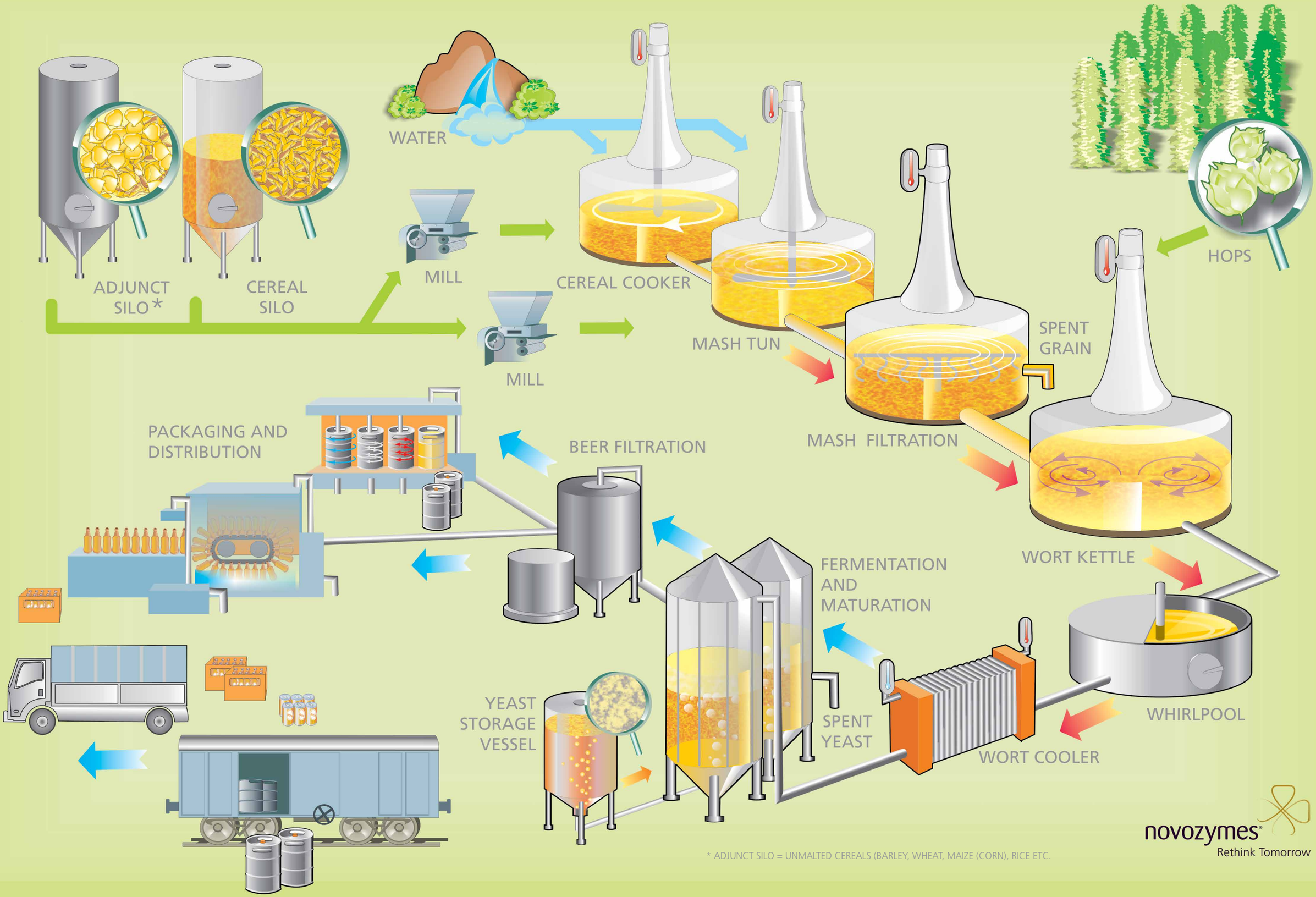
BREWING

novozymes®
Food & Beverages

Brewing Handbook

Version 1 2013

Rethink Tomorrow



* ADJUNCT SILO = UNMALTED CEREALS (BARLEY, WHEAT, MAIZE (CORN), RICE ETC.

novozymes®
Rethink Tomorrow

Foreword

Over 30 years ago Novo Nordisk A/S (today Novozymes) introduced industrial, microbially produced enzymes for the brewing industry. The first products were a bacterial protease and a bacterial alpha-amylase. Our offering for the brewing industry has since evolved into a comprehensive portfolio of enzymes combined with an extensive range of services to meet your needs, whether it is optimizing your products and production processes or developing innovative new products. Technical information on these enzyme products and how they can be used in brewing is available in a number of separate information brochures, lectures, and published articles. The Brewing Handbook brings the most relevant information together in one single publication for easier access and reference.

The purpose of this publication is to support breweries to improving the beer to improving the production economy, process control or beer quality.

Our belief is that quality solutions require both the product and the service to be outstanding. In line with globalization and the trend for customizing solutions, the demand for great service is steadily growing. And as that demand grows, our support is growing to ensure that we can continue to meet the needs of the brewing industry – and we see this handbook as an integral part of our support for the brewing industry.

CHAPTER 1.

INTRODUCTION

1.0 Enzymes in brewing

Our brewing solutions reduce costs, accelerate production processes and achieve consistently high beer quality while combining profitability with sustainability.

By enabling flexible raw material use and lowering energy consumption, enzymes are a tool for breweries to reach their strategic business goals. Novozymes' brewing solutions offer new opportunities to secure processes that are right the first time and that enable the creation of tasty and inviting brews for beer lovers around the world.

Our solutions cover a wide range of brewing applications:

- Raw material optimization
- Cost-effective cereal cooking
- Efficient wort separation and beer filtration
- Attenuation control and light beer production
- Fermentation control with Free Amino Nitrogen (FAN) optimization
- Diacetyl control

Our handbook examines each application area; introduces the benefits, background and mode of action of each solution, and provides practical advice and real examples. We hope it will become an invaluable aid for you when brewing!

1.1 Meeting your needs

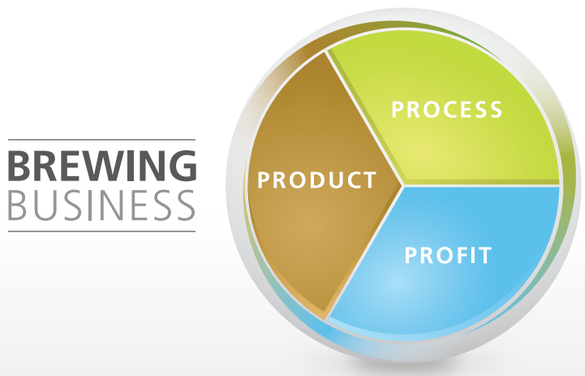
We offer you a comprehensive portfolio of enzymes combined with an extensive range of services with a shared goal – to support you to innovative ways to optimize your products, processes and profits. Working together, we can help your current product portfolio cater more distinctly to local consumer needs. We can also help secure right-first-time processes with a variety of raw materials, and ensure the most profitable route to your high quality beer.

Together we can unlock opportunities that secure the future of your brewing business.

- **Optimize your process**
  Throughout the brewing process there are many opportunities to optimize without compromising quality. We offer a variety of solutions that ensure your processes are right the first time and assist you in finding excellent new ways to utilize your capacity. At the same time, we can help you save energy and water – no matter which raw materials you're using.

- **Optimize your profits**
  Enzymes are much more than a process aid and can also become a strategic tool. For example, enzymes make it possible to utilize local raw materials, which can not only reduce your input costs but can also support the local economy. Enzymes give you the flexibility to rethink the brewing process, including in regions where alternative local raw materials present tough processing challenges.

- **Optimize your products**
  Enzymes make it possible to efficiently produce a variety of great-tasting beers and other cereal-based beverages. Collaborate with us to explore the possibilities for current product optimization and new product development.



1.2 Quality matters

Novozymes is committed to exceeding your expectations. Reliability and consistency are an integral part of who we are. We have systems for assessing and approving suppliers, and our IT systems ensure traceability of our products from supplier to you. Our long tradition of working actively with health and safety issues ensures that our products are safe to use – and safe to handle. We use safe production strains and development programs, including the toxicological testing of our enzymes which minimize any risk. We have acquired our safety expertise through decades of producing enzymes and share it with our customers through safety and warning labels and Material Safety Data Sheets (MSDS).

Our global business is covered by ISO 9001, and we also hold the ISO 22000, FSSC and AIB certifications for plants producing a wide range of food and beverage enzymes, including internationally recognized kosher and halal compliant products. Our solutions are approved by all relevant authorities and international committees.

As brewing is a sensory business where consumers judge beer one serving at a time, we ensure the consistent quality of your brands by producing the majority of our brewing enzymes in compliance with BrewQ specifications. This means that they are additionally analyzed according to Analytica-Microbiologica-EBC 2005 Section 4.6.1.

1.3 Sustainability – truly better business

Novozymes' solutions deliver savings – whether it is raw materials, time, energy or water, and can empower you to upgrade your social sustainability profile too. We believe that you, like us, understand that true progress cannot be achieved at the expense of the world around us. That is why sustainability is at the core of everything we do: our solutions and our business strategy. We strive to lead by example by integrating sustainable solutions into our own organizational practices as well as those of our external partners, seeking innovative partnerships with our customers, NGOs, governments, and the general public.

Novozymes also applies efficient technology to manufacture food and beverage enzymes because it provides benefits over traditional enzyme solutions. Not only do these benefits include reduced energy and water consumption, but they also ensure consistent quality, better use of raw materials, and less waste. As a result, people around the globe can benefit from better and safer products produced with far less impact on the environment.

In the brewing industry, solutions such as Novozymes Maturex® secure shorter maturation time which in turn leads to energy savings – as does Novozymes Termamyl® through facilitating shorter cereal cooking cycles. With Novozymes Ondea® Pro or Novozymes Ceremix®, you can instantly achieve excellent raw material flexibility and more sourcing options through benefiting from brewing with alternative raw materials. Depending on your brewery's location and local raw material availability, you could have the option of sourcing barley, cassava, sorghum etc. Not only does this deliver cost savings, it also enables you to support local farmers, local communities and their economies.

1.4 Why Novozymes?

With a solid, global base of experts, there is always someone close by to support you in implementing and optimizing our solutions to fit your needs, conditions and raw materials. We have large Technical Services centers in Denmark, Switzerland, Russia, South Africa, Malaysia, USA, India, Japan and China; bases from which trial support and application recommendations can be offered. Our unique global distribution set-up secures product availability in any location. We're looking forward to working with you to meet the future needs of the beverage markets.

For information about our solutions and services, visit:

- **Our microsite – www.brewingwithenzymes.com**
- Our company website – www.novozymes.com
- Your Customer Center – www.mynovozymes.com
- Novozymes' Food & Beverages Focus magazine – www.focusonline.novozymes.com
- Novozymes' Food & Beverages app – http://app.novozymes.com/mobile
- foodandbeverages@novozymes.com, or speak to your Novozymes representative.

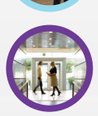
Production

R&D

Sales offices

Major distribution partners

Technical Service centers



Global marketing

CHAPTER 2.

RAW MATERIAL OPTIMIZATION
PART 1

2.0 Introduction to segment and key benefits

Regional availability, cost and grade, as well as brewer and consumer expectations have always influenced the selection of the brewing raw materials. However, increasing cost pressure in the industry has led to further constrained adjustments in beer recipes over the last couple of years, with more focus on cost effective and sustainable alternatives. The industry is also challenged by seasonal and regional availability, fluctuation in price and quality caused by climatic conditions during cultivation and harvest. As a consequence, there is generally a need for stronger strategic focus on raw materials sourcing. Exogenous enzymes have regularly been established in brewing to balance processability, increase yield and assure wort and beer specifications. Broadly speaking, even higher flexibility in the raw material sourcing is desirable to compensate for variability as well as fluctuations in the grain market and raw material quality.

Novozymes' products are developed to work either in synergy with the existing enzyme systems in the various grains (barley, malted barley, wheat etc.), or to enable the degradation and utilization of cereals beyond the traditional malt-based enzyme configuration. To ensure optimal processability and fermentability, different enzyme products containing glucanases, xylanases, proteases, amylases, pullulanases (limit dextrinase) and lipase activities are optimally combined according to the properties of the relevant raw materials. The individual enzymes in Novozymes' products are developed to fill in what the natural enzymatic environment is lacking under the specific brewing conditions (substrate specificity, pH and temperature).

Key benefits

Utilizing maize (corn), rice and sorghum in a cereal cooking setup

- Achieve faster and advanced viscosity reduction and increased extract yield in your cereal cooking step with Termamyl
- Optimize your liquefaction temperature and reduce your energy consumption
- Guarantee an efficient sorghum utilization and extract yield by combining Termamyl with Novozymes Neutrase® before cooking

Processing malted cereals and barley, wheat and other alternative raw materials

- Improve mash separation and beer filtration with the use of Novozymes Ultraflo®
- Optimize your processability, starch degradation and FAN release with Ceremix
- Adjust your fermentability regardless of the raw material choice with Novozymes Attenuzyme® for attenuation control and Neutrase to optimize your FAN levels
- Add Novozymes Fungamyl® BrewQ to the mash or at the beginning of fermentation to control your degree of fermentation, primarily due to an increase of maltose
- Utilize the full potential of alternative raw materials without compromising processability and fermentability with Novozymes Ondea® Pro

The limitations to raw material choice and processability have expanded significantly over the last years by the use of exogenous enzymes. Traditionally, high portions of well modified malted barley needed to dominate the brewing recipes to achieve sufficient yield, efficiency and quality. Novozymes' exogenous enzymes are selected according to cereal-specific substrates and the relevant pH and temperature optima.

Processing up to 100% under modified malt, barley or sorghum, as well as including more than 60% wheat, rice and maize (corn), are globally well-established approaches today. However, raw material optimization is not only about including more un-malted cereal in the recipes, but rather about achieving high consistency and efficiency in production and beer specifications without compromising quality. In general, Novozymes works to address customer needs and enable the brewing industry to drive a raw material agenda.

2.1 Core enzyme application

The quick recipe guide for your raw material optimization

Table 2.1-1 shows an overview of recipe opportunities and the recommended enzyme application to reach standard processability and fermentability. The focus of the enzyme application is to support the cytolytic, amylolytic and proteolytic degradation within an efficient mashing process and without compromising yield. If, on top of the raw material optimization, the Real Degree of Fermentatiion (RDF) specification is increased, a dosage of 0.05 to 0.1 kg of Attenuzyme Pro per ton of grist enables an RDF increase by approx. +1% (Max 72-74% RDF).

Malted barley	Barley	Wheat	Rice/ Maize (corn)	Sorghum	Ultraflo® Max	Ceremix® Plus MG	Ondea® Pro	Termamyl® SC DS	Attenuzyme® Pro	Neutrase® 1.6 L BrewQ
100					0.10-0.15					
80	20				0.12-0.18	0.10-0.25		Optional		
60	40				0.12-0.18	0.25-0.60			0.10-0.35	
40	60						0.6-1.2			
20	80						1.2-1.8			
0	100						1.8-2.2		Optional	
80			20		0.12-0.15			0.17-0.25*		
60			40		0.12-0.15			0.17-0.25*		0.25-0.50
80		20			0.12-0.18	0.10-0.25				
60		40			0.15-0.25	0.25-0.70				
40		60			0.15-0.25		1.2-1.5			
60				40				0.17-0.25*	0.20-0.50 or Fungamyl BrewQ 0.5-1.0 kg/ton	0.15-0.30*
20				80				0.17-0.25*	0.20-0.50 or Fungamyl BrewQ 0.5-1.0 kg/ton	0.15-0.30*
50	20		30		0.12-0.18	0.25-0.50		0.17-0.25*		
30	50		20				0.6-1.2	0.17-0.25*		
30	40	30					1.2-1.8			

* Dosage per ton of adjunct in the cereal cooker

Table 2.1-1. Examples of potential recipes in % and recommended enzyme application in kg/ton of grist

2.2 Opportunities for individual raw material optimization

Malt-based recipes with minor barley inclusion

To improve lautering performance and beer filtration also on well-modified malt and to increase the extract yield by approximately 1%, Ultraflo Max is recommended in all recipes.

For recipes containing 100% malt or small replacements by barley or wheat of up to 20%, the main focus of the enzyme application is on the cyctolytic degradation of cell wall components like β-glucans and arabinoxylans. Ultraflo Max contains highly efficient glucanase and GH-10 family xylanase activity. A dosage of 0.10 to 0.15 kg/ton of total grist is sufficient.

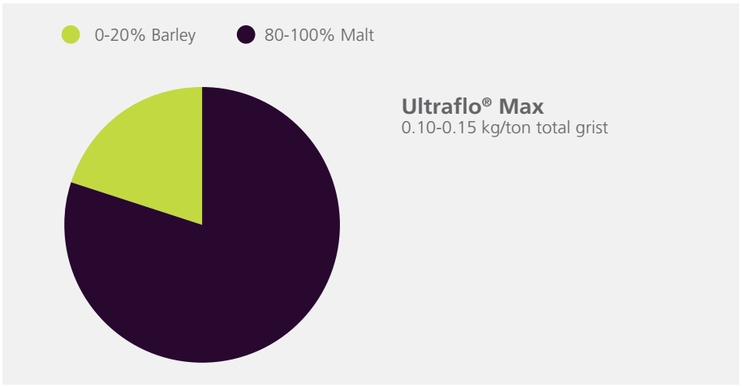


Fig. 2.2-1. Enzyme recommendation for malt-based recipes with minor barley inclusion

Depending on the malt quality and wort specifications, small dosages of Ceremix Plus MG and Attenuzyme Pro can already significantly improve the overall brewing performance as shown in Table 2.2-1 and 2.2-2.

Raw materials: 85% malted barley + 15% raw barley	Ceremix® Plus MG (kg/ton grist)	Ultraflo® Max (kg/ton grist)	Attenuzyme® Pro (kg/ton grist)
Reference	-	-	-
Application example	0.10	0.12	0.05

Table 2.2-1. Example of effective enzyme treatment on 85% under modified malt and 15% barley

Raw materials: 85% malted barley + 15% raw barley	Filtration (ml/10')	Extract (°P)	β-Glucan (16.0°P)	FAN (16.0°P)	Viscosity (16.0°P)	DP 1 (%)	DP 2 (%)	DP 3 (%)	DP 4/DP4+ (%)	Ferment-ables (%)	Expected RDF
Reference	43	15.86	1049	192	2.516	17.8	42.5	13.9	25.8	74.2	66.8
Application example	67	17.15	139	213	1.951	22.6	42.4	13.5	21.5	78.5	70.7

Table 2.2-2. Results of wort analysis after applying Novozymes Ultraflo® Max, Novozymes Ceremix® Plus MG and Novozymes Attenuzyme® Pro on 85% under-modified malt and 15% barley

Malt-based recipes including rice or maize (corn)

Processing high gelatinizing adjuncts like maize (corn) and rice in a cereal cooker with 0.18-0.20 kg of Termamyl SC DS per ton of adjunct provides a fast and effective viscosity break and forms the basis for effective starch saccharification. The Termamyl SC DS amylase is not dependent on the calcium concentration as alternative heat-stable amylases. Further, there is the opportunity to optimize the liquefaction temperature to ca. 85°C and still increase your extract yield. In combination with Ultraflo Max, at a dosage of 0.10 to 0.15 kg/ton of malt, you can achieve high processability and a very robust brewing set-up.

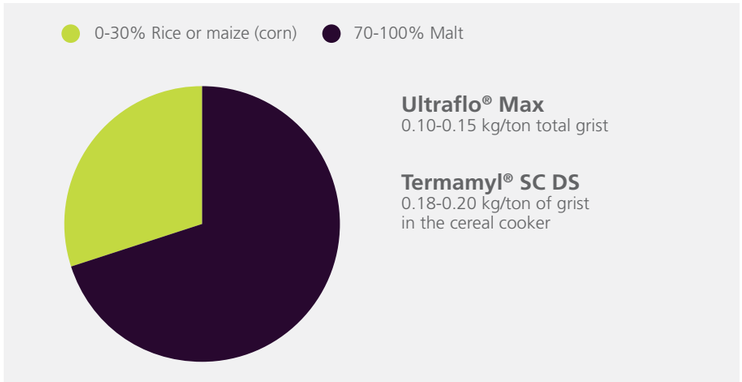


Fig. 2.2-2. Enzyme recommendation for malt – based recipes including rice or maize (corn).

Malt based recipes with high levels of alternative raw materials and adjuncts

Utilizing high amounts of under modified malt, or malt in combination with high portions of barley, rice or maize (corn) can impact sufficient FAN supply for the yeast as well as lead to limited diastatic power during mashing. This would lead to extract losses and poor fermentability. On top of Ultraflo Max at 0.10 to 0.15 kg/ton of malt and barley and 0.18-0.20 kg of Termamyl SC DS per ton of adjunct in the cereal cooker, it is recommended to use approximately 0.25-0.70 kg of Ceremix Plus MG per ton of barley.

Depending on the malt quality an additional dosage of 0.25 kg Ceremix Plus MG per ton of malt compensates a lack in malt modification and assures high processability and fermentability.

