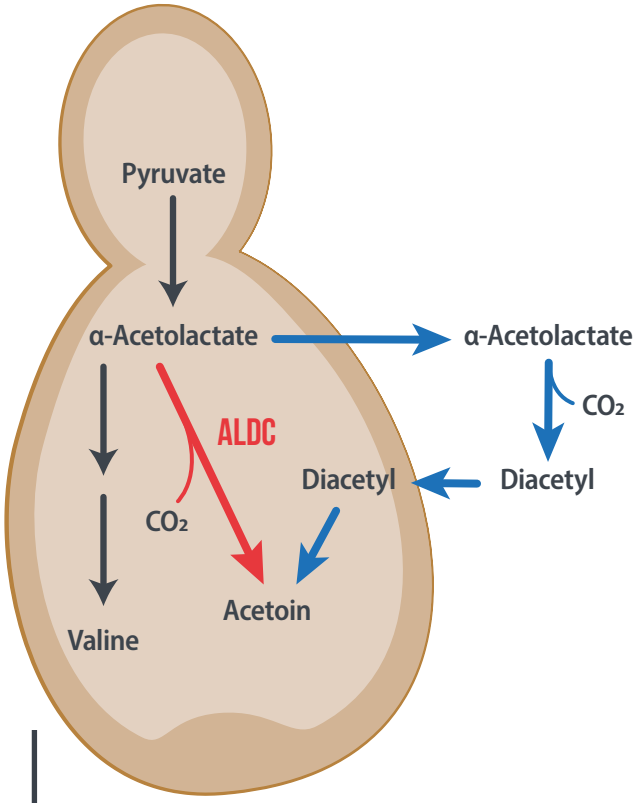


FIGURE 3
The addition of α-acetolactate decarboxylase (ALDC) enzyme allows the direct breakdown of α-acetolactate into flavorless acetoin (red lines) and prevents the formation and normal metabolism of diacetyl by the yeast cell (blue lines).

The formation of diacetyl can be inhibited by using an **acetolactate decarboxylase enzyme (ALDC)**, which converts α-acetolactate directly to acetoin (Figure 3). The reduced formation of diacetyl results in a reduced maturation time and an increased maturation capacity in the brewery. The activity of ALDC is pH dependant and will not work efficiently at the pH of a fully fermented beer (3.9-4.2), so it cannot remove diacetyl from a finished beer. For this reason, ALDC must be added to the wort at the start of fermentation when the pH is higher and valine biosynthesis is occurring during yeast multiplication. This enzyme is very expensive, but only low addition rates are required (Table 1).

Attenuation / Stuck Fermentations

Using low quality, poorly modified malt or mashing at very high temperatures can produce wort with low fermentability due to the presence of greater amounts of larger, unfermentable sugars. The addition of a **fungal α-amylase** can rescue a stuck fermentation by breaking down these larger sugars into fermentable maltose. Fungal α-amylase is preferable to glucoamylase when added to the FV because it only produces maltose, whereas glucoamylase produces glucose which can inhibit the fermentation of other types of sugars. Also, lower dosage rates are required for fungal α-amylase compared to glucoamylase (Table 1).

Biotransformation

Different hop varieties contribute a myriad of different flavor compounds to the beer. The most abundant class of hop flavor compounds is terpenes, which are present either as aromatic free volatiles or non-aromatic glycosides where they are bound to a sugar molecule. Some yeast strains produce **β-glucosidase enzymes** that can cleave the sugar molecule from the terpenyl glycoside to release the aromatic terpene (Fig. 4.1). β-Glucosidase can also be added as a pure enzyme, which gives the brewer more control since the enzyme can be used during fermentation using any yeast strain and the enzyme activity is not coupled to yeast metabolism. The application of a β-glucosidase enzyme during fermentation improves hop utilization by releasing additional volatile aroma compounds, allowing the brewer to decrease overall hop quantities or express more character from less sophisticated hop varieties. Some yeast strains also produce **β-lyase enzymes** that can liberate free aromatic thiols from a cysteine-bound precursor (Fig. 4.2), but no commercial exogenous β-lyase enzymes are currently available for brewing applications.

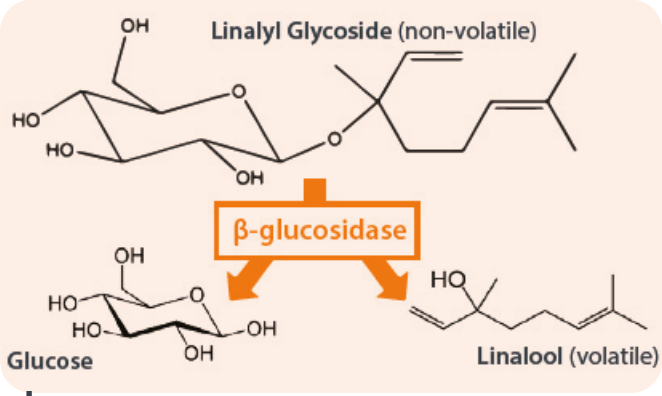


FIGURE 4.1 - β-glucosidase enzyme mechanism: Linalool (an aromatic terpene) and a glucose molecule are released from a non-aromatic linalyl glycoside.