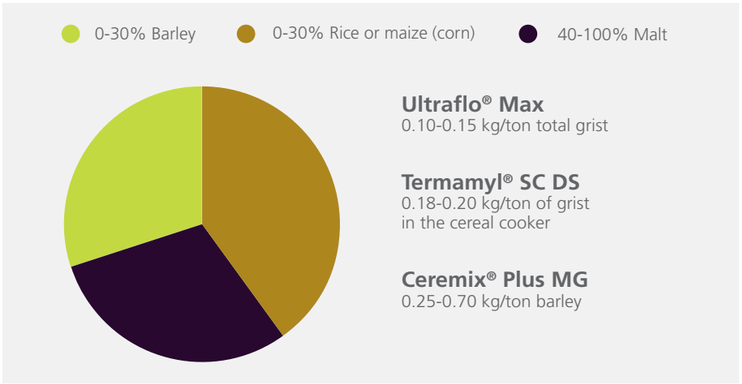


Fig. 2.2-3. Enzyme recommendations for malt-based recipes with higher portions of alternative raw materials and adjuncts

Barley based recipes

Using the full potential of exogenous enzymes you can create recipes with up to 100% barley. However, any ratio of barley, wheat and malt can be processed efficiently. Ondea Pro enables brewers to brew maltose-based wort with standard fermentability and similar processability compared to using high portions of malt. The present pullulanase, amylase and protease activities in Ondea Pro ensures sufficient starch and protein degradation in synergy with the β-amylase and peptidases of the barley. The glucanase and xylanase components enable sufficient cell wall degradation and low viscosity. The lipase activity significantly improves the turbidity during lautering.

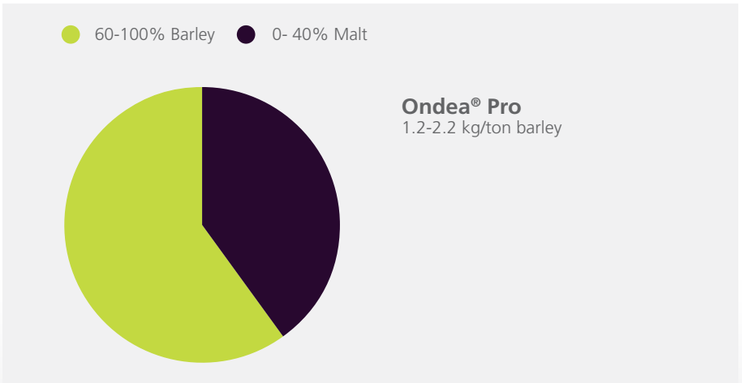


Fig. 2.2-4. Enzyme recommendation for barley-based recipes

Depending on the final raw material choice, it is recommended to use 1.2 to 2.2 kg of Ondea Pro per ton of barley. Tables 2.2-3 and 2.2-4 show three different recipes that use the full potential of Ondea Pro for highly cost-effective and good quality wort production.

	Barley (%)	Wheat (%)	Malted barley (%)	Ondea® Pro (kg/ton of grist)
Application example A	100	0	0	2000
Application example B	50	35	15	1500
Application example C	35	50	15	1500

Table 2.2-3. Different recipes using the full potential of Novozymes Ondea® Pro for highly cost-effective and good quality wort production

	Filtration (ml/10')	Extract (°P)	Turbidity (NTU)	β-Glucan (16.0°P)	ar-Xylan (16.0°P)	Viscosity (16.0°P)	DP 1 (%)	DP 2 (%)	DP 3 (%)	DP 4/ DP4+ (%)	Fermentables (%)	Expected RDF
Application example A	42	15.03	12	64	215	1.942	8.8	46.7	18.5	26.0	74.0	66.6
Application example B	55	15.67	14	56	236	1.937	10.0	50.4	17.2	22.4	77.6	69.9
Application example C	54	15.88	17	58	240	1.944	9.5	53.1	17.1	20.2	79.8	71.8

Table 2.2-4. Results of wort analysis after applying Novozymes Ondea® Pro on mixes of barley, wheat and malt

Use the Novozymes' enzyme toolbox to drive your individual aw material agenda

In general, Novozymes can support you in creating individual recipes with any raw material set-up to increase flexibility. The unique components in Ultraflo, Termamyl, Ceremix, Attenuzyme, Neutrase and Ondea Pro are designed to enable the utilization of wheat, rye, oat and triticale with up to 20-40%, or even higher in some cases. In these cases the remaining part is not limiting either and can be based on various mixtures of malt and barley.

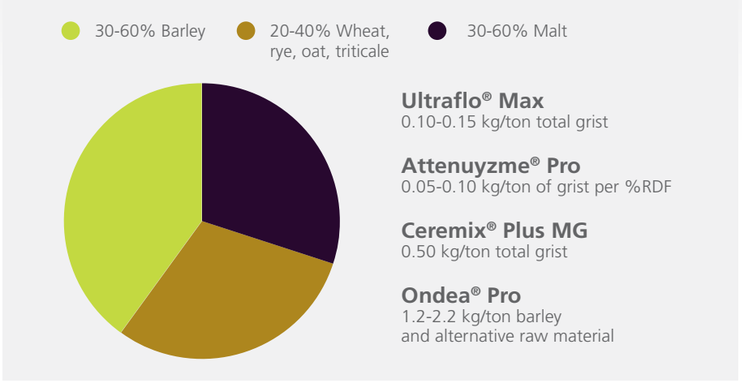


Fig. 2.2-5. Example of how to use Novozymes' enzymes to utilize individual raw material agenda

Additionally, Novozymes can provide ready to use solutions for cassava and sorghum. Depending on the available brewing equipment, these raw materials can be processed up to 100% and still achieve standard beer specifications. It might be necessary to include Neutrase and Fungamyl to support proteolysis and saccharification.

Recommended products	Benefits	Main enzyme activities
Termamyl® SC DS	• 0.5-2% higher extract yield • Faster viscosity break • No risk of starch retrogradation • Reduced risk of haze in final beer	α-amylase
Ultraflo® Max	• Best filtration with any malt and adjunct • Low viscosity • Reduced costs • High throughput	β-glucanase Xylanase
Ceremix® Plus MG	• High flexibility in malt, adjunct choice and adjunct inclusion rate • Low viscosity • High extract yield • Efficient filtration • High fermentability	β-glucanase Xylanase α-amylase Protease
Ondea® Pro	• High flexibility in malt, adjunct choice and adjunct inclusion rate • Low viscosity • High extract yield • Efficient filtration • High fermentability	β-glucanase Xylanase α-amylase Pullulanase Protease Lipase
Attenuzyme® Pro	• Consistent RDF control • Faster mashing	Glucoamylase Pullulanase
Neutrase® 1.6 L	• FAN optimization • Better starch degradation	Protease

Table 2.2-5. Recommended product range for raw material optimization

2.3 Background to application

To seize the cost saving opportunities that come with alternative raw materials and adjuncts in brewing, to drive sustainability in terms of local raw material sourcing, to create specific beer properties by using individual raw materials characteristics, or to level out inconsistencies in the raw material quality (including malt), the traditional enzyme source, malt, and the process that is based on it, can be the limiting factor. Either the enzymes are not sufficient in terms of temperature or pH characteristics, or the amount and function do not support the set-up of a modern raw material agenda. The following section describes the different enzyme systems used in brewing to fulfill the required processability and fermentability, and to reach the target quality specifications.

Cytolytic degradation to improve mash filtration performance, yield and beer filtration

The husk of barley and barley malt contains approximately 5-6% cellulose which works as a structure substance, but is widely inaccessible during the brewing process. However, the hemicellulose as principal matrix element of the cell walls in the endosperm consists of approximately 65% β-glucan and 25% pentosans. Both substances are critical to the brewing process in terms of starch utilization, viscosity and filterability, but this can effectively be addressed by using exogenous β-glucanases and GH-10 family xylanases. Fig. 2.3-1 shows the structure of the cell walls linked together by proteins in the middle lamella.

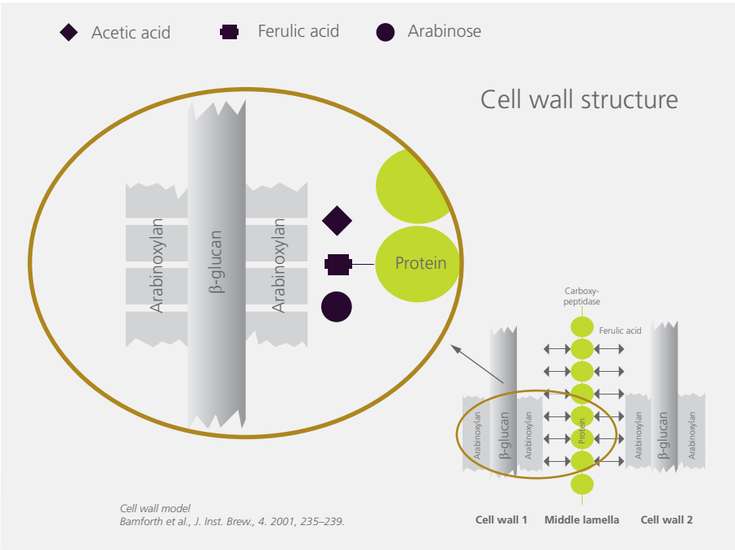


Fig. 2.3-1. Barley cell wall model

β-glucan degradation

β-glucan is a polysaccharide composed of D-glucose molecules with β-1,3 – and more frequently β-1,4-glucosidic bonds. The characteristic of the bond makes the β-glucan inaccessible for amylolytic enzymes like amylases or amyloglucosidases. The basic structure is shown in Fig. 2.3-2.

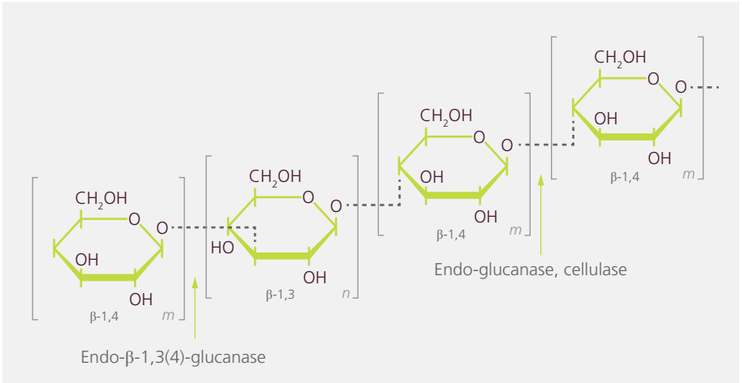


Fig. 2.3-2. Chemical structure of β-glucan

During the brewing process β-glucan with high water binding capacity is released. If not degraded sufficiently, β-glucan causes high wort and beer viscosity. β-glucan with increasing chain length in particular, causes a significant decreased mash and beer filtration performance. Amongst others long chain β-glucan underlies stretching during the process, for instance in pumps, which can result in additional windings into micelles that punctiliously block the filtration steps.

The relevant β-glucan degrading enzymes are the β-glucan-solubilase, the endo- and exo-β-glucanases as well as cellulases. The β-glucan-solubilase belongs to the enzyme class of esterase and dissociates the high molecular hemicellulose β-glucan from the proteins in the cell wall matrix. The optimum temperature of the endogenous β-glucan-solubilase is around 62-65°C and it is deactivated at 72-73°C.

The group of in-malt endo-β-glucanases consist of endo-β-1,4-glucanase, endo-β-1,3-glucanase and an unspecific endo-β-glucanase. The enzymes convert the insoluble β-glucan into soluble glucan and finally into glucan dextrins. The in-malt enzymes have a pH and temperature optima of 4.5-4.8 and 40-50°C. Higher temperature leads to a fast deactivation of this enzyme system. The exo-β-glucanases cut the β-glucan from the non-reducing end and form cellobiose. This reaction reduces the viscosity slowly.

The enzyme only degrades β-1,4-linkages and is already deactivated at temperatures above 40°C and therefore not relevant under normal brewing conditions.

The activity characteristics of the individual enzymes in terms of temperature and pH optima indicate that the in-malt cytolytic enzyme system does in most cases not have the optimal properties for the brewhouse process working at a pH range of 5.2-5.6 and mashing-in temperatures of 50-63°C.

Like the α-amylase, some of the cytolytic enzymes are not present in barley and are formed during the malting process. Before malting the barley contains the endo-β-1,4-glucanase, β-glucan-solubilase and the exo-β-glucanase. Even though the β-glucan-solubilase is present in the barley, the activity is significantly increased (up to five times) during malting, and will release more critical β-glucan at higher temperatures during mashing. Also the exo-β-glucanase activity is decupled during malting. The increase of the exo-β-glucanase, however, depends on the variety and climate conditions.

The exogenous β-glucanses in Ultraflo products can support or even substitute the enzyme system present in barley and malt in a significantly more sophisticated manner. Independently from the raw material set-up, a faster and advanced viscosity reduction results in high performing mash and beer filtration.

Arabinoxylan degradation

Similar to the β-glucan, the pentosans, especially the arabionoxylans, significantly impact the wort and beer viscosity and the performance in mash and beer filtration. The barley contains an analogue to the glucanases prevalent pentosan-solubilases, endo- and exo-xylanases.

The endo-xylanase cuts β-1,4-bonds independently of arabinose side chains and reduces the wort viscosity within an intensive mash regime. However, the temperature optimum is around 45°C, making the activity nearly irrelevant for modern brewing conditions. The exo-xylanase degrades the xylan from the end, but only if the substrate was already released because of the endo-activity. The remaining in-cereal cytolytic activities are limited and the activity increase minor during the malting process.

However, the effective degradation of the arabinoxylans by GH-10 family xylanases can in particular lower the viscosity and, in addition to better mash filtration, boost the beer filtration both in kieselguhr and membrane filtration systems.

Amylolytic degradation for maximum yield and controlling the degree of fermentation

The primary focus of the brewing process is starch conversion into fermentable sugar and dextrin. The amount of extract released from degradation of mainly starch and the final degree of fermentation forms the basis for the produced beer volume. Generally, cereal starch consists of 75% frequently branched amylopectin and 25% linear amylose, Fig.2.3.3. In traditional malt-based brewing, the starch hydrolysis are mainly transformed by the α and β-amylases. While in unmalted conditions, the β-amylase is already sufficiently present in most cereals like barley, wheat and sorghum, the α-amylase is formed de novo during malting. The intensity of the formation is highly dependent on the variety and malting conditions.

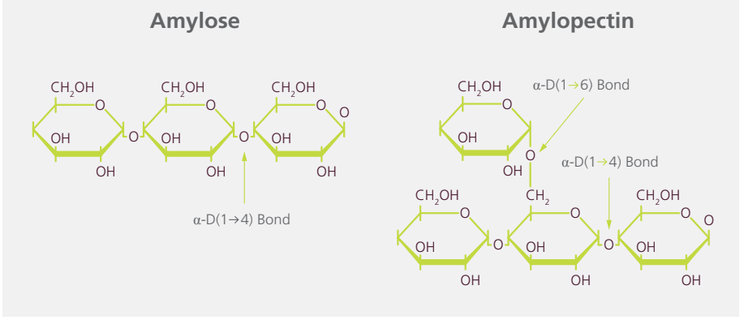


Fig. 2.3-3. Chemical structure of amylose and amylopectin

The two in-malt endo-α-amylases cut down the α-1,4-glucosidic bonds of amylose and amylopectin from the inside. The major products are dextrins. However, with increasing mashing time, the α-amylase can degrade the polysaccharides further to mono and disaccharides. In brewing, the temperature optimum of the α-amylase is in the range of 70-75°C. That is above the optimum of the β-amylase and partly below the gelatinization temperature of maize (corn), sorghum, cassava and rice. The pH optimum is at 5.6-5.8.

In contrast to the α-amylase, the β-amylase belongs to the exo-enzymes. The β-amylase degrades the amylose from the non-reducing end and cuts off maltose. If the glucose chain is unequal, the last three glucose units are not attacked and stay in the wort as maltotriose. The temperature and pH optima of the β-amylase under brewing conditions are 60-65°C and pH 5.6-5.8. Because of the conformation and properties of both amylases it is not possible to degrade all the dextrin into fermentable sugars. Even with a highly modified, enzyme rich malt and intensive mashing, the real degree of fermentation is limited to approximately 72%.

The gelatinization temperature of brewing raw materials and adjuncts is decisive for using either the infusion or decoction process. Raw barley and wheat, as well as triticale, oat and rye, have a similar gelatinization temperature to barley malt and can be liquefied and saccharified in infusion mashing. However maize (corn), rice, sorghum and cassava need to be gelatinized and liquefied at higher temperatures in a separate cereal cooking process. Table 2.3-1 shows the gelatinization temperatures of the common brewing raw materials and adjuncts.

Raw material	Barley	Barley malt	Wheat	Maize/ corn	Rice	Sorghum	Cassava
Gelatinization temperature (°C)	60-65	61-65	55-65	64-82	68-84	68-75	64-76

Table 2.3-1. Gelatinization temperatures of brewing raw materials and adjuncts

Traditionally, high gelatinizing cereal processing is conducted by using a part of the malt loading in the decoction step, conducting an intensive rest at 72°C and a "cooking" step between 90-100°C.

Exogenous enzymes can certainly support and partly substitute the malt-based enzyme set-up in the amylolytic degradation. The α-amylase activity can be totally replaced by Novozymes' Termamyl solutions, both in the cereal cooking step and in the mashing process. Because of specific screening for enzymes with an activity optima relevant for brewing, these heat-stable amylases even lead to a faster viscosity break and yield increase. The properties of the exogenous enzymes also provide the opportunity to lower the maximum adjunct liquefaction temperature and optimize the temperature profile and time in the cereal cooker.

The β-amylases in a standard brewing process are not economically substitutable with exogenous enzymes because the activity in the cereal is more than sufficient. However, the exogenous enzyme tool box can add functionality. In general, the applications provide enhanced consistency in regular beer production, the opportunity to brew low carb or strong beers with the raw material load similar to regular lager beers.

More specifically, glucoamylases like the ones from Novozymes' Attenuzyme solutions help increase and control the RDF beyond the malt-based limits for production of light or strong beer. In fact, the resultant wort is based on glucose instead of maltose. However, applying a pullulanase (limit dextrinase) like the Novozym 26062 can both increase the amount of maltose in wort in synergy with the cereal β-amylase and speed up the saccharification process significantly for optimal capacity utilization.

Proteolytic degradation for high fermentability, yield generation and improved processability

One brewing priority of the protein hydrolysis is to secure the fermentability. Especially when using large amounts of alternative raw materials and adjuncts, the free amino nitrogen (FAN) content becomes critical even if an advanced yeast management system is in place. However, the proteolytic degradation during malting and mashing not only releases amino acids and dipeptides as yeast nutrients, but also enables and support access to starch. The protein in the endosperm is linked to the β-glucan and pentosans in the cell walls surrounding the starch kernels. This becomes most relevant for high protein wheat and especially sorghum processing, which has large amounts of kafarin in the endosperm.

The various proteolytic enzymes can also be grouped into endo-peptidases and exo-peptidases. While the endo-peptidases break down high molecular oligopeptides from the inside, the exo-peptidases are responsible for releasing single amino acids and dipeptides. Fig. 2.3-4 shows the principle in protein hydrolysis.

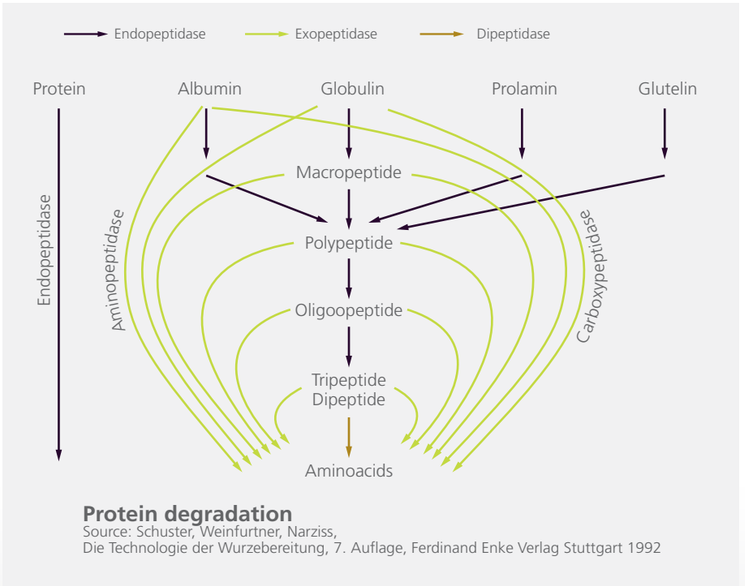
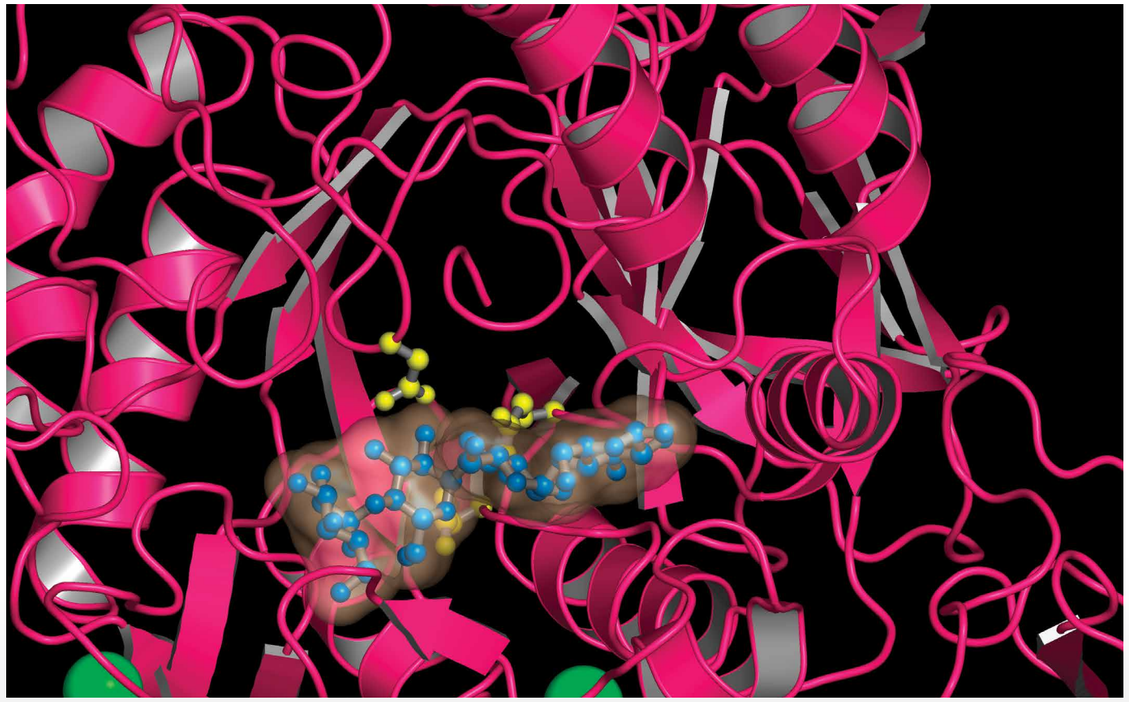


Fig. 2.3-4. The schematic protein degradation

The bulk of the endo-peptidases belong to the group of sulfhydryl peptidases while the minor part is activated by metal. The individual peptidases work specifically on certain amino acid bonds. Partly the endo-peptidase activity is already present in the raw barley. However, the activity is increased approximately five times during germination, which indicates a bigger need for exogenous proteases when high amounts of alternative un-malted raw materials are processed.

The exo-peptidase can be separated into carboxypeptidases cutting off amino acids from the carboxyl end of the proteins and aminopeptidases attacking the proteins from the end of the free amino group. While the carboxypeptidase activity is increased during malting, the aminopeptidases are to a large extent already present in the un-malted cereal.

The traditional way to increase FAN is to use over modified malt and a long protein rest during mashing. Both methods, however, have often shown to be insufficient to give an acceptable FAN level when using high amounts of adjuncts. Novozymes' Neutrase products are working in synergy with the in-cereal amino and carboxypeptidases to provide more amino acid during an efficient mashing.



2.4 Action of the enzymes

The provided endo-β-1,3(4)-glucanases (E.C. 3.2.1.6) in Ultraflo solutions hydrolyze β-1,3- or β-1,4-linkages in β-D-glucans as shown in Fig. 2.4-1. These enzymes are more heat-stable than the malt glucanases. This allows sufficient β-glucan degradation during the saccharification rest at 63°C and a further hydrolysis when the malt β-glucan-solubilase is still active at higher mashing temperatures.

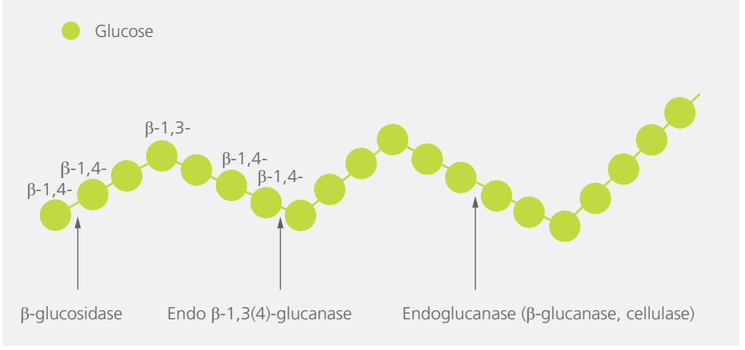


Fig. 2.4-1. Structure of β-glucan and the effect of β-glucanases

The provided endo-1,4-xylanases (E.C. 3.2.1.8) in the Ultraflo products hydrolyze β-1,4-D-xylosidic linkages in arabinoxylans as shown in Fig. 2.4-2. In this respect, it is important to utilize the full potential of the GH-10 family xylanases provided in Ultraflo Max. This xylanase breaks down the xylose backbone even if arabinose units are collaterally linked. This enables a faster and more far-reaching viscosity reduction for a significantly improved beer filtration of up to 30% compared to standard GH-11 family xylanases.

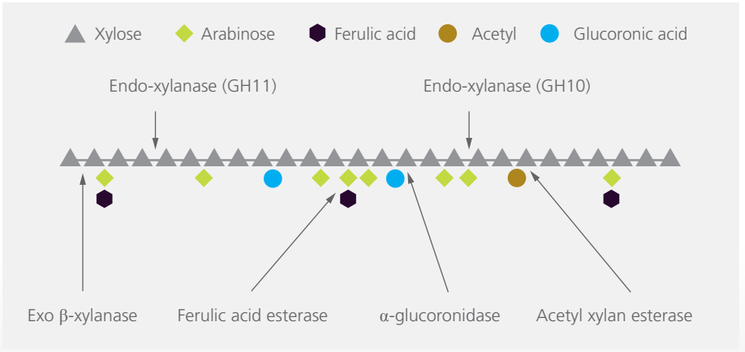


Fig. 2.4-2. Structure of arabinoxylan and the effect of xylanases

The amylolytic enzymes provided in the Termamyl, Ceremix, Attenuzyme and Fungamyl products contain three major activities:

- The endo-α-amylase (E.C. 3.2.1.1) hydrolyzes α-1,4-D-glucosidic linkages in starch polysaccharides
- The glucoamylases (E.C. 3.2.1.3) hydrolyze α-1,4- and α-1,6-D-glucosidic linkages at the non-reducing ends of polysaccharides
- The pullulanase (E.C. 3.2.1.41) hydrolyzes α-1,6-D-glucosidic linkages in pullulan, amylopectin and glycogen. The enzyme activity is basically similar to plant-derived limit-dextrinase.

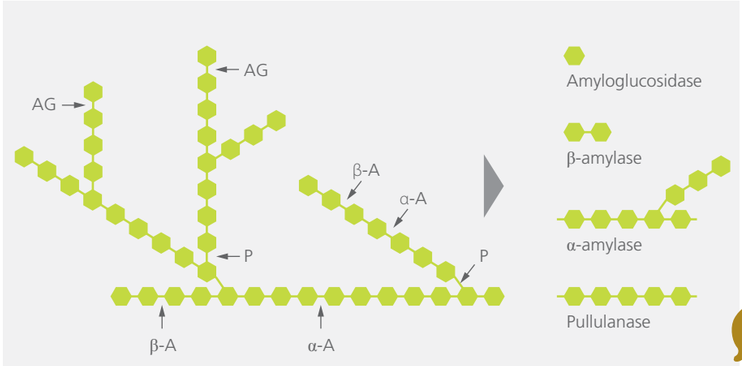


Fig. 2.4-3. Schematic reaction of enzymatic starch hydrolysis

The metallo endo-protease (E.C. 3.4.24.28) provided in Novozymes' Neutrase solutions hydrolyze internal peptide bonds as shown in Fig. 2.4-4. This reaction generates more substrate for the in-cereal peptidases releasing higher amounts of FAN (Free Amino Nitrogen).

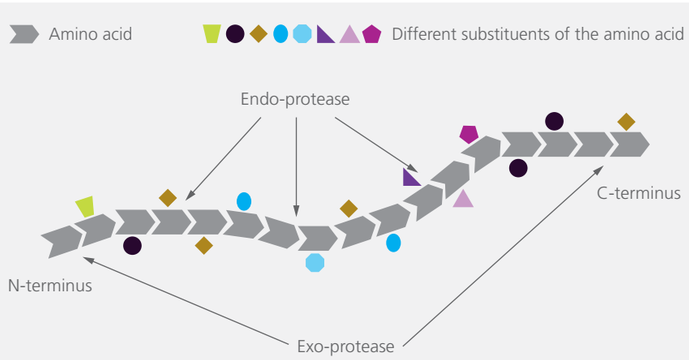


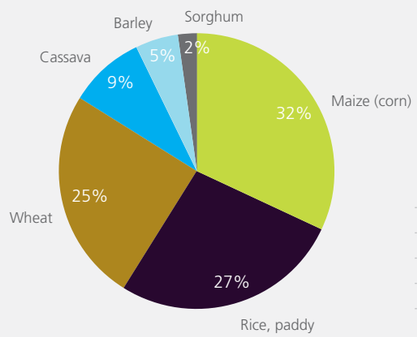
Fig. 2.4-4. Protein structure and the effect of endo and exo-proteases

CHAPTER 3.

RAW MATERIAL OPTIMIZATION
PART 2

3.0 Agricultural overview on brewing raw materials and adjuncts

Beside the major brewing raw materials barley and barley malt, various starch sources like maize (corn), rice, wheat, sorghum, rye and cassava, as well as syrups and sucrose from both sugar cane and sugar beet, are widely used in the brewing industry. Raw material crops are handled on a global trade market. Price and availability are greatly influenced by an increasing demand owed to the growing population and beer consumption worldwide. Crop distribution is regionally diverse, as described in the following sections, but on a global scale, the barley crop of approximately 125 million MT p.a. accounts for only 5% of the global production of relevant grains. Fig. 3.0-1. shows the global production of potential raw materials in the brewing industry.



Raw material

Raw Material	Production in Mio. MT (2010)
Maize (corn)	840
Rice	700
Wheat	650
Cassava	230
Barley	120
Sorghum	60

Fig. 3.0-1. Global production of potential brewing raw materials

Distribution is dominated by the production of maize (corn), rice and wheat. These grains are mainly used for food, feed and partly for the production of bioethanol. Sugar cane and sugar beet are not displayed. However, with approximately 2 billion MT, this crop is the largest source of carbohydrate globally. While sugar beet is also grown in Europe, sugar cane is mainly planted and harvested in South America and Asia.

Regionally the dominating raw materials are changing significantly. As displayed in Fig. 3.0-2, in Europe the major crops are wheat, maize (corn) and barley while the Americas, and in particular USA, grow maize (corn) in significant amounts.

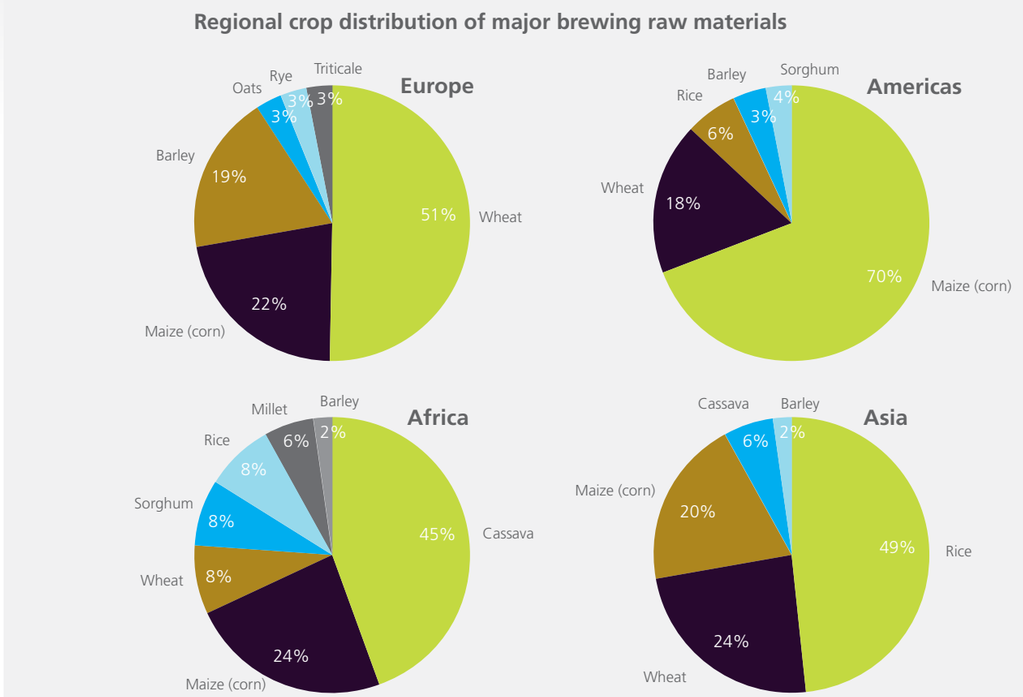


Fig. 3.0-2. Regional crop distribution of major brewing raw materials

With 120 million MT, Africa represents approx. 50% of the global production of cassava and this accounts for 45% of the starch source produced on the African continent. Other major producers of cassava include USA and India. Furthermore, maize (corn), sorghum, millet, wheat, rice and smaller quantities of barley are relevant in Africa to cover the demand for food, feed and beverage industries. Various crops like barley, rice and sorghum are mostly limited to specific countries or small regions. Sorghum is mainly produced in Nigeria, Ethiopia and in the Sudan area, whereas barely is grown in significant amounts in Morocco, Algeria and Ethiopia. In contrast, South Africa's main crop is maize (corn). Rice is the most important food source in Asia followed by wheat and maize (corn).

The cost of rice has increased significantly over the last decade. Rice has been used in large quantities for beer production. However, cost pressure has led producers to look for alternative brewing materials. Even though cassava and barley play a minor role in the Asian agriculture sector, they have become more in focus in the brewing supply chain. To cover the demand of the Asian brewing industry, Oceania and Europe are important sources of barley, barley malt and wheat which are all imported in a significant amount.

3.1 Individual grain considerations and characteristics

Barley, malt and wheat

Barley, either as a direct ingredient used in the brewing process or as a raw material for malting, is the most important source for the brewers. Fig. 3.1-1 illustrates the ten largest barley producers globally, with a production of more than 80 million MT of barley. These countries are harvesting approximately two-thirds of the worldwide barley production. Fig. 3.1-1 shows that Europe is the largest producer of barley. Together with Canada and Australia, Europe represents the heart of the global barley supply for the brewing industry.

Fig. 3.1-1. Barley crop distribution (2010)

Malting barley

The difference between barley growing countries and main malting barley suppliers is marginal. Still Europe is accountable for approximately 44% of the worldwide supply see: Fig. 3.1-2. South America, with 13%, has a larger share of the malting barley market although it is not among the top 10 barley growing nations. The largest beer market, Asia, is only producing approximately 6% of the global malting barley.

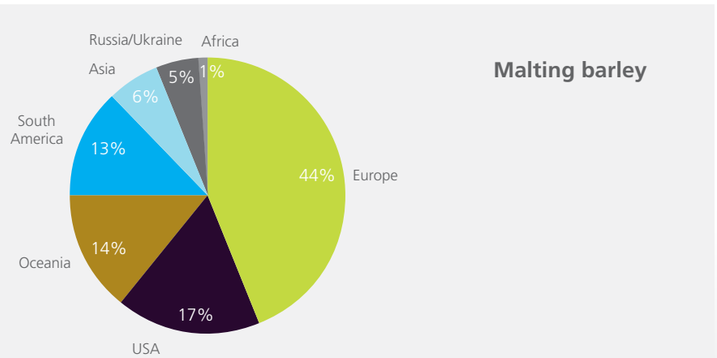


Fig. 3.1-2. Malting barley crop distribution (2010)

Despite the large amounts of adjuncts in beer recipes in Asia the brewing industry has to deal with a regional undersupply and needs to import barley and barley malt from Europe, Oceania and North America.

The barley malt trade market reflects the malting barley agricultural situation. Traditional barley growing countries also have major malting capacity. Main exporters are France, Canada, Australia and Belgium. In contrast, Brazil and Japan are the main importers of malt. This is demonstrated in Fig. 3.1-3.

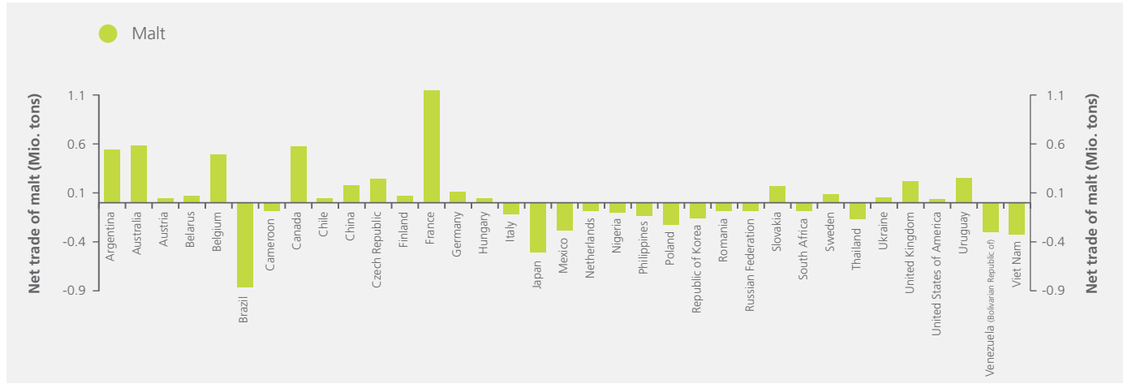


Fig. 3.1-3. Net malt trade of major brewing countries

Fig. 3.1-3 also shows that China is neither importing high quantities of malt nor growing sufficient amounts of barley. Furthermore, the local barley crop might be partly inaccessible because of distance and infrastructure.

The Chinese malt supply is not directly linked with malt imports, but malting is conducted locally with imported barley. Approximately 2.4 million MT of barley is imported to address the demand of 445 million hectoliters. This is mainly due to the fact that the barley husk can be used as a natural filter cake for the traditional lautering process. Barley is characterized by a complex composition of starch, proteins, lipids and cellulosic components as well as pentosans, in particular arabinoxylans β-glucans as demonstrated in Table 3.1-1.

Barley composition*

Starch	Protein	Pentosans & β-glucans	Cellulose	Lipids	Ash
69-73%	9-13%	10%	5-6%	2.5%	3%

*Table 3.1-1. Average composition of brewing barley (*on dry matter)*

The gelatinization temperature of barley is in the range of 60-65°C. Both barley and produced malt belong to the fraction of low gelatinizing starch sources. In combination with the natural enzyme system which is present in the raw barley, and additionally produced during malting, these properties are the foundation for the common temperature profile in an infusion mashing process.

With more than 635 million MT p.a., wheat is the third largest global grain crop. The wheat crop shows an opponent agricultural distribution in terms of major growing areas. In the wheat market, the ten biggest wheat producers are accountable for more than 450 million MT, growing approximately 70% of the crop worldwide. Asia, and in particular China and India, with their high population, play an important role in global wheat production. This is demonstrated in Fig. 3.1-4.

Despite the apparent availability in this region, utilization in brewing is limited, even though process adjustments and enzymatic treatments have made it possible to use. USA, Russia, France and Germany complete the list of major players. In general, wheat or malted wheat is traditionally used for brewing in France, Germany and Belgium.

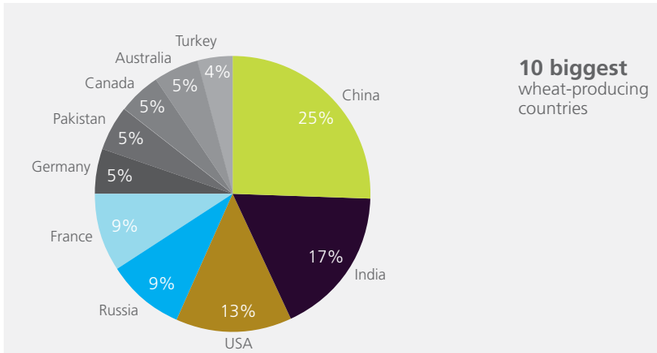


Fig. 3.1-4. Wheat crop distribution (2010)

To plant and breed wheat for brewing is not a local point of the agricultural agenda. The amount of wheat that is used in brewing is marginal compared to the food sector. The main challenge of insourcing wheat for beer production is the food industry's deviating focus on protein levels. While for the food industry, high protein content is equal with high, first grade quality, brewers are looking for wheat with less proteins – which in the sense of food production, is not first grade quality. However, this is an opportunity for economically attractive sourcing on the regular wheat market. Wheat has a similar composition to barley, but does not contain husks after threshing.

Therefore the starch content per ton of traded material is higher and the cellulosic components are significantly decreased. Table 3.1-2 shows the average composition of brewing wheat.

Wheat composition*

Starch	Protein	Pentosans & β-glucans	Cellulose	Lipids	Ash
72-77%	11-15%	7-8%	2-3%	2%	2%

*Table 3.1-2 Average composition of brewing wheat (*on dry matter)*

Compared to barley, the pentosans in wheat contain higher amounts of arabinoxylans. Combined also with higher protein content, these components increase the need for exogenous xylanase and protease activity during mashing to ensure processability and yield.