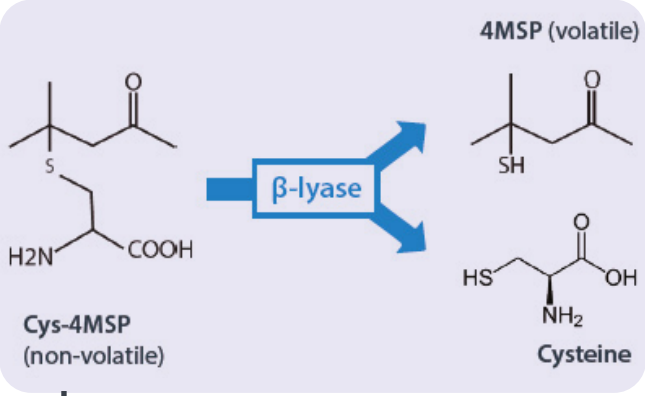


FIGURE 4.2 - β-lyase enzyme mechanism: 4MSP (an aromatic thiol) and cysteine are released from a non-aromatic cysteinylated precursor.

Stabilization

Beer shelf life is influenced by the potential for haze to form in the packaged product. Historically, beer was stabilized through a long period of cold conditioning, allowing time for sensitive protein to bind to polyphenols (tannins) forming colloidal particles, which were then removed by sedimentation or filtration. The addition of process aids such as PVPP or silica gel accelerates this process by binding to and efficiently removing polyphenols or proteins and preventing the formation of colloidal particles. A detailed discussion of these process aids is included in the ABV Process Aids document.

Formation of colloidal haze can also be prevented by adding protease enzymes to break down proteins. Papain is a cysteine protease enzyme that is a more affordable option for preventing haze formation compared to other process aids. The activity of papain is non-specific, so it is important to remove yeast and precipitate some protein by cooling the beer to near or below 0°C to facilitate lower enzyme dose rates during maturation. Higher dose rates of papain may negatively impact foam potential. Proline-specific protease enzymes are more costly than papain but can be added at the start of fermentation since they target the polyphenol binding site of sensitive proteins and so do not require maximum cooling to remove yeast and other proteins prior to application.

Filtration

Addition of enzymes can also help downstream beer processing. The presence of unconverted starch in the fermented beer can cause problems with haze and filtration. The addition of a **fungal α-amylase** into the maturation tank can break down the starch into maltose, which will not sweeten the beer to the same degree as the glucose produced from the addition of glucoamylase.

β-Glucanase can also be used in maturation tanks to improve filtration, but it is more common to add this enzyme in the brewhouse to avoid carry-through of active enzyme into the packaged product. It is always a good idea to check the specifications of your enzyme to ensure it can be added during fermentation or to the finished beer. When adding any enzymes that produce fermentable sugars to a maturation tank it is important that all active yeast is removed by filtration or pasteurization in order to prevent additional fermentation in the packaged product.