Rice, maize (corn) and sorghum belong to the high temperature gelatinizing starch sources which are extremely relevant to global production and/or as regionally dominating crops. These grains are regularly processed in a cereal cooking step using heat stable α-amylases for liquefaction. Maize (corn) contributes to global cereal production as a major crop with approximately 840 million MT. This is not only due to the very high yields farmers can achieve by planting it. Compared to wheat or barley, the yield is nearly double and has increased significantly over the last few years, indicating that grain breeders have an enormous focus on this raw material. Maize (corn) is also the raw material for bioethanol production, especially in USA. This is reflected in the crop distribution of the 10 biggest maize (corn) producers globally who are accountable for approximately 80% of global maize (corn) production; see Fig. 3.1-5. Out of these countries, USA is harvesting 47% followed by China and Brazil with 26% and 8%. In Europe, France is the largest maize (corn) producer, but only contributing to 1.5% of global production.

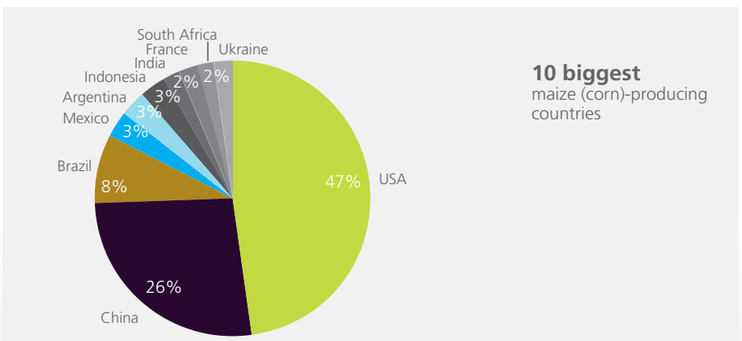


Fig. 3.1-5. Maize (corn) crop distribution (2010)

Maize (corn) is regularly harvested with a moisture content of 25-30% and subsequently dried to <15% moisture for storage and transportation to minimize metabolic losses, equal to other cereals. The high lipid content of maize (corn) can impact the beer quality negatively in terms of foam and flavor stability. Most of the oil is located in the embryo. So for brewing, the maize (corn) kernel is usually de-germinated. The composition of untreated maize (corn) is displayed in Table 3.1-3.

Maize (corn) composition*

Starch	Protein	Pentosans & β-glucans	Cellulose	Lipids	Ash
73-77%	8-11%	5-6%	4%	5-6%	1.5%

*Table 3.1-3. Average composition of maize (corn) (*on dry matter)*

The protein content of maize (corn) is not significantly accessible during mashing and it doesn't contribute to the nitrogen supply of the yeast during fermentation. The pentosan and β-glucans (0.5-1.3%) is not extracted during the brewing process. That makes the amount of corn in brewing recipes limited to 50-60%. In breweries, maize (corn) can be used as corn grits, flakes, pre-gelatinized grits or in the form of maize (corn) syrup and starch.

Rice is the most widely consumed staple food for a large part of the world's population, especially in Asia and the West Indies. Worldwide rice production is close to 700 million MT p.a. More than 85% of annual production is grown by the 10 biggest producers.The major contributors in the Asian region are China and India (see Fig. 3.1-6). The next largest producers are Indonesia, Vietnam and Myanmar. The only two non-Asian countries in the top ten are Brazil and USA, accountable for less than 3.5% of global rice crops. Alongside food production, broken rice is usually used for beer production. Rice is the adjunct with the highest gelatinization temperature; up to 85°C. However, rice also has the highest naturally occurring starch content; 84-88%.

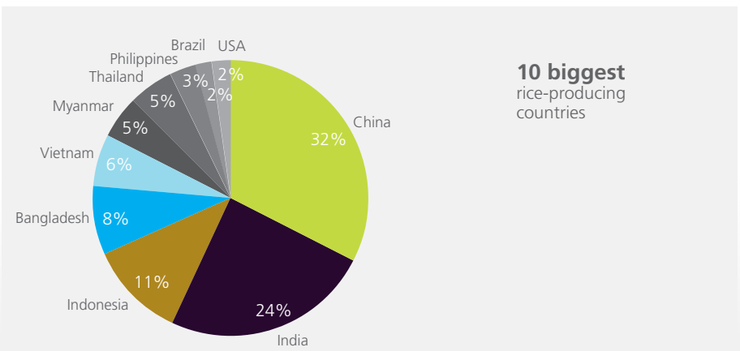


Fig. 3.1-6. Rice crop distribution (2010)

Table 3.1-4 displays an average composition of rice. Similar to maize (corn), rice protein is not accessible during mashing and the nitrogen nutrients need to be sourced from barley, barley malt or wheat.

Rice composition*

Starch	Protein	Pentosans & β-glucans	Cellulose	Lipids	Ash
84-88%	5-9%	2%	2.0-2.5%	0.5%	0.5%

*Table 3.1-4. Average composition of rice (*on dry matter)*

Sorghum is cultivated in warm climates. For the brewing industry, you mainly find this in Africa. Nevertheless, the biggest producers of Sorghum are USA, Mexico and India, Fig. 3.1-7. As for Africa, this genus of grass species is mainly grown in Nigeria, Ethiopia and Sudan and is greatly important for beer production there. Sorghum accounts for only 2% of the worldwide grain crop production. Approximately 55 million MT of Sorghum are produced globally. Sorghum can generally be separated in two groups: the white sorghum and the yellow or colored sorghum species. Colored sorghum is rich in polyphenols making it bird resistant. It is becoming uninteresting for the brewers in term of taste and quality. White sorghum is used for malting or directly for beer production. Pure sorghum beers are produced in Africa. The gelatinization temperature is comparable with maize (corn) and is in the range of 68-75°C. It is normally processed in a cereal cooking step.

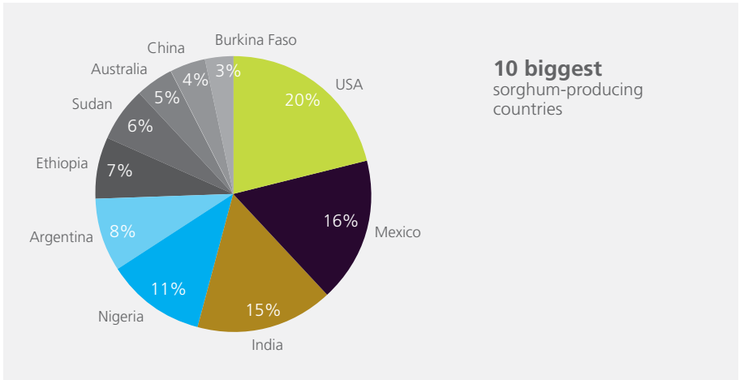


Fig. 3.1-7. Sorghum crop distribution (2010)

The 11-12% protein in sorghum and sorghum malt can be solubilized during mashing and is available as yeast nutrients in fermentation. Approximately 110 mg/100 ml FAN, which is half of regular malt brews, can be achieved. Table 3.1-5 shows the composition of sorghum.

Sorghum composition*

Starch	Protein	Pentosans & β-glucans	Cellulose	Lipids	Ash
78-80%	11-12%	2%	6.8%	3.7%	1.5%

*Table 3.1-5. Average composition of sorghum (*on dry matter)*

It is important to use an exogenous protease to increase the utilization of the starch in cereal cooking. The starch content is comparable with barley, but the diastatic power is slightly low, Table 3.1-6.

Amylase activity in unmalted and malted cereal grains:

Grain	α-amylase*	β-amylase*
Barley	0.62	350
Cassava	nd	nd
Corn	nd	nd
Malted barley	280	920
Rice	0.12	57.4
Sorghum	0.36	252
Wheat	0.42	454

* Units/g nd – not detected.

Table 3.1-6. Average amylase activity in brewing raw materials

Oats, rye and triticale belong to the so called secondary crops that are not yet in the focus of the brewing industry. However, these grains are today used for some special beer brands that use the properties of these raw materials to position the beer with healthy attributes. Oat and rye can be traced back to the Stone Age and are well known for their modesty in terms of soil and weather. These crops obtain reasonable yields even in cooler regions. However, triticale is a hybrid based on rye and wheat. Breeders combined the modesty of rye with the agriculture yield and quality of wheat. In recent years, a considerable amount of new triticale varieties has been evaluated and registered.

Based on their characteristics oats, rye and triticale could be utilized as raw materials for brewing, especially in Northern and Eastern Europe. Fig. 3.1-8 shows that the crop is actually dominated by Poland, Germany and Russia which are accountable for 50% of the global production. France, Canada, Australia and Belarus are also growing a significant amount of these grains.

This makes local, sustainable sourcing an opportunity for either specific brands, or for part of the extract in overall production.

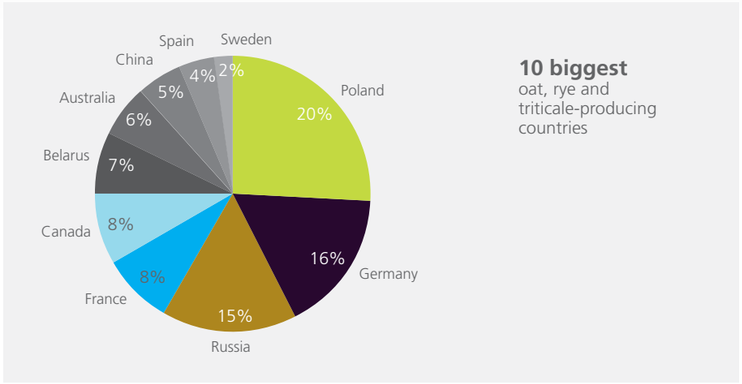


Fig. 3.1-8. Oat, rye and triticale crop distribution (2010)

Today, oats are mainly used for animal feed and breakfast cereals, while rye and triticale are already widely used in distilling and the production of bioethanol. Table 3.1-7 shows an overview of the average composition of these grains.

Oat composition*

Starch	Protein	Pentosans & β-glucans	Cellulose	Lipids	Ash
72-76%	12-14%	5-6%	4-5%	7%	3%

Rye composition*

Starch	Protein	Pentosans & β-glucans	Cellulose	Lipids	Ash
72-74%	11-14%	6-7%	5-6%	2%	1.5%

Triticale composition*

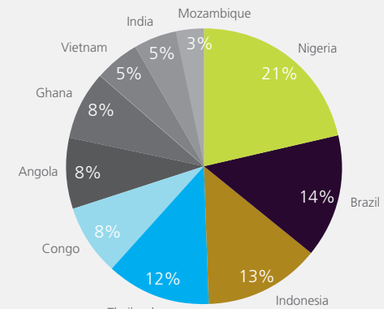
Starch	Protein	Pentosans & β-glucans	Cellulose	Lipids	Ash
68-72%	11-13%	8-9%	4-5%	1-2%	2.1%

*Table 3.1-7. Average composition of oat, rye and triticale (*on dry matter)*

The Cassava root can be seen as the rising star of raw material for brewing due to its economic attractiveness in the substitution of other starch sources and to the ability to support brewing groups reaching their social sustainability targets. Cassava can support production for low cost segments, or replace expensive sugar or syrups in all beer segments.

Cassava accounts for 45% of the relevant crops in Africa and 6% of the agriculture production in Asia, but it is not relevant to the Americas or Europe. However, in addition to Nigeria, Indonesia and Thailand, Brazil is the second largest cassava producer globally (see Fig. 3.1-9).

In the case of cassava, global production does not mirror the global trade market. Thailand is the dominant supplier to world markets accounting for approximately 80% of global trade. Vietnam, Indonesia and a few countries in Africa and Latin America share the remaining market. This situation is mainly caused by the lagging behind of industrial cassava manufacturers rather than local processing in Africa.



10 biggest
cassava-producing countries

Fig. 3.1-9. Cassava crop distribution (2010)

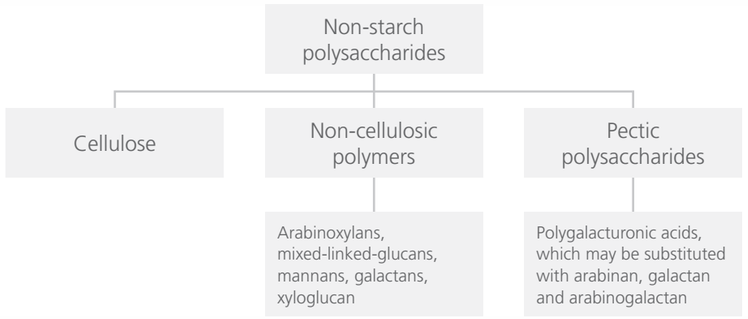
Cassava is already widely used within many large industries in food, feed and bioethanol production. However, the processing of cassava needs to start straight after the harvest to avoid rotting. Processed starch or cake can then easily be integrated into the brewing supply chain. The gelatinization temperature is slightly higher than that of barley. Therefore, the cassava needs to be liquefied in a cereal cooking step beforehand. Table 3.1-8 shows a typical composition of cassava chips.

Cassava chips*

Starch	Protein	Pentosans & β-glucans	Cellulose	Lipids	Ash
86-90%	3-5%	1.1%	2.8%	1.3%	1.5%

*Table 3.1-8. Average composition of cassava chips (*on dry matter)*

The content of Non-Starch Polysaccharides (NSP), after Mingan Choct et al unpublished data 2013, in the table below, is also very valuable when deciding which enzyme-solution to choose:



Parameters	Chips	Pellets	Pulp
Total starch (g/kg)	751.4	678.3	373.5
Amylose (g/kg)	173.6	180.2	113.2
Amylopectin (g/kg)	578.2	497.6	260.9
Amylose / Amylopectin	0.29	0.36	0.43
Resistant starch (g/kg)	389.7	310.8	592.1
Free sugars (up to 10 monosaccharides) (g/kg)	18.89	25.69	12.96
Soluble NSP (g/kg)	8.28	8.27	27.90
Insoluble NSP (g/kg)	42.19	53.30	97.37

Table 3.1-9. The carbohydrate contents and property of starch granule for the cassava products (Choct et al unpublished data 2013)

CHAPTER 4.

COST-EFFECTIVE CEREAL COOKING

4.0 Introduction to segment and key benefits

Starch containing adjuncts and cereals must be processed in such a way that the starch is gelatinized and liquefied. Gelatinization is the swelling of the starch granules whereas liquefaction is the debranching process that breaks down the intermolecular bonds of starch; both amylose and amylopectin, in the presence of excess water and heat in order to engage more water, also known as hydrolysis. The gelatinization process is necessary for liquefaction in order to reduce the viscosity, and to make the starch susceptible to the enzymatic hydrolysis taking place during saccharification with malt enzymes and/or exogenous enzymes.

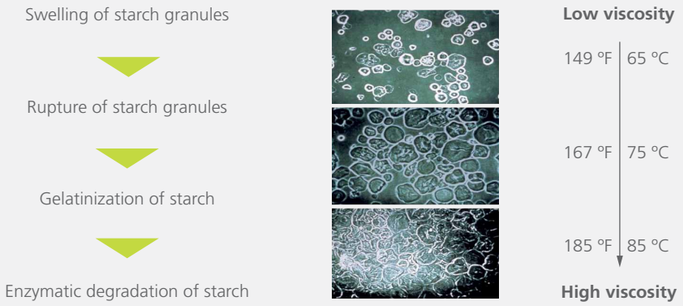


Fig. 4.0-1. The gelatinization and liquefaction process – a schematic approach. After gelatinization, the viscosity is lowered due to the action of α-amylases resulting in an enzymatic degradation of starch, also known as liquefaction

The gelatinization temperature is, for example, dependent on the cereal or adjunct type, variety and growing conditions. When the starch gelatinizes, the starch granules rupture, releasing the dextrins for enzyme attack. This is demonstrated in Fig. 4.0-1. If the starch is not properly hydrolyzed during this process, there is a risk that the starch molecules will retrograde, or reform into a crystalline structure, on cooling and the starch which is not sufficiently broken down in the brewhouse, will cause yield decrease, problems during mash filtration and beer filtration.

Adjuncts containing starch with low gelatinization temperatures, < 65°C, such as barley, wheat and oats can be mashed together with the malt in the mash-tun. Other adjuncts, such as maize (corn), rice, cassava and sorghum, have significantly higher gelatinization temperatures, and therefore must be processed in a separate vessel, a cereal cooker, for gelatinization and liquefaction.

This is of course very dependent on the actual starch type and quality. Typically, temperatures between 85°C and 100°C are used in the cereal cooker. At these temperatures, malt α-amylases are deactivated. Therefore, exogenous heat-stable α-amylases are frequently used in brewing with these types of adjuncts. Novozymes offers four heat-stable alpha-amylase products:

Termamyl Classic, Termamyl BrewQ, Termamyl SC and Termamyl SC DS.

Key Benefits

- Faster and more consistent liquefaction
- Lower mash viscosity, which means easier wort production
- No danger of resistant or retrograded starch formation, or insufficient saccharification
- Reduced processing costs through more efficient liquefaction and increased yield of up to 1%
- Improved flexibility in using various cereal grain adjuncts

4.1 Core enzyme application

Termamyl is added to the cereal cooker with the adjunct at the start of liquefaction, or to the mash-tun with the adjunct at the start of liquefaction in a single-vessel brewhouse. Standard dosages to be applied are as follows, dependent of the liquefaction time:

- Termamyl Classic – 0.50 kg/ton adjunct; 50-150 ppm Ca^{2+} needed
- Termamyl BrewQ – 0.25 kg/ton adjunct; 50-150 ppm Ca^{2+} needed
- Termamyl SC – 0.37 kg/ton adjunct; No calcium dependency (<20 ppm)
- Termamyl SC DS – 0.19 kg/ton adjunct; No calcium dependency (<20 ppm)

4.2 Background to application

As mentioned above, all cereals /adjunct types have different gelatinization temperatures. Table 4.2-1 summarizes the gelatinization temperatures of most common cereal grains.

Cereal/adjunct:	Gelatinization temperature		To be mashed in the	
	°C	°F	mash tun	cereal cooker
Barley	60-65	140-150	x	
Cassava	64-76	147-169		x
Maize (corn)	64-82	147-180		x
Oat	53-60	127-140	x	
Rice	68-84	154-183		x
Rye	57-70	135-158	x	
Sorghum	68-75	154-167		x
Triticale	61-64	142-147	x	
Wheat	55-65	131-149	x	

Table 4.2-1. Gelatinization temperatures of common brewing cereal grains; average values and process

4.3 Action of the enzymes

The viscosity of gelatinized starch is reduced through the action of an endo-α-amylase, which breaks down the α-1,4- linkages in amylose and amylopectin (liquefaction). α-amylase is an endo-enzyme that specifically "attacks" α-1,4 glucose linkages and is thermostable. The -1,6- linkages are bypassed and are not hydrolysed. This enzyme also reduces the viscosity of starch suspensions and produces dextrin's which are compounds that contain up to twelve glucose units. α-amylase is largely absent from unmalted barley and wheat. Review the raw material optimization section on page 46. Table 3.1-6, for further information.

Reducing the concentration of these solubilized, large molecules reduces the viscosity of the resulting wort. If the chains remain long, the chance of retrogradation is higher. The retrograded starch precipitate represents a loss in extract, and can appear in the finished beer as haze.

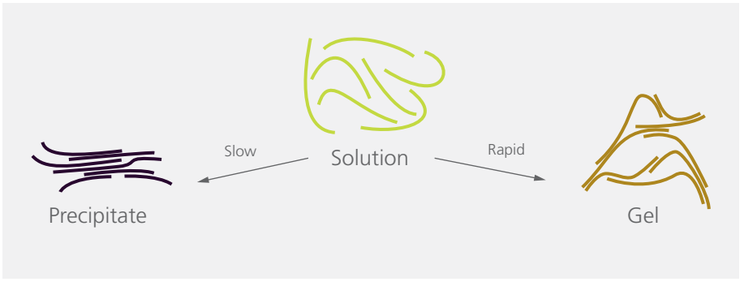


Fig. 4.3-1. Mechanisms of retrogradation of the linear starch fraction

In the brewing industry, liquefaction is traditionally done using the α-amylase of the malt enzyme complex in the following way:

- Part of the malt (5-10% of the total malt quantity in the cereal cooker) is mashed together with the adjuct
- The water-to-grist ratio should be between 3:1 and 4:1
  1. Mashing in at 60-65°C
  2. Rest at 72-75°C for 15 minutes
  3. Heating to 100°C
  4. Rest at 100°C for 20 minutes before cooling and mixing into the malt mash

Because malt α-amylases are not active at temperatures higher than approximately 75°C, it is quite common practice to introduce a break before 75°C for approximately 15 minutes to allow for enzymatic activity to occur. This gives the mashing/time profile, in the cereal cooker, as shown in Fig. 4.3-2.

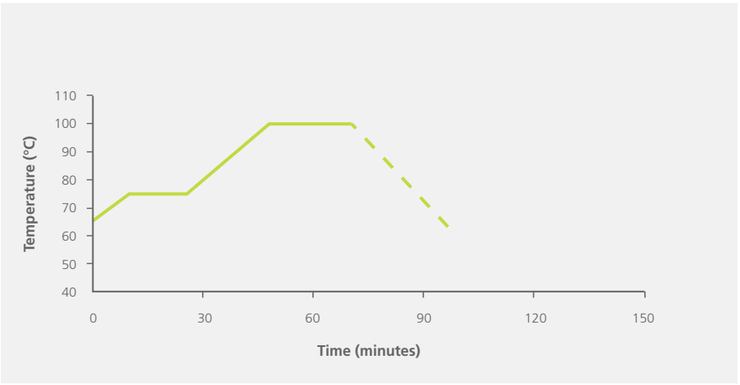


Fig.4.3-2. Adjunct liquefaction with malt

Due to the low heat stability of the malt α-amylase, relatively high viscosities due to inadequately liquefied starch are observed. It should be noted, that if malt is used for adjunct liquefaction, all the other enzymes (β-amylase, α-amylase, β-glucanase, limit dextrinase, protease, peptidase) are destroyed very quickly during this process and are lost for later utilization during mashing and mash filtration.

Liquefaction process with Novozymes Termamyl®

Liquefaction with Termamyl is a simpler and faster process when compared to liquefaction with malt enzymes. The rest at ca. 72°C can be omitted, allowing for rapid heating and shorter overall cereal cooking time.

Using Termamyl, the cereal mash viscosity is greatly reduced, thereby preventing formation of retrograded starch. This means that the yield from using adjuncts are ensured when comparing to using malt as the liquefaction material.

The yield can be increased by more than 1%. While it is necessary for malt α-amylase to work at its limit for temperature stability, Termamyl maintains very high stability throughout the temperature range applied.

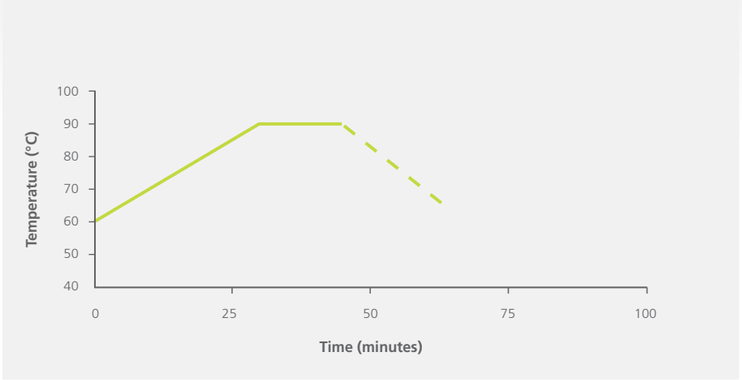


Fig.4.3-3. Liquefaction with Novozymes Termamyl®

Fig. 4.3-3 is showing a normal mashing regime in the cereal cooker with the addition of Termamyl instead of malted barley. In Fig. 4.3-4 a normal viscosity graph is shown during the cereal cooking process with a peak during gelatinization.

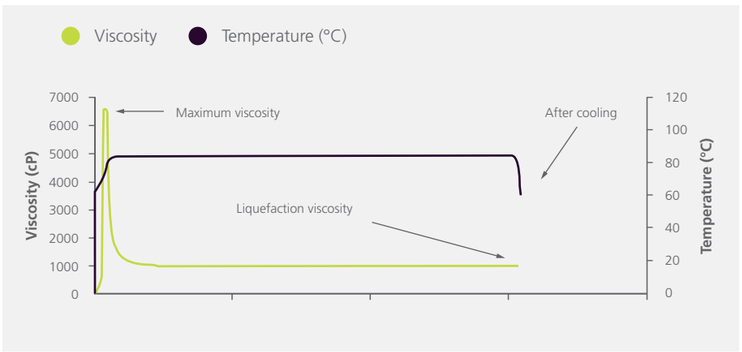


Fig. 4.3-4. demonstrates the viscosities during a cereal cooking step after the addition of Novozymes Termamyl®

It is also a versatile solution in terms of the thickness of the mash, because of Termamyl's exceptional liquefaction power which is approximately 200 to 300 times higher per kg than that of malt. Thicker mashes can be operated without the risk of working with high viscosities. This, in combination with the fact that 100 kg of malt is replaced with 0.19 kg Termamyl SC DS, enables smaller mashes, which is invaluable when balancing volumes and temperatures while working with high proportions of adjuncts. This versatility can also be used to increase brewhouse capacity. In addition to what is achieved by working with thicker mashes, the malt is replaced with adjuncts with higher extract values.

The process is also a more cost effective one, as all the malt can be utilized in the main mash. This safeguards the mashing operation and provides an improved wort, which reaches the correct and higher side of the desired attenuation range and increased FAN content.

Even so, there are still brewers who maintain that with good malt and standard amounts of adjuncts, the amount of malt enzymes available is so high that it does not matter that some are destroyed in the adjunct liquefaction process. However, the combined effect of efficient liquefaction and saccharification paving the way for better yield and brewhouse control should be sufficient arguments for exchanging malt with thermo-stable amylase.

4.4 pH and temperature curves

Fig. 4.4-1 and 4.4-2 show the activity of Termamyl BrewQ and Termamyl SC DS as a function of pH and temperature. The corresponding curves for Novozymes BAN® (a bacterial alpha-amylase from Bacillus Amyloliquefaciens) are shown for comparison:

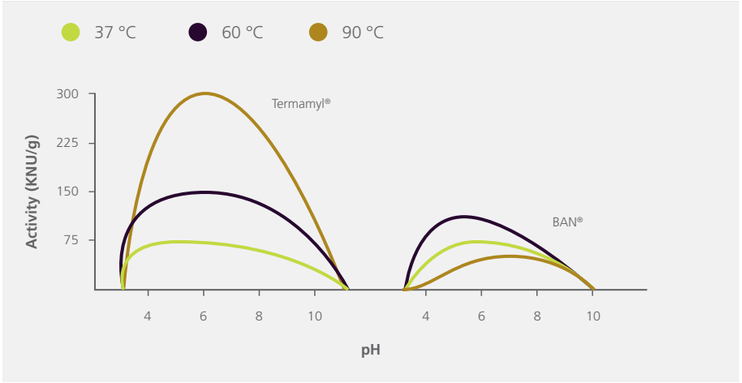


Fig. 4.4-1. Influence of pH on the activity of Novozymes Termamyl® BrewQ at different temperatures. (Activity curves for the conventional alpha-amylase Novozymes BAN® shown for comparison) Substrate: 0.5% soluble starch Stabilizer: 30-60 ppm calcium

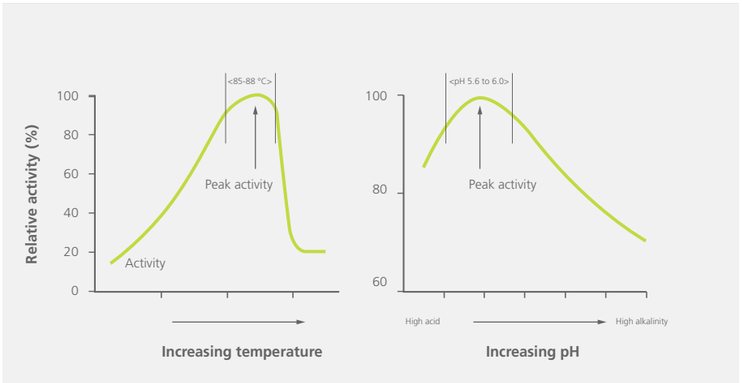


Fig. 4.4-2. pH and temperature curve for Novozymes Termamyl® SC

4.5 Practical applications

Rice and maize (corn) grits and purified starch from maize (corn) and cassava
Rice and maize (corn) are widely used adjuncts that require separate liquefaction. From laboratory liquefaction trials – see Fig.4.5-1 and Table 4.5-1 below – very low viscosities can be achieved with Termamyl.

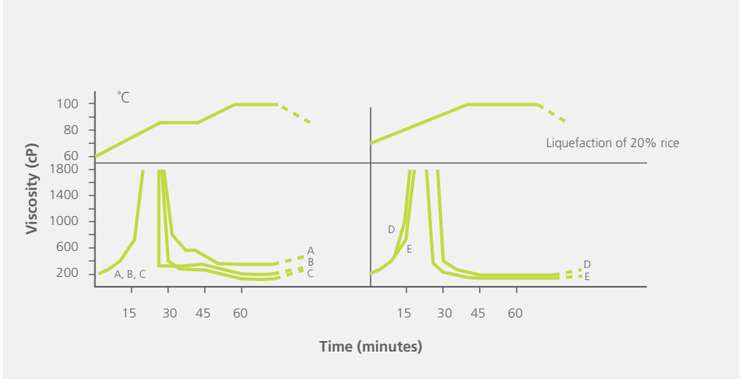


Fig. 4.5-1. Illustrates calcium concentrations utilized as a function of the enzyme dosage and the resulting viscosities

	A	B	C	D	E
Termamyl® 120 L, Type L*	0.025	0.05	0.025	0.025	0.05
$Ca(OH)_2$ concentration*	0	100	0	100	100
** % of adjunct*	6.1	6.1	6.5	7.3	6.5

Table 4.5-1.

Based on this and other experiences, our recommendations for rice and maize (corn) liquefaction are as follows as an initial trial:

- Follow the liquefaction profile for maize (corn) with a minimum 15-30 minutes in the temperature range 85-95°C
- A Termamyl SC DS dosage of 0.2 kg/ton adjunct depending on the mill setting/grits size and liquefaction regime
- pH 5-6
- A water-to-adjunct ratio of 2.2:1 – 4:1
- For purified starch made of corn or cassava it is recommended to use the same process as for grits.

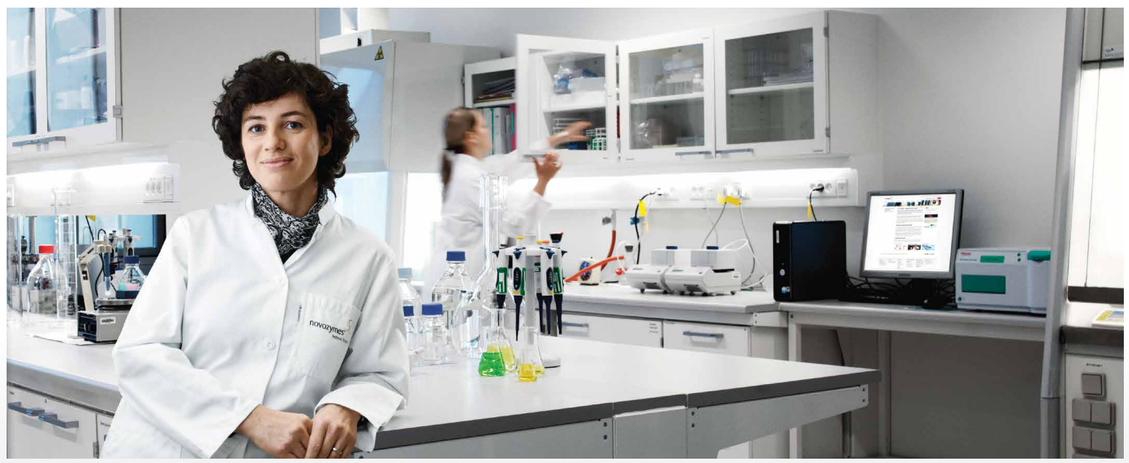
For the various forms of cassava (e.g. pellets and cake) please contact Technical Services at Norvozymes as the solution is dependent on the process conditions and equipment in use and on the starch quality delivered.

Sorghum
When using sorghum as an adjunct, higher dosages of Termamyl are recommended. The best choice for sorghum liquefaction is Termamyl SC DS, as it works at a much lower Ca^{2+} level, below 20 ppm, and offers better performance with respect to mash viscosity reduction and filtration compared to Termamyl Classic or Termamyl BrewQ.

Sorghum is characterized by having stronger cell walls captured in a protein layer and a higher content of glucans than most of the other cereal grains utilized for brewing. Based on the composition of the sorghum it is recommended to add 0.4 – 0.6 kg/ton sorghum of Neutrase 0.8 L together with Termamyl at the beginning of the liquefaction process, and include a 30 minute rest at 54°C before boiling, to aid the breakdown of the cell wall material in the sorghum. It is optional to use a β-glucanase, like Ultraflo Max, in case of reduced extract yield or insufficient liquefaction.

Nitrogen
When using higher amounts of adjuncts (>20%), worts with insufficient nitrogen (FAN) yeast nutrients may be the result. This can be counteracted by using a protease, such as Neutrase 0.8L /1.6 L, in the malt mash in order to extract more nitrogenous compounds from the malt. This topic will be discussed further in the relevant chapter of "Fermentation control with FAN optimization".

Inactivation
Ultraflo Max, Neutrase 0.8 L/1.6 L, Termamyl Classic, Termamyl BrewQ and Termamyl SC/DS will all be deactivated during a typical wort boil.



4.6 Enzyme data table

Novozymes Termamyl® Classic	
Declared enzyme	Thermostable α-amylase
Catalyzes the following reaction:	Hydrolyzes 1,4-α-glucosidic linkages in amylose and amylopectin. Gelatinized starch is rapidly broken down into soluble dextrins and oligosaccharides.
Declared activity	120 KNU_T/g
E.C/ I.U.B. no:	3.2.1.1
Physical form	Brown liquid
Production method	Submerged fermentation of a non-genetically modified microorganism of the Bacillus type. The enzyme protein, which in itself is not genetically modified, is separated and purified from the production organism.

Novozymes Termamyl® BrewQ	
Declared enzyme	Thermostable α-amylase
Catalyzes the following reaction:	Hydrolyzes 1,4-α-glucosidic linkages in amylose and amylopectin. Gelatinized starch is rapidly broken down into soluble dextrins and oligosaccharides.
Declared activity	240 KNU_T/g
E.C/ I.U.B. no:	3.2.1.1
Physical form	Brown liquid
Production method	Submerged fermentation of a genetically modified microorganism of the Bacillus type. The enzyme protein, which in itself is not genetically modified, is separated and purified from the production organism.

Novozymes Termamyl® SC or SC DS (double strength)	
Declared enzyme	Thermostable α-amylase
Catalyzes the following reaction:	Hydrolyzes 1,4-α-glucosidic linkages in amylose and amylopectin. Gelatinized starch is rapidly broken down into soluble dextrins and oligosaccharides.
Declared activity	120 KNU_S/g & 240 KNU_S/g
E.C/ I.U.B. no:	3.2.1.1
Physical form	Brown liquid
Production method	Submerged fermentation of a genetically modified microorganism of the Bacillus type. The enzyme protein, which in itself is not genetically modified, is separated and purified from the production organism.

Table 4.6-1. Enzyme data

CHAPTER 5.

EFFICIENT WORT SEPARATION AND BEER FILTRATION

5.0 Introduction to segment and key benefits

Efficiency and time in wort separation and beer filtration are key brewing parameters to secure capacity optimization.

Novozymes' range of filtration enzymes provide consistent, fast and efficient wort separation and beer filtration, resulting in maximizing the number of brews per day, and ensuring high volumes of beer per filter run.

The filtration enzymes include currently Ultraflo Max, Ultraflo L, Ultraflo XL and Finizym 250 L.

Key benefits

- Consistent and high speed of wort filtration
- Consistently high utilization of beer filtration equipment
- Consistently high brewhouse capacity
- Possibility to eliminate production variations due to varying quality of raw materials
- High flexibility in choice of mashing temperature profile
- Possibility to use High Gravity Brewing and Very High Gravity Brewing
- Secure minimal investment in brewhouse and beer filtration capacity
- Higher extract yield

5.1 Core enzyme application

The optimal working conditions for the Ultraflo series and Finizym 250 L are 45-70/75°C and pH 4.0-6.5.

Ultraflo is added to the mash-tun during mash-in, starting when ca. 1/3 of the grist has been loaded into the mash tun.

Finizym 250 L is added to the fermentor at the start of fermentation. Although, Finizym does not work at its optimal temperature the solution can still be effective due to the longer time of action in the fermenter.

- The recommended dosages for Ultraflo Max are:
  - 0.1 kg/ton when using well modified malt and 0.25 kg/ton when using barley (< 14°Plato)
  - 0.15 kg/ton when using well modified malt and 0.3 kg/ton when using barley (>14°Plato)
  - If very short mash filtration time is requested, trials have shown that higher doses can be effective
- The general dosage recommendations for Ultraflo L and Ultraflo XL are 25-50 % higher than for Ultraflo Max, but the exceptional low viscosity levels achieved when using Ultraflo Max will not be reached.
- Malt based on wheat, rye and sorghum will need up to 50% higher dosages of Ultraflo Max to deal with the high xylan content in both wheat and rye, and the more or less undegraded cell walls in sorghum malt. Raw grains from these cereals will also need up to 50% higher dosages of Ultraflo Max when compared with raw barley.
- The dosage recommendation for Finizym 250 L is 0.5 to 1.0 g/hl beer, when the treatment time is 2-5 days.

5.2 Background to application

Filtration and cell wall components

The efficiency of separating wort from the mash, and later on the efficiency of beer filtration, is highly dependent on the large molecules dissolved in the liquid. The high molecular weight molecules in question, mixed-linked 1,3-1,4 β-glucans and arabinoxylans, are constituent components of barley cell walls, as can be seen in Fig. 5.2-1. They are also present in other cereal grains, but in different amounts and ratios. Barley, oats and sorghum have more than twice as much β-glucan compared with xylans, while it is the opposite with wheat and rye. Maize (corn) and rice have only limited amounts of these compounds, so their contribution to filtration issues is not a factor. Please see the raw material optimization section for more information.

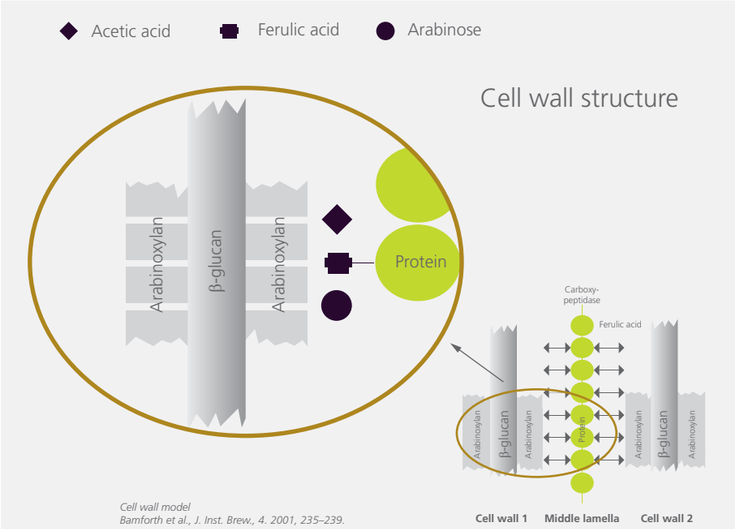


Fig.5.2-1. Barley cell wall model

β-glucans and arabinoxylans are very hydroscopic; they absorb water readily. They create high wort viscosity, reducing mash filtration speed dramatically. These components also become rather greasy when they absorb water, so they stick to other grain components and to filter aids and filter membranes. β-glucans and arabinoxylans can also stick to starch molecules, making them less available for enzymatic degradation, thus resulting in a lower brewhouse yield or can cause haze.

During the malting of barley, the cell walls are broken down, and most of the β-glucans are degraded to lower molecular weight, less viscous polysaccharides, as seen in Fig. 5.2-2. Arabinoxylans are not broken down to the same degree as β-glucans, so viscous polysaccharides from xylans still remain in wort and beer. The malt derived from β-glucanases and xylanases are not very heat stable as they are. They are inactivated at temperatures above 50˚C and will therefore not be active during saccharification". Solubilization of the cell wall components however, continues during saccharification, resulting in some high molecular weight, highly viscous β-glucans, as well as highly viscous xylans in the wort and beer. The lower the modification of the malt, the higher the amount of solubilized high molecular weight β-glucans and xylans, giving rise to inefficient and long lasting wort separation, and rapid pressure build-up during beer filtration.

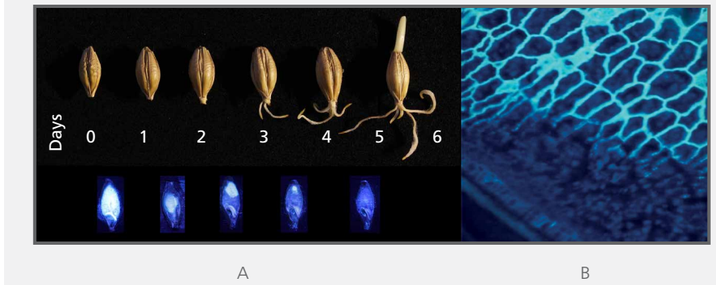


Fig. 5.2-2.

A. Barley grains malted for 6 days showing sprout and acrospire development and half cut kernels stained with the fluorescent dye Calcofluor making the cell wall degradation visible.

B. Close up of Calcofluor stained thin section of barley grain showing cell wall degradation in detail. Intact cell walls show light blue fluorescence. Degraded cell walls have no fluorescence.

Wheat, rye and sorghum are cereal grains that are also regularly malted. For wheat and rye malt, the modification pattern is similar to that of barley malt, where the arabinoxylans and β-glucans are broken down to minor and less viscous components. Sorghum, however, is different, leaving almost intact cell walls after malting.

5.3 Action of the enzymes

Novozymes' filtration enzymes hydrolyze mixed linked 1,3-1,4 – β-glucans, as seen in Fig. 5.3-1 and arabinoxylans , as seen in Fig. 5.3-2 to low viscosity polysaccharides. The enzymes are more heat stable than malt enzymes. The enzymes will only be inactivated at 70-75°C, so they will stay active during the entire mashing, resulting in improved wort separation and beer filtration compared with no use of external enzymes.