TABLE 1: AB VICKERS ENZYME SOLUTIONS REFERENCE

PROBLEM / TARGET	SOLUTION	ENZYME CLASS	APPLICATION POINT	DOSE RATE	TEMPERATURE / PH
Starch positive worts	ALPHA**MYLASE LT30 EXTRA**	Bacterial α-amylase	Mash conversion	0.01-0.05 kg/MT	77-90°C pH 5.8-6.6
	ALPHA**MYLASE TH PLUS**	UHT α- amylase	Mash conversion / Cereal cooker	0.15-0.7 kg/MT	85-97°C pH 5.5-7.0
	ALPHA**MYLASE FA**	Fungal α-amylase	FV	2-7 ml/hL	40-60°C pH 4-6
Increase extract	ALPHA**MYLASE LT30 EXTRA**	Bacterial α-amylase	Mash conversion	0.01-0.05 kg/MT	77-90°C pH 5.8-6.6
	ALPHA**MYLASE TH PLUS**	UHT α-amylase	Mash conversion / Cereal cooker	0.15-0.7 kg/MT	85-97°C pH 5.5-7.0
Increase wort fermentability	ALPHA**MYLASE FA**	Fungal α-amylase	Mash conversion, FV	1-10 kg/MT 2-7 ml/hL	40-60°C pH 4-6
	GLUCO**AMYLASE 400**	Glucoamylase	Mash conversion, FV	1-10 kg/MT 2-7 ml/hL	55-75°C pH 3.5-5.5
Use of high levels of adjuncts / Brewing with raw barley	ALPHA**MYLASE FA**	Fungal α-amylase	Mash conversion	0.5-1 kg/MT	45-60°C pH 4-6
	ALPHA**MYLASE TH PLUS**	UHT α-amylase	Mash conversion / Cereal cooker	0.15-0.7 kg/MT	85-97°C pH 5.5-7
	PROTO**ZYME NP**	Neutral protease	Mash conversion	0.3-1 kg/MT	>55°C
	GLUCANASE PREMIER	β-Glucanase	Mash conversion	200-500 ml/MT	55-70°C
Low carbohydrate beers / Brut style	GLUCO**AMYLASE 400**	Glucoamylase	Mash conversion, FV	1-10 kg/MT 2-7 ml/hL	55-75°C pH 3.5-5.5
	ALPHA**MYLASE FA**	Fungal α-amylase	FV	2-7ml/hL	40-60°C pH 4-6
Poor wort run off / filtration	**GLUCANASE** PREMIER	β-Glucanase	Mash conversion	200-500 ml/MT	40-70°C pH 4-6.7
	GLUCANASE PLUS	Enzyme blend (β-glucanase, xylanase, α-amylase)	Mash conversion	50-300 ml/MT	40-70°C pH 4-6.7
Improve beer filtration / yield	**GLUCANASE** PREMIER	β-Glucanase	Mash conversion	200-500 ml/MT	40-70°C pH 4-6.7
	GLUCANASE PLUS	Enzyme blend (β-glucanase, xylanase, α-amylase)	Mash conversion	50-300 ml/MT	40-70°C pH 4-6.7
Increased FAN	PROTO**ZYME NP**	Neutral Protease	Mash conversion	0.3-1 kg/MT	<55°C
Diacetyl reduction	**ALDC**	α-Acetolactate decarboxylase	FV	1-2 ml/hL	>25°C pH 3.9-4.2
Chill haze	CHILL**ZYME**	Papain (plant-derived protease)	Maturation or bright beer tank	3-6 ml/hl for cold conditioning tank 1-2 ml/hl for bright beer tank	pH 4-6
Increased or more diverse hop aroma	**Aromazyme**	β-Glucosidase	FV	5 g/hL	15-65°C pH 3.5-6.5

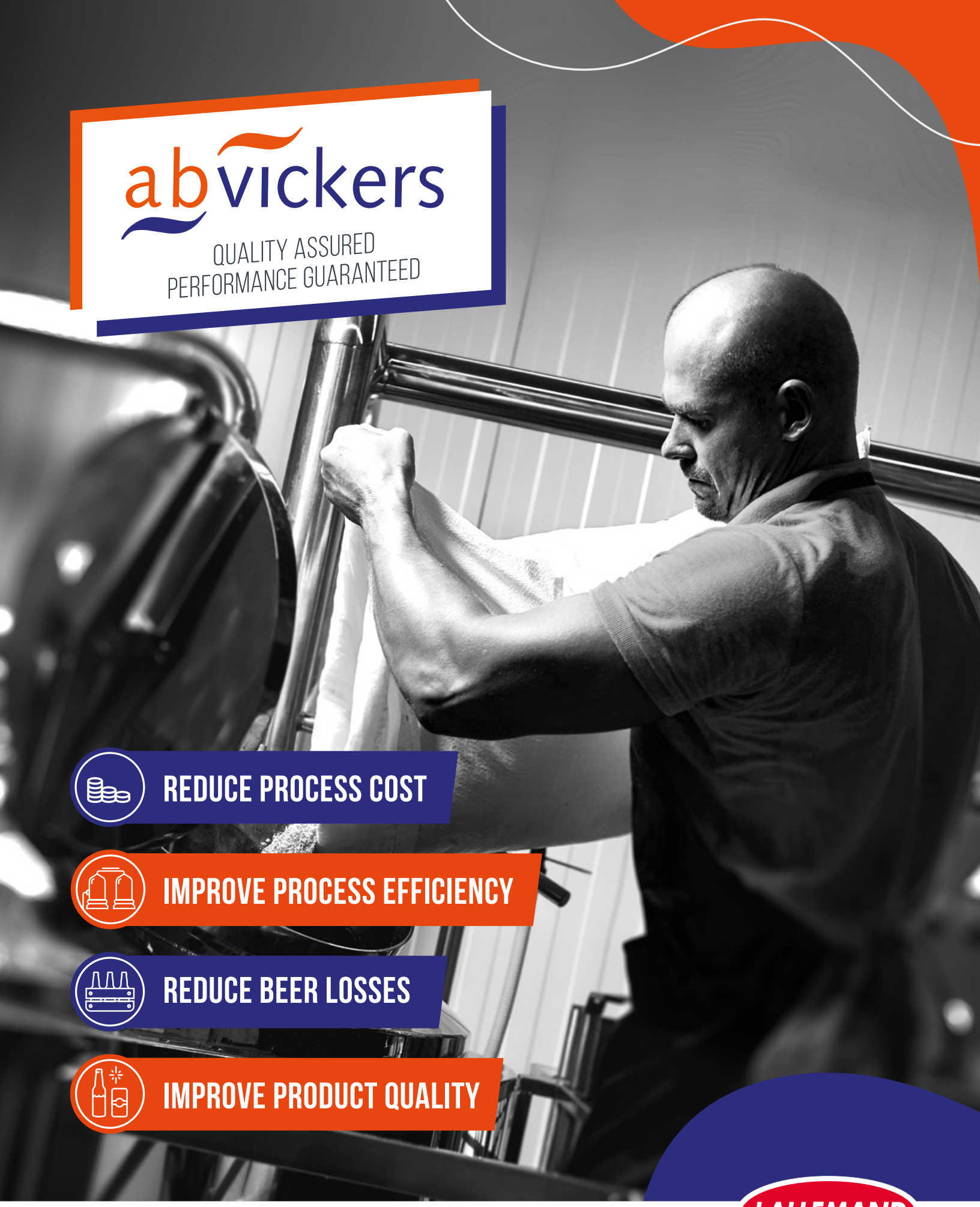
MT = metric ton (for use during mash conversion)
hL = hectoliter (for use in FV)