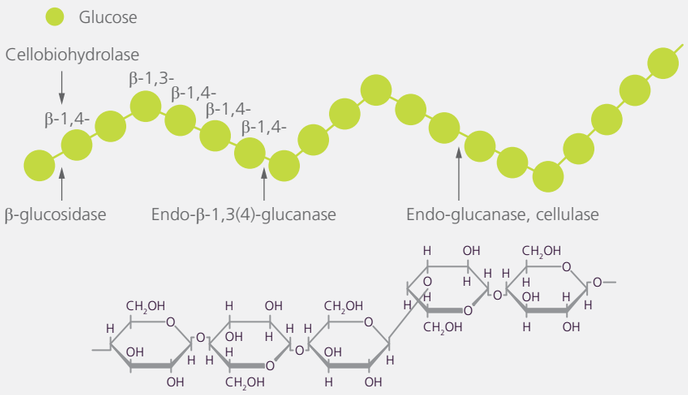


Fig. 5.3-1. Structure of mixed-linked 1,3-1,4 β-glucans, also showing the action points of various glucanases

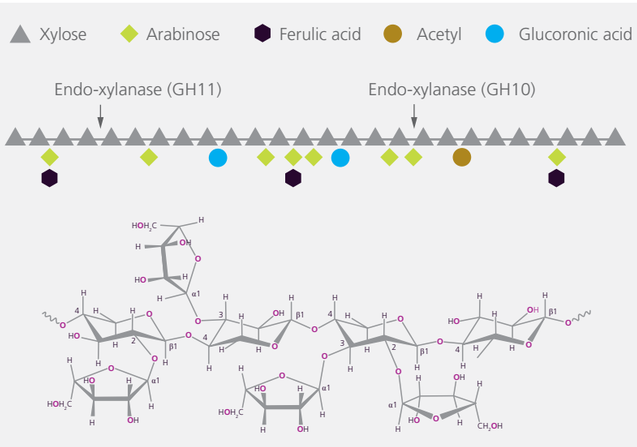


Fig. 5.3-2. Structure of arabinoxylans, showing the action points of various endo-xylanases. Two types of endo-xylanases are present in filtration enzymes: the GH-10 family (Glucoside Hydrolase) can cut the xylose backbone into the right chain lengths for improved filtration better than the GH-11 family, resulting in lower viscosity of wort and beer

All enzymes in the Novozymes' Ultraflo series contain both β-glucanases and xylanases, but of different types. Only Ultraflo Max contains the GH-10 family xylanase, which very effectively breaks down arabinoxylans to non-viscous polysaccharides, resulting in viscosity reduction that cannot be matched by standard filtration enzymes. This can be seen in Fig. 5.3-3.

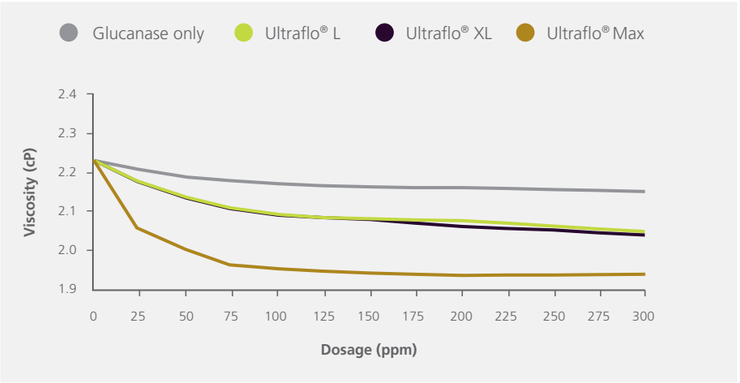


Fig. 5.3-3. Lowest viscosity level delivered by Novozymes Ultraflo® Max at all dosage levels

The use of Ultraflo Max as a filtration enzyme makes it possible to combine High Gravity Brewing, and Very High Gravity Brewing, with efficient mash filtration, as demonstrated in Fig. 5.3-4.

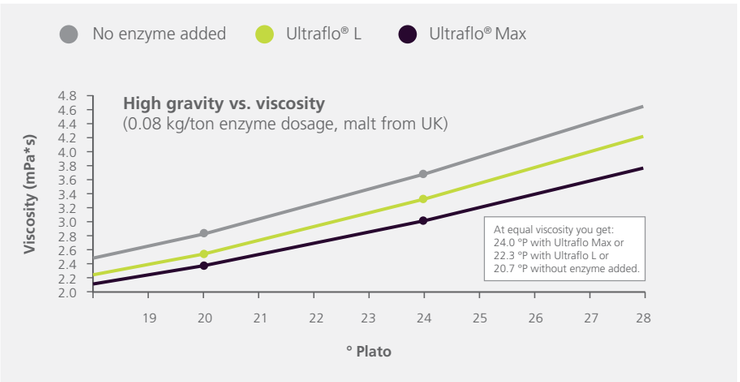


Fig.5.3-4 Wort viscosity as function of gravity when no enzymes, Novozymes Ultraflo® L and Ultraflo Max are added

At lower gravity the difference in mash separation performance among the two enzymes is less pronounced, but for beer filtration Ultraflo Max is always superior. This can be seen in Fig. 5.3-5.

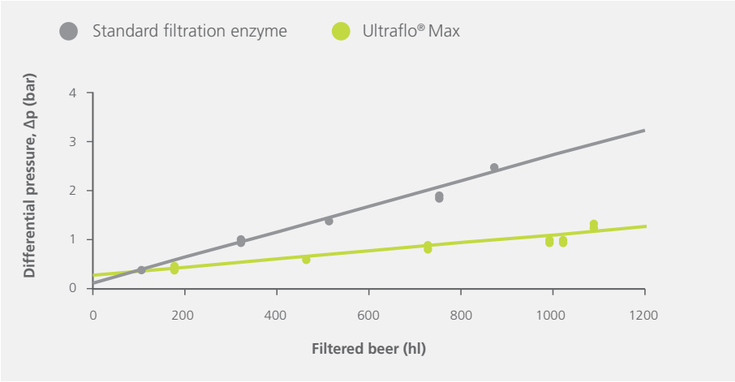


Fig. 5.3-5. Volume beer filtered as function of differential pressure showing significant improved beer filtration when using Novozymes Ultraflo® Max compared with standard filtration enzyme containing β-glucanase + GH-11 family xylanase

The low wort and beer viscosity results in significantly slower differential pressure increase across the filter over time, resulting in more filtered beer per filter run and less beer loss. Compared with no enzyme use, up to 50% longer beer filtration cycles can be achieved, and compared with filtration enzymes having the family GH-11 xylanase, 25-30% more beer through the filter can been achieved.

The effective breakdown of the cell walls accomplished by the Ultraflo enzymes allows for higher extract yield in the order of 0.5 to 2.0% depending on the raw material quality.

5.4 pH and temperature curves

Fig. 5.4-1 – 5.4-3 show the influence of temperature and pH on Ultraflo Max, Ultraflo L and Ultraflo XL performance under brewing conditions. Fig. 5.4-4 shows the influence of temperature and pH on Finizym 250 L activity under analytical conditions.

Fig. 5.4-1 A and B show the influence of temperature and pH on the performance of Ultraflo Max.

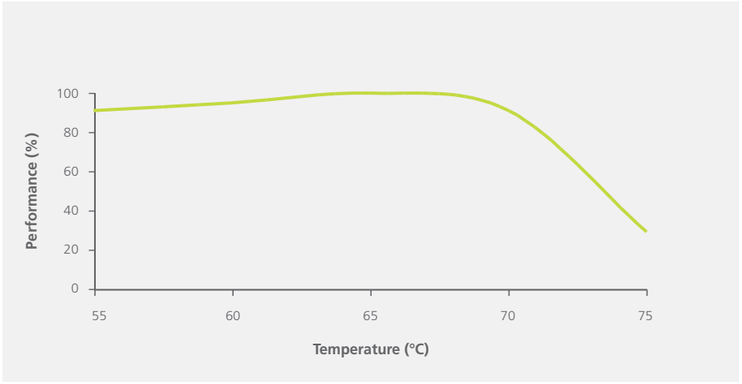


Fig. 5.4-1 A. Temperature dependency of Novozymes Ultraflo® Max

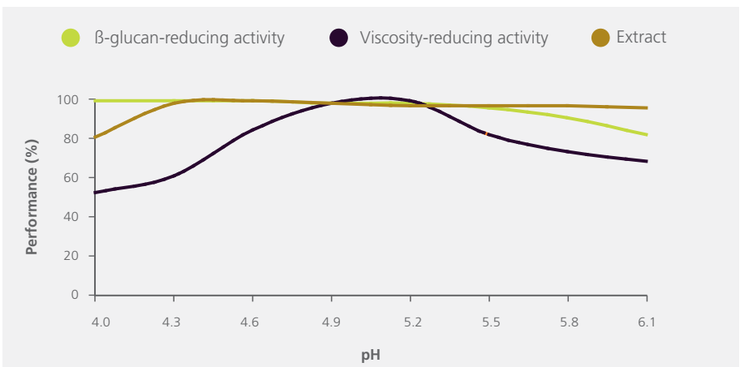


Fig. 5.4-1 B. pH dependency of Novozymes Ultraflo® Max

Fig. 5.4-2 A and B show the influence of temperature and pH on the performance of Ultraflo L.

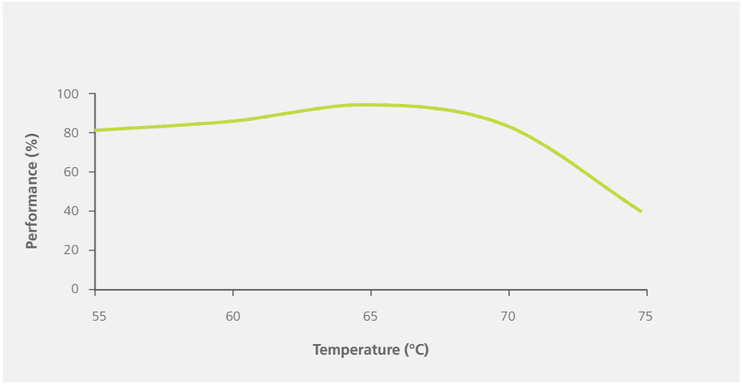


Fig. 5.4-2 A. Temperature dependency of Novozymes Ultraflo® L

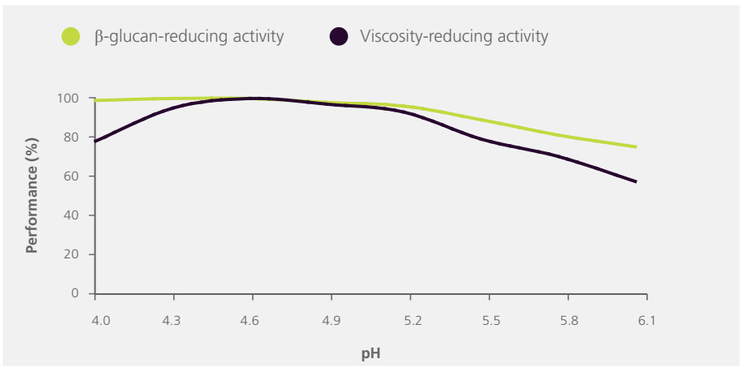


Fig. 5.4-2 B. pH dependency of Novozymes Ultraflo® L

Fig. 5.4-3 A and B show the influence of temperature and pH on the performance of Ultraflo XL.

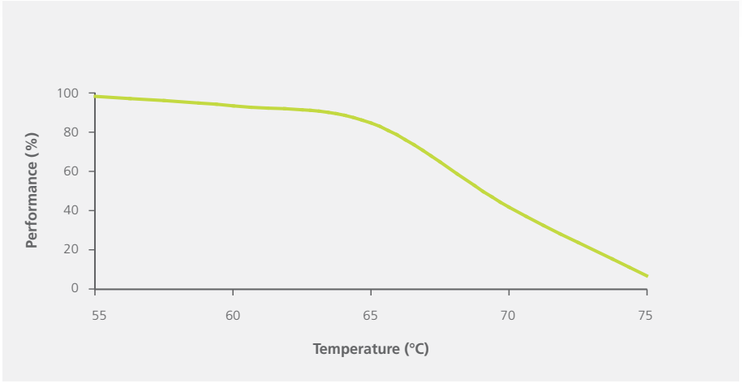


Fig. 5.4-3 A. Temperature dependency of Novozymes Ultraflo® XL

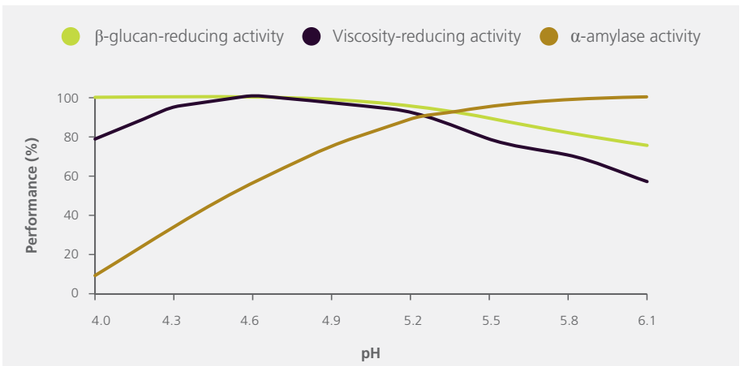


Fig. 5.4-3 B. pH dependency of Novozymes Ultraflo® XL

Fig. 5.4-4 A and B show the influence of temperature and pH on the activity of Finizym 250 L.

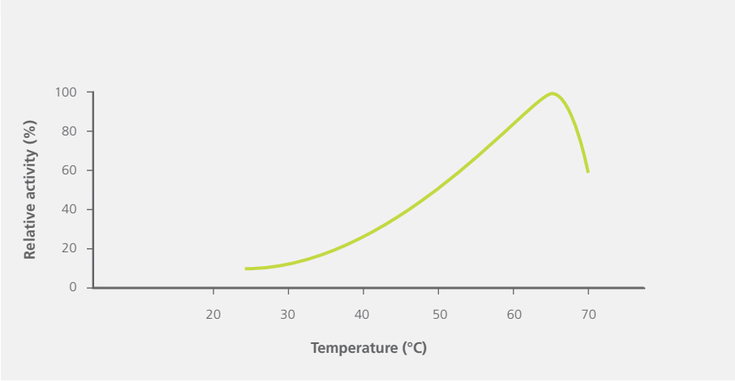


Fig. 5.4-4 A. Temperature dependency of Novozymes Finizym® 250 L

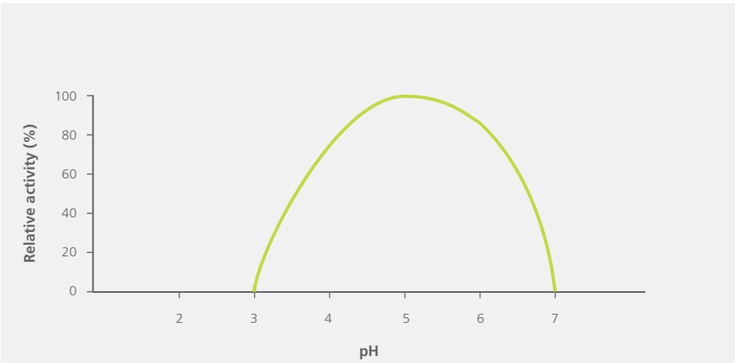


Fig. 5.4-4 B. pH dependency of Novozymes Finizym® 250 L

5.5 Practical applications

Use of exogenous enzymes for the reduction of wort and beer viscosity is the most widespread enzyme application in the brewing industry, and it is one of the first to have been regularly used throughout the years. The first filtration enzymes only contained β-glucanase activity, but today most filtration enzymes contain both β-glucanase and xylanase activities. The most advanced enzymes have the xylanase of the GH-10 family, which secures the lowest wort and beer viscosity.

Filtration enzymes are often added to all brews to level out fluctuations in brewing raw materials, to secure consistently high brewhouse performance, and to reach consistently high beer filtration rates.

All Novozymes' filtration enzymes break down the unmodified cell walls from barley malt or from unmalted barley. The more intact the cell wall materials, the higher the dosage of enzymes required to attain acceptable brewhouse performance and beer filtration. The most advanced filtration enzymes, like Ultraflo Max, provide significantly better performance, especially for beer filtration, compared with even the best well modified malt.

Choice of enzyme

The correct enzyme solution should always fulfill the needs of the brewer. Evaluation of cost versus benefit is very important! If capacity and time is the brewer's focus, there will be a need for higher gravity, shorter mash separation time, and longer beer filtration cycles. In this case the lowest possible viscosity is highly desirable, and Ultraflo Max is the answer. Ultraflo Max is well suited for well modified malt, moderately modified malt, and blends of barley and well modified malt, up to 25% barley.

If gravity is relatively low (< 14 °Plato), and the number of beer filtration cycles is not critical, Ultraflo L or Ultraflo XL can fulfill the brewer's needs. The choice between Ultraflo L and Ultraflo XL is related to the quality of the malt and the grist. Ultraflo XL is a more robust enzyme that can deal with moderately modified malt, inhomogeneous malt, and barley and malt blends up to 25–30 % barley. Ultraflo L is more suited for use with well-modified malt, which is demonstrated in Fig. 5.5-1.

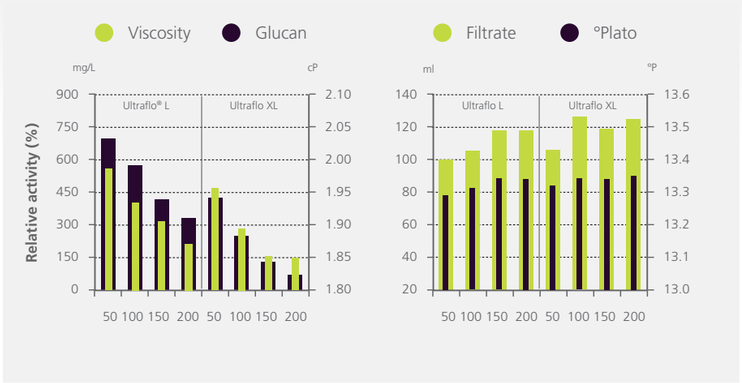


Fig.5.5-1. Laboratory mashing test showing the higher efficiency of Novozymes Ultraflo® XL on under modified malt versus Ultraflo L, due to the broader range of activities in the former.

For non-barley cereals and their respective malts containing significant levels of β-glucan and xylan, Novozymes' filtration enzymes can also be employed for wort and beer filtration improvements.

For wheat and rye with arabinoxylans as the main cell wall components, Ultraflo Max is absolutely the preferred enzyme. In the case of sorghum, both raw and malted, Ultraflo Max is also recommended.

Finizym 250 L is a filtration enzyme used in fermentation and maturation of beer to prevent filtration difficulties and haze caused primarily by β-glucans. This product is typically used when the brewer knows in advance the presence of un-filtered wort with high β-glucan levels that will give rise to problems in filtration and may manifest as haze in the packaged beer. Some brewers prefer to combine filtration enzymes in the mashing with filtration enzymes in the cellar. When using difficult raw materials, this has shown to be valuable for preventing haze and improving colloidal stability.

Monitoring the effect of filtration enzymes

The comparison of different filtration enzyme solutions and evaluation of their effect in daily brewing can be a challenge with variations in raw materials, recipes and brewing diagrams.

A good indication is a simple laboratory test, as demonstrated in Fig.5.5-2, where β-glucan, wort viscosity, extract yield and filtration are measured.

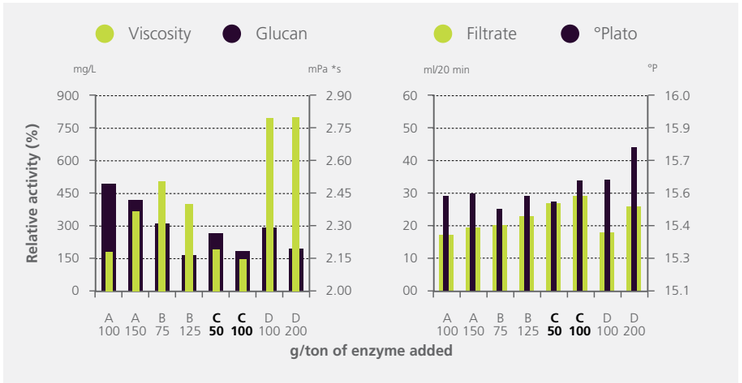


Fig. 5.5-2. Simple laboratory test showing the difference in performance for 4 different filtration enzymes. A: Novozymes Ultraflo® L, B: Ultraflo XL, ***C: Ultraflo Max****; D: Standard β-glucanase with α-amylase side activity*

For an industrial scale evaluation it makes sense to test the various enzyme solutions over a period of time, for example,1-3 months, and collect data such as:

- Wort viscosity, β-glucan content and arabinoxylan content
- ΔP during mash separation and beer filtration
- First run time
- Total lautering time
- Extract yield
- Brewhouse efficiency
- Extract loss
- Beer volume per filter run
- Beer loss and kieselguhr consumption

5.6 Practical examples

Benefits of external enzyme addition compared with no enzyme use
Two types of filtration enzymes, A: β-glucanase and GH-10 family xylanase and B: β-glucanase and GH-11 family xylanase, were tested against no enzyme use, control, for a period of 3 weeks in a brewery using well modified malt and maize (corn) grits. High gravity brewing was performed with first wort at > 20°Plato, and final wort at 17°Plato. The dosage was 0.15 kg/ton of both enzymes.

The main benefits, demonstrated in Table 5.6-1, were:

- Extract yield increased by 1.0% for both enzymes versus control
- Mash filtration was significantly improved for both enzymes versus control
- Greatest improvement was observed for enzyme A:
  - 10% faster filtration time
  - 7% higher flow
- Beer filtration only improved when using enzyme A:
  - 0.2 bar/2000 hl beer lower pressure versus B and control.

	Filtration enzyme with GH-10 family xylanase	Filtration enzyme with GH-11 family xylanase	No enzyme
Extract yield (%)	76	76	75
Mash filtration time (min.)	59	62	65
Mash filtration flow (Hl/h)	170	165	160
Pressure increase in final beer filtration (bar/2000Hl)	0.65	0.85	0.85

Table 5.6-1. Brew house performance and beer filtration improvements by use of exogenous filtration enzymes

Standard filtration enzyme vs. Ultraflo Max
Ultraflo Max was evaluated against a standard β-glucanase in a trial series of 30 brews. The trials were carried out in a brewery using 12 MT moderately modified malt per brew. Dosages were 1.8 kg of Ultraflo Max versus 2.5 kg of standard β-glucanase per brew. The average trial data are summarized in the tables 5.6-2 and 5.6-3 below. Analyses of the first worts showed low β-glucan values for both wort types, as expected. However, Ultraflo Max treated wort was significantly lower in arabinoxylans than the wort treated with the traditional β-glucan product, as seen in Table 5.6-2.

Analysis of first worts
Treated with a traditional filtration enzyme and Novozymes Ultraflo® Max. These results are based on an average of thirty brews:

Enzyme	°Plato	Arabino-xylan (ppm)	β-glucan (mg/l)
Traditional filtration enzyme	25,4	1045	< 15
Ultraflo® Max (0.15 kg/ton)	25,8	145	< 15

Table 5.6-2.

Significant improvements using Ultraflo Max were seen in brewhouse performance and beer filtration. This is demonstrated in Table 5.6-3. Total beer volume per filtration cycle went from 3.800 hl to 4.900 hl, resulting in kieselguhr savings of 15%.

Brew house performance and beer filtration improvements by exchanging traditional filtration enzymes by Novozymes Ultraflo® Max

Parameter	Average improvement (30 brews)
Extract yield	0.5%
Brewhouse capacity	15 minutes per brew
Beer filtration cycles	30% more throughput

Table 5.6-3.

5.7 Enzyme data table

Novozymes Ultraflo® Max	
Declared enzyme	β-glucanase (endo-1,3(4)-) and Xylanase (endo-1,4-)
Catalyzes the following reaction:	endo-β-glucanase that hydrolyzes (1,3) – or (1,4)-linkages in β-D-glucans xylanase that hydrolyzes (1,4)-beta-D-xylosidic linkages in xylans
Declared activity	700 EGU/g 250 FXU-S/g
E.C/ I.U.B. no:	3.2.1.6 and 3.2.1.8
Physical form	Liquid
Production method	Submerged fermentation of genetically modified microorganisms. The enzyme proteins, which in themselves are not genetically modified, are separated and purified from the production organisms.

Novozymes Ultraflo® L	
Declared enzyme	β-glucanase (endo-1,3(4)-)
Catalyzes the following reaction:	endo-β-glucanase that hydrolyzes (1,3) – or (1,4)-linkages in β-D-glucans
Declared activity	45 FBG/g
E.C/ I.U.B. no:	3.2.1.6
Side activities	The product contains activity of Cellulase and Xylanase
Physical form	Liquid
Production method	Submerged fermentation of a microorganism. The enzyme protein is separated and purified from the production organism.

Novozymes Ultraflo® XL	
Declared enzyme	β-glucanase (endo-1,3(4)-)
Catalyzes the following reaction:	endo-β-glucanase that hydrolyzes (1,3) – or (1,4)-linkages in β-D-glucans
Declared activity	45 BGU/g
E.C/ I.U.B. no:	3.2.1.6
Side activities	The product contains activity of Xylanase and α-amylase
Physical form	Liquid
Production method	Submerged fermentation of a microorganism. The enzyme protein is separated and purified from the production organism.

Continue on next page

Novozymes Finizym® 250 L	
Declared enzyme	β-glucanase (endo-1,3(4)-)
Catalyzes the following reaction:	endo-β-glucanase that hydrolyzes (1,3) – or (1,4)-linkages in β-D-glucans
Declared activity	250 FBG/g
E.C/ I.U.B. no:	
Side activities	The product contains activity of Cellulase and Xylanase
Physical form	Liquid
Production method	Submerged fermentation of a microorganism. The enzyme protein is separated and purified from the production organism.

Table 5.7-1. Enzyme data

CHAPTER 6.

ATTENUATION CONTROL AND LIGHT BEER PRODUCTION

6.0 Introduction to segment and key benefits

Globally, one of the fastest growing beer styles in recent years has been the light, or low-calorie, beer.

Producing this type of beer requires an increase in the degree of attenuation of the wort, thus decreasing the proportion of non-fermentable and short-chain dextrin material. The result is a highly attenuated beer. A beer made this way will have 25-30% fewer calories than a normally attenuated beer, assuming the same alcohol content in both beers.

The ability of brewers to achieve predictable and targeted attenuation specifications can be hampered by variability in raw material quality and inherent variability in the mashing process. Furthermore, where non-traditional raw materials are used as adjuncts, there may be the need to add exogenous saccharifying enzymes to achieve a sufficiently high degree of attenuation for proper fermentation.

Novozymes offers a broad range of attenuation control products to allow brewers to create highly attenuated beers, or to control attenuation fluctuation due to raw material deficiencies, in a simple and cost-effective manner.

Attenuation enzymes include: Novozymes AMG® 300 L BrewQ, Attenuzyme Core, Attenuzyme Pro, Novozym® 26062 and Fungamyl BrewQ.

Key benefits

- Produce highly attenuated beers in a cost-effective manner
- Maintain consistent fermentability, regardless of varying raw material qualities
- Produce super-attenuated malt base for flavored alcoholic beverage production
- Increasing the attenuation level by 4-5% utilizing the same amount of raw materials

6.1 Core enzyme application

Preferably, all attenuation enzymes should be added to the mash tun at mash-in. Alternatively, these enzymes can either be added to a separate process tank prior to the kettle or into fermentation. Please note that when fermentation addition is considered, additional heat treatment must be incorporated prior to packaging to ensure that no residual enzyme activity exists in the beer, or that no "substrate" is left in the final beer. Dosage is calculated based on total grist (ton) and on the degree of attenuation desired and is a function of conversion time and temperature. For example:

- RDF of 75-80%, Attenuzyme Pro dosage is 0.2 to 0.5 kg/ton
- RDF of 80-90%, Attenuzyme Pro dosage is 0.3 to 5.5 kg/ton

6.2 Background to application

Malt worts produced under standard brewing conditions with traditional raw materials typically yield a real degree of fermentation (RDF) of 67-72% or apparent degree of fermentation (ADF) of 80-85%. Both RDF and ADF are used to describe the "degree of attenuation" of the wort (the latter (ADF) does not take into account the lower density of alcohol compared to water in the final gravity of the fermented beer). Attenuation is a measure of the degree to which sugars (i.e. glucose, fructose, maltose, maltotriose) in the wort can be fermented into alcohol. Approximately 25% of the carbohydrate material will remain as non-fermentable, short-chain dextrins (i.e., panose, isomaltose, isomaltotriose, DP4/DP4+) in the beer. The basic premise of controlling attenuation of wort is to increase, or maintain at a specified level, the percentage of fermentable sugars from derived starch. Starch is composed of amylose and amylopectin. This is illustrated in Fig. 6.2-1. Natural starch (such as from cereal grains) is typically 10-25% amylose and 75-90% amylopectin.

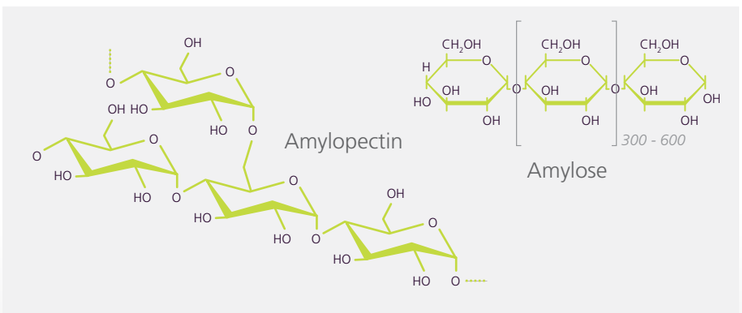


Fig. 6.2-1. Amylopectin and amylose

6.3 Action of the enzyme

Diluting the excess alcohol formed during fermentation, of highly attenuated beers with water will result in beer with lower alcohol, less residual extract, and fewer calories compared to a beer of standard attenuation. For attenuation control, different enzymes are employed at either mashing or fermentation to produce the desired degree of attenuation and carbohydrate profile.

Amyloglucosidase (glucoamylase)

Glucoamylases are typically the first choice for a brewer to produce highly attenuated beers, or to make small adjustments in attenuation. These enzymes break α-1,4-glucosidic linkages at the non-reducing ends of starch (amylase and amylopectin) as depicted in Fig. 6.3-1.

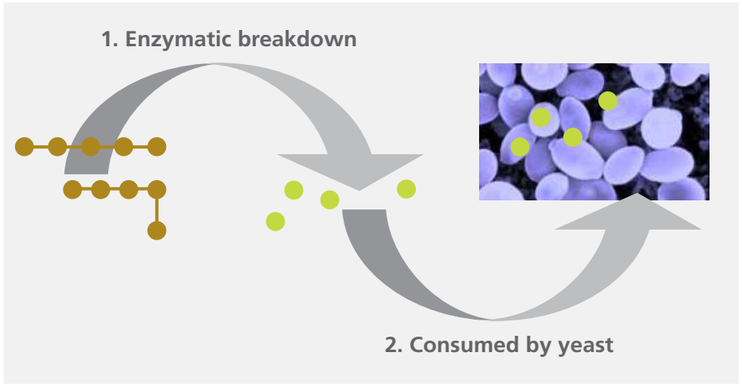


Fig.6.3-1. Starch breakdown by glucoamylase

Glucoamylases release glucose as the main fermentable sugar. Glucoamylases are efficient enzymes that produce a strong effect on wort attenuation at even relatively low dosages.

α-amylase

α-amylases cleave α-1,4-glucosidic linkages in starch, as do glucoamylases, but act upon random locations on the starch molecule. They yield maltotriose and maltose from amylose and maltose, glucose, and limit-dextrin from amylopectin. As α-amylases can act upon any 1,4-glucosidic linkage in starch, they are relatively fast-acting enzymes. Fig. 6.3-2 illustrates the action of α-amylase on amylopectin.

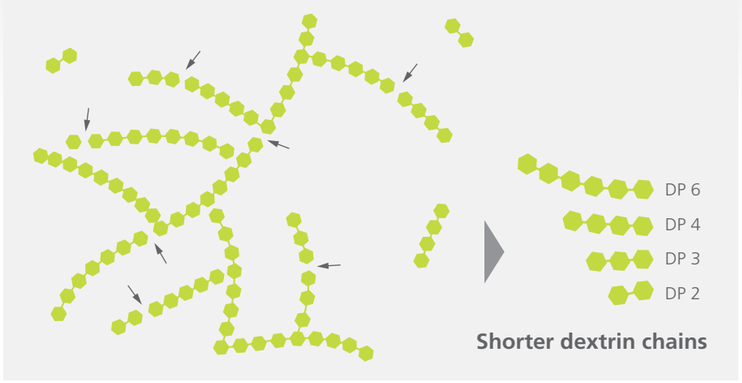


Fig.6.3-2. Action of α-amylase on amylopectin

Pullulanase

Pullulanases are de-branching enzymes used in conjunction with glucoamylases and/or α-amylases to increase the rate of starch breakdown. This allows for attenuation targets to be reached in shorter conversion times, or with lower dosages of glucoamylase. Pullulanases cleave α-1,6-glucosidic bonds in amylopectin. They work in synergy with malt β-amylase and can be used alone for small attenuation adjustments via maltose formation. Fig. 6.3-3 illustrates the action of pullulanase, glucoamylase, and α-amylase on amylopectin, producing glucose and maltose.

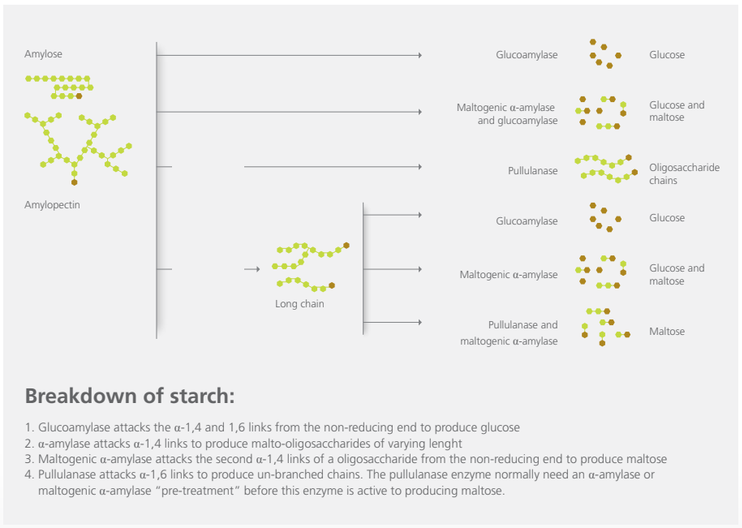


Fig. 6.3-3. Amylopectin breakdown by glucoamylase, α-amylase and pullulanase to glucose and maltose

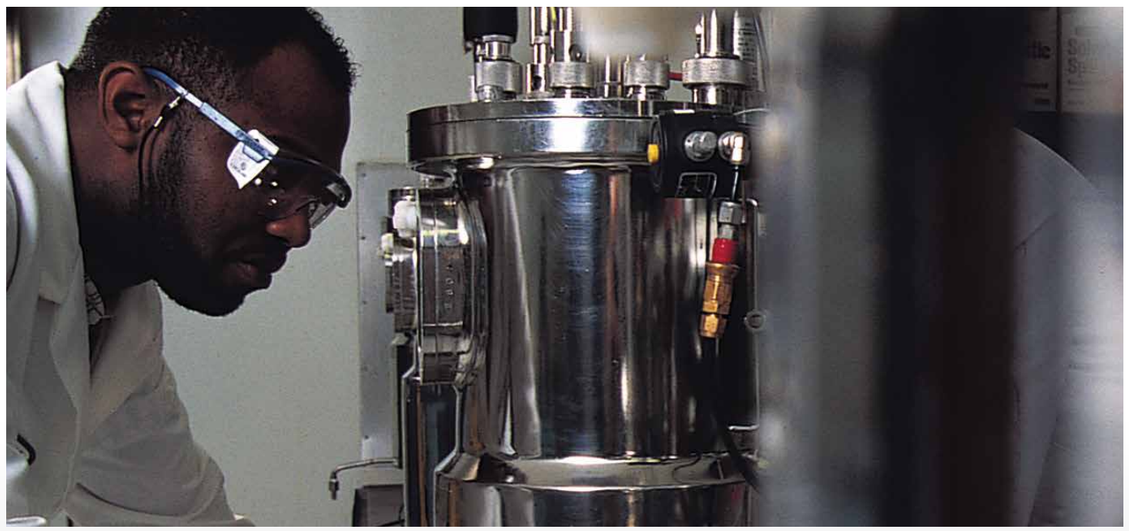
6.4 pH and temperature curves

Attenuation enzymes can be used in the brewhouse or possibly during fermentation.

The degree of attenuation desired is governed by the choice of attenuation enzyme (glucoamylase, α-amylase, pullulanase or combination), enzyme stability (temperature and pH), enzyme dosage, conversion temperature and conversion time.

When choosing an enzyme solution for attenuation control, it is important to look at the activity curves for each based on temperature and pH. Select an enzyme solution that has significant activity and stability where you want to use it – in either mashing or fermentation.

Fig. 6.4-1 illustrates the temperature and pH activity curves for Novozymes' attenuation enzymes. It is clear that from a pH point of view, all enzymes have significant activity in the typical pH ranges encountered during brewing. From a temperature perspective, Attenuzyme Core (and Attenuzyme Pro) and AMG 300L BrewQ have high activity between 60°C and 70°C and would be more suitable for mashing application than Fungamyl BrewQ, which undergoes significant denaturation in this temperature range. Therefore, Fungamyl BrewQ may be of more use in fermentation applications.



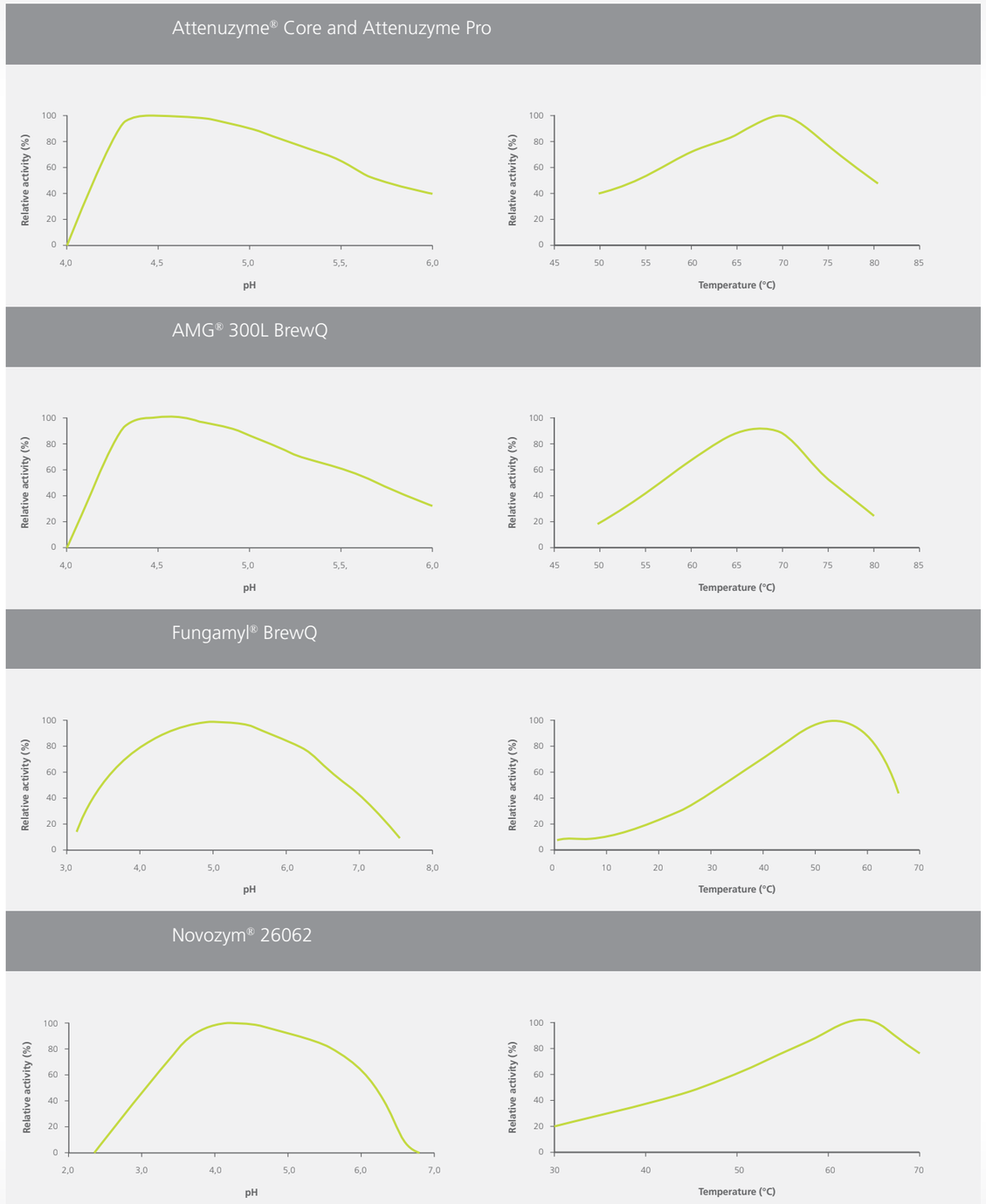


Fig.6.4-1. Temperature and pH activity curves for Novozymes' attenuation enzymes

A second very important consideration is inactivation of the attenuation enzyme selected or making sure that no more substrate is left in the final beer. Typically heat is used to inactivate (or denature) the enzyme after its activity is no longer needed in the process.

From a quality point of view, glucoamylase will continue to react with remaining dextrin material in the beer, giving a "sweet" off-taste in the product.

For use in the brewhouse, wort boiling will completely eliminate any remaining enzymatic activity that may be present. For use in fermentation, typical pasteurization (tunnel or flash) conditions will inactivate only Fungamyl BrewQ. Limited activity will remain from Novozym 26062, but significant activity will remain from Attenuzyme Core, AttenuzymePro and AMG 300 L BrewQ if a secondary heat treatment step is not employed in addition to standard pasteurization.