Temperature and pH recommendations are for optimal enzyme activity. Using conditions outside of this range will result in reduced enzyme activity and may require higher dose rates or longer reaction times.

TABLE 2: AB VICKERS ENZYME BENEFITS

PRODUCT	DESCRIPTION	BENEFITS
ALPHA**MYLASE LT30 EXTRA**	Bacterial alpha-amylase from *Bacillus subtilis*. Liquefying enzyme: produces a decrease of viscosity on starch	• **Liquefies starch substrates** • **Facilitates the use of starches with higher temperature gelatinisation** • **Allows the use of high levels of adjunct** • **Increases the level of attenuation** • **Reduces the potential for starch positive worts**
ALPHA**MYLASE FA**	Fungal alpha-amylase from *Aspergillus oryzae* (EC 3.2.1.1)	• **Increased level of fermentable sugars in wort** • **Eliminates residual starch in wort** • **Better control of attenuation level** • **Facilitates removal of starch haze in beer**
GLUCANASE PREMIER	Beta-glucanase that breaks down celluloses and hemicelluloses in wheat, barley and other cereals	• **Improves lautering and wort filterability** • **Guards against β-glucan induced beer hazes** • **Improves beer filterability**
GLUCANASE PLUS	Enzyme blend derived from fungal organisms. Primary activities: betaglucanase, xylanase and alpha amylase	• **Improves lautering and wort filterability** • **Increased extract recovery** • **Efficient final beer filtration**
GLUCO**AMYLASE 400**	Saccharifying glucoamylase or amyloglucosidase from *Aspergillus niger*	• **Maximizes the conversion of starch into fermentable sugars** • **Reduces residual carbohydrates** • **High degree of attenuation**
PROTO**ZYME**	Bacterial Neutral Protease from *Bacillus subtilis*	• **Allows the use of higher levels of adjuncts in the mash** • **Increases soluble protein** • **Enhance yeast vitality during fermentation** • **May improve downstream processing efficiencies**
CHILL**ZYME**	Protease from the plant *Carica papaya*, Best added to the cold conditioning tank	• **Prevents the formation of protein-tannin complexes** • **Reduces the risk of chill hazes in packaged beers leading to longer shelf-life**
ALPHA ACETOLACTATE DECARBOXYLASE - **ALDC**	Decarboxylase from *Bacillus licheniformis*, should be added at the start of fermentation	• **Reduces cold conditioning time** • **Prevents the formation of diacetyl**
Aromazyme	Food-grade enzyme preparation from *Aspergillus niger* containing β-glucosidase enzymes that are capable of hydrolyzing the glycosidic bonds, liberating aromatic monoterpenes and glucose.	• **Increase the diversity of hop flavors and aroma** • **Enhance beer mouthfeel and drinkability** • **Slightly increase wort fermentability** • **Express more character from less sophisticated hop varieties**

Brewing beer is a complex process with many complex biochemical processes to control. Some of the largest and smallest brewing companies benefit from using **AB Vickers® enzymes** and tapping into the significant expertise and resources offered by our team of brewmasters, industry experts, and R&D capabilities. **For every step in the brewing process, there is an AB Vickers® enzyme available to improve process efficiency and consistency.**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REDUCE PROCESS COST
IMPROVE PROCESS EFFICIENCY
REDUCE BEER LOSSES
IMPROVE PRODUCT QUALITY