6.5 Practical applications

How to adjust fermentability

If normal attenuation of 67-74% RDF is not achieved with the available mashing methods and raw materials, and if corrections to these are not possible or desired, then the addition of Fungamyl BrewQ to fermentation is the easiest way to smooth out small fluctuations in attenuation. Alternatively, a small dosage of AMG 300L BrewQ, Attenuzyme Core or Attenuzyme Pro at mashing into the mash tun can smooth out small variations in attenuation. Table 6.5-1 outlines recommended starting points for enzyme dosages to alter attenuation, with respect to the degree of attenuation desired.

Desired Attenuation (%)		Option	Enzymes	Dosage Range	Units (per ton grist or hL beer)	Point of addition
RDF	ADF					
70-75	85-90	A	Fungamyl® BrewQ	0.5 to 5.0	g/hL	Start of fermentation
75-80	90-95	A	AMG® 300L BrewQ	1.2 to 3.5	kg/ton	Mashing-in
			+ Novozym® 26062	2.4 to 3.6	kg/ton	
		B	Attenuzyme® Core	0.35 to 1.0	kg/ton	Mashing-in
		C	Attenuzyme® Core	0.25 to 0.75	kg/ton	Mashing-in
			+ Novozym® 26062	1.2 to 2.4	kg/ton	
		D	Attenuzyme® Pro	0.15 to 0.5	kg/ton	Mashing-in
80-90	95-100	A	Fungamyl® BrewQ	4.0 to 8.0	g/hL	Start of fermentation
			+ Novozym® 26062	1.2 to 3.6	kg/ton	Mashing-in
		B	Fungamyl® BrewQ	2 to 5	g/hL	Start of fermentation
			+ Novozym® 26062	12 to 20	g/hL	
		C	AMG® 300L BrewQ	6.0 to 18	kg/ton	Mashing-in or hot wort (63°C)
			+ Novozym® 26062	6.0 to 18	kg/ton	
		D	Attenuzyme® Core	2.0 to 6	kg/ton	Mashing-in or hot wort (63°C)
		E	Attenuzyme® Core	1.5 to 5	kg/ton	Mashing-in or hot wort (63°C)
			+ Novozym® 26062	2.4 to 4.8	kg/ton	
		F	Attenuzyme® Pro	0.25 to 5.0	kg/ton	Mashing-in or hot wort (63°C)

Table 6.5-1. How to adjust fermentability

It can be seen from Table 6.5-1 that the most efficient methods for producing a super-attenuated beer in terms of both economy and achieved fermentability is to use Attenuzyme Core or switch to Attenuzyme Pro or AMG 300 L BrewQ + Novozym 26062 with addition to the mash tun at mashing-in. Fig.6.5-1 below illustrates the broad range of attenuation targets that can be reached with Attenuzyme Core and Pro in relatively short conversion times, as a function of enzyme dosage.

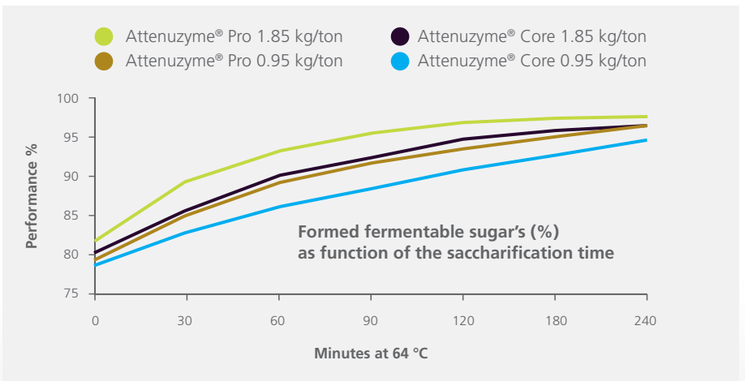


Figure 6.5-1. RDF development as a function of Novozymes Attenuzyme® dosage

In practice, a high dosage of glucoamylase is often associated with a decrease in lautering performance. Should lautering and/or filtration issues arise when producing super-attenuated worts, it is recommended to also use Novozymes Ultraflo Max to bring lautering performance back to normal. This will ensure that lautering and/or mash filtration times are as short as possible with good performance.

The increased attenuation and increased amount of alcohol formed should be taken into account when calculating the amount of raw materials used. For a given strength of alcohol in the beer, lower amounts of raw materials are needed. This will manifest in less free amino nitrogen (FAN) in the wort. The levels of FAN should be measured in the wort, and if on the low side, should be supplemented. For good fermentation performance in an all-malt wort, FAN should be at ca. 15-18 mg/L/°P. If FAN is low, use of Neutrase 0.8 L BrewQ or Neutrase 1.6 L during mashing can be beneficial for fermentation performance.

Which attenuation solution is best for me?
When choosing an attenuation solution, there are different decision factors a brewer can consider to select the most appropriate product.

For example, Attenuzyme Core is a straightforward glucoamylase product with limited α-amylase activity. Attenuzyme Pro, meanwhile, is a high-performing, fast-acting combination of glucoamylase, α-amylase and de-branching enzyme (pullulanase) that enables production of highly attenuated beers with greater ease, including shorter mashing times, lower enzyme dosages and the ability to produce super-high attenuated beers.

In fact, Attenuzyme Pro has been found to shorten mashing times by up to 50%, increasing brewhouse capacity while saving time and energy. If the brewer wishes to address attenuation adjustment in fermentation, the best solution is Fungamyl BrewQ and possibly Novozym 26062.

6.6 Enzyme data table

Novozymes AMG® 300L BrewQ	
Description	A classic heat-stable amyloglucosidase (glucoamylase) used for production of highly fermentable, glucose-based worts.
Declared enzyme	Glucoamylase (glucan 1,4-α-glucosidase)
Catalyzes the following reaction:	Hydrolyzes (1, 4)- and (1, 6)-α-D-glucosidic linkages at the non-reducing ends of polysaccharides to produce glucose.
Declared activity	300 AGU/mL
E.C/ I.U.B. no:	3.2.1.3
Physical form	Liquid
Production method	Produced by submerged fermentation of a microorganism. The microorganism is not genetically modified. The enzyme protein is separated and purified from the production organism.

Novozymes Attenuzyme® Core	
Declared enzyme	Glucoamylase (glucan 1,4-alpha-glucosidase)
Catalyzes the following reaction:	Hydrolyzes (1,4)- and (1,6)-α-D-glucosidic linkages at the non-reducing ends of polysaccharides to produce glucose
Declared activity	1600 AGU/g
E.C/ I.U.B. no:	3.2.1.3
Physical form	Liquid
Production method	Submerged fermentation of a genetically modified microorganism. The enzyme protein, which in itself is not genetically modified, is separated and purified from the production organism.

Continue on next page

Novozymes Attenuzyme® Pro	
Declared enzyme	A multi-component enzyme solution comprised of a fungal α-amylase, glucoamylase, and pullulanase for accelerated production of highly fermentable glucose-based worts
Catalyzes the following reaction:	Glucoamylase that hydrolyzes (1, 4)- and (1, 6)-α-D-glucosidic linkages at the non-reducing ends of polysaccharides to produce glucose. Pullulanase that hydrolyzes (1,6)-α-D-glucosidic linkages in pullulan, amylopectin and glycogen to produce smaller fragments of linear dextrin.
Declared activity	1300 AGU/g & 315 PUN/g
E.C/ I.U.B. no:	3.2.1.3 & 3.2.1.41
Physical form	Liquid
Production method	Submerged fermentation of a genetically modified microorganism. The enzyme protein, which in itself is not genetically modified, is separated and purified from the production organism.

Novozymes Novozym® 26062	
Description	A heat-stable pullulanase which accelerates production of highly fermentable worts when used in conjunction with a glucoamylase.
Declared enzyme	Pullulanase
Catalyzes the following reaction:	Hydrolyzes (1,6)-α-D-glucosidic linkages in pullulan, amylopectin and glycogen to produce smaller fragments of linear dextrin.
Declared activity	400 PUN/g
E.C/ I.U.B. no:	3.2.1.41
Physical form	Liquid
Production method	Produced by submerged fermentation of a genetically modified microorganism. The enzyme protein, which in itself is not genetically modified, is separated and purified from the production organism.

Novozymes Fungamyl® BrewQ	
Description	A classic fungal α-amylase used for increased starch breakdown, facilitating higher alcohol output.
Declared enzyme	α-amylase
Catalyzes the following reaction:	Endo-amylase that hydrolyzes (1,4)-α-D-glucosidic linkages in starch polysaccharides
Declared activity	800 FAU-F/g
E.C/ I.U.B. no:	3.2.1.1
Physical form	Liquid
Production method	Produced by submerged fermentation of a microorganism. The microorganism is not genetically modified. The enzyme protein is separated and purified from the production organism.

Table 6.6-1. Enzyme data

CHAPTER 7.

FERMENTATION CONTROL WITH FAN OPTIMIZATION

7.0 Introduction to segments and key benefits

To ensure proper fermentation, yeast needs to be provided with sufficient Free Amino Nitrogen (FAN) for growth, which translates into acceptable and reproducible beer quality.

For FAN increase, Novozymes offers brewers Neutrase 0.8 L BrewQ and Neutrase Xtra 1.6 L.

Key benefits

- FAN control for improved yeast growth and stable fermentation
- FAN optimization in high barley/adjunct brewing
- Improvement of mash filtration
- Yield improvement

7.1 Core enzyme application

The optimal working conditions for Neutrase are 45-55°C and pH 5.5-7.5. It is typically used at mashing-in during protein rest and is completely inactivated during wort boiling.

Recommended dosages for high adjunct ratios or under modified malt for FAN generation:

- Neutrase 0.8 L BrewQ0.4 – 2.5 kg/ton of grist
- Neutrase Xtra 1.6 L 0.2 – 1.3 kg/ton of grist

Novozymes offers two types of Neutrase preparations for this application:

- Neutrase 0.8 L BrewQ: a non-GMM derived preparation
- Neutrase® Xtra 1.6 L: a GMM-derived variant and cost effective alternative and with performance on pair with Neutrase 0.8 L BrewQ (unit based)

7.2 Background to application

The FAN recommendation for all-malt wort is 180 to 220 mg/L (at 12 °P) or 15 to 18 mg/L/°P. If under-modified malt is used for brewing, or high levels of adjunct (e.g. barley, corn, sorghum or rice) are employed, low FAN levels in the resultant wort can occur.

Neutrase products provide consistent, higher levels of FAN, when the brewer requires it, based on malt modification and choice of raw materials. These proteases do not adversely affect beer foam stability. Modification of the protein matrix by these solutions can also have a positive impact on wort filtration and extract yields in the brewhouse.

7.3 Action of the enzymes

Neutrase is a neutral protease produced by submerged fermentation of selected strains of Bacillus strains.

The key enzyme activity is provided by an endo-protease that hydrolyzes internal peptide bonds. With normal malt, no more than 30-40% of the protein is solubilized. With Neutrase, solubilization of protein can be increased by up to 30%.

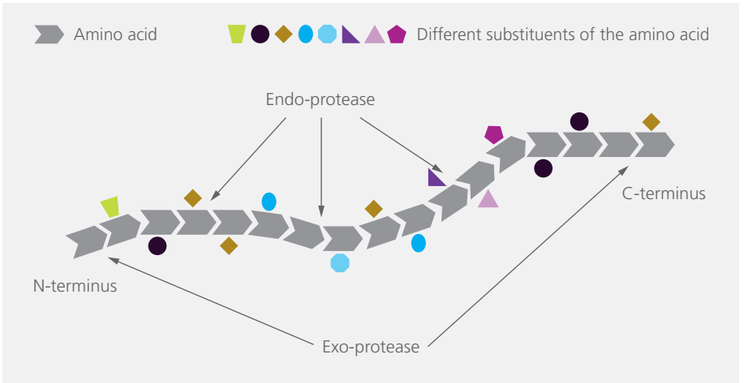


Figure 7.3-1. Protein structure and the effect of endo and exo-proteases

7.4 pH and temperature curves

Fig. 7.4-1 – 7.4-3 show the influence of temperature and pH on Neutrase activity under analytical conditions without the stabilizing effect of proteinaceous substrates.

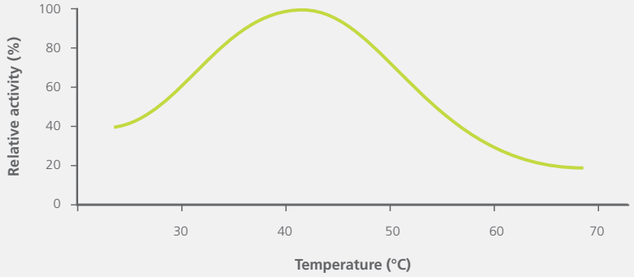


Fig. 7.4-1. Influence of temperature on the activity of Novozymes Neutrase® at pH 6.0

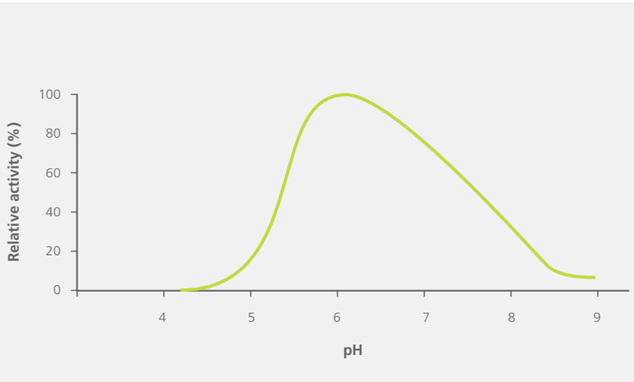


Fig. 7.4-2. Influence of pH on the activity of Novozymes Neutrase® at 45°C

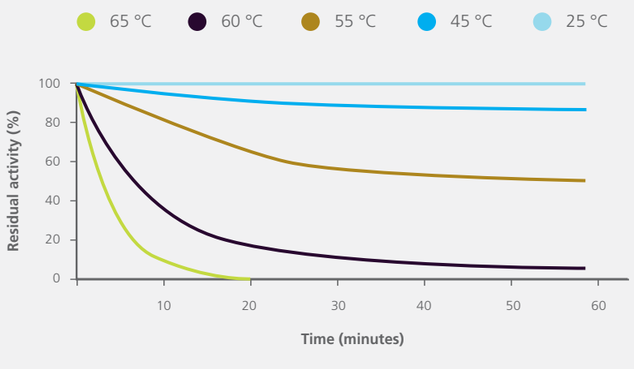


Fig. 7.4-3. Stability of Novozymes Neutrase® at pH 6.0 and different temperatures

7.5 Practical applications

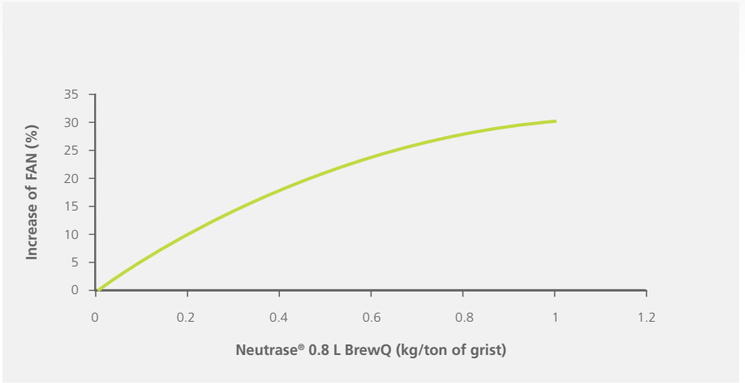


Fig. 7.5-1. %-Increase of Free Amino Nitrogen by Novozymes Neutrase® 0.8 L BrewQ addition

Example 1:
Brewing with adjuncts in a decoction process of 60% malt and 40% rice
Liquefaction with Termamyl BrewQ and FAN adjustment with Neutrase in main mash during protein rest.

The result show an increase of around 13-20% in FAN level with the addition of 0.4 kg/ton of Neutrase BrewQ, and 20-26% with the addition of 0.8 kg/ton of Neutrase 0.8 L BrewQ.

Rice (%)	40	40	40	40	40	40
Malt (%)*	14 (CC) + 46 (MT)	14 (CC) + 46 (MT)	14 (CC) + 46 (MT)	60 (MT)	60 (MT)	60 (MT)
Termamyl® 0.8 L BrewQ® (kg/ton of rice)	-	-	-	0.25	0.25	0.25
Neutrase® BrewQ (kg/ton of malt)	-	0.40	0.80	-	0.40	0.80
Lab filtration performance (ml after 30 min)	90	100	135	190	180	180
Extract (°P)	12.0	12.1	12.2	12.3	12.3	12.3
FAN (mg/liter wort)	133	151	154	156	188	197
**CC = Cereal Cooker; MT = Mash Tun*						

Table 7.5-1. Trial design and analytical results

Example 2:
Brewing with 60% malt and 40% barley
Main mash regime: 52°C/30' – 64°C/35' – 67°C/15' – 73°C/10' – 78°C/05'
Cereal cooker regime: 55°C/15' – 75°C/10' – 85°C

The result show an increase of around 30% in FAN level with the addition of 0.4 kg/ton grist of Neutrase 0.8 L BrewQ

Cereal cooker: Barley				
Enzymes (kg/ton)	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**
Termamyl® SC (on barley)	0.60	0.60	0.60	0.60
Main mash: Malt + Barley				
Enyzmes (g/ton)	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**
Ultraflo® Max (on total grist)	0.30	0.30	0.30	0.20
Attenuzyme® Core (on malt)	0.20	0.20	0	0
Attenuzyme® Pro (on malt)	0	0	0.20	0.20
Neutrase® 0.8 L Brew Q (on total grist)	0	0.40	0	0.40
Analytics (16°P)	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**
FAN (mg/l)	149	196	151	195
β-glucan (mg/l)	52	51	53	103
Viscosity (mPa*s)	1.950	1.937	1.938	1.985

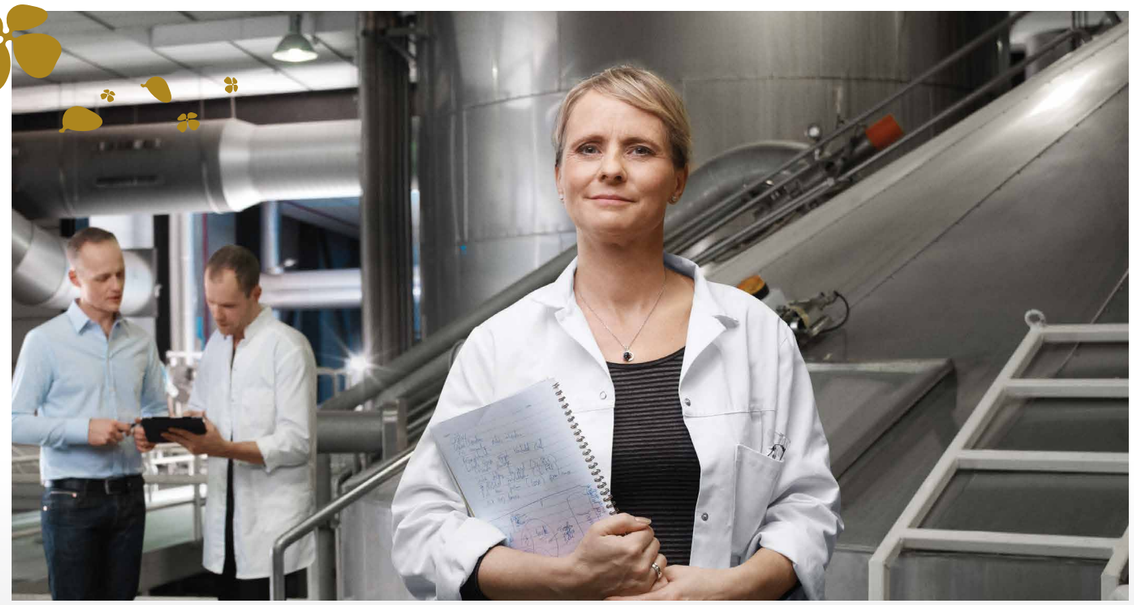
Table 7.5-2. Trial design and analytical results

7.6 Enzyme data table

Novozymes Neutrase® 0.8 L BrewQ	
Declared enzyme	Neutral proteinase
Catalyzes the following reaction:	Protein to free amino acids
Declared activity	0.8 AU_NH/g
E.C/ I.U.B. no:	3.4.24.28
Physical form	Liquid
Production method	Submerged fermentation of a non-genetically modified microorganism.

Novozymes Neutrase® Extra 1.6 L	
Declared enzyme	Neutral proteinase
Catalyzes the following reaction:	Protein to free amino acids
Declared activity	1.6 AU_NH/g
E.C/ I.U.B. no:	3.4.24.28
Physical form	Liquid
Production method	Submerged fermentation of a genetically modified microorganism.

Table 7.6-1. Enzyme data



CHAPTER 8.

DIACETYL CONTROL

8.0 Introduction to segment and key benefits

Diacetyl causes a butterscotch or buttery flavor in beer, and it is ranked as one of the most offensive off-flavors in Pilsner-type beer, based on the taste threshold 0.02 to 0.15 mg/L depending on beer style, brand and taster. Maturex 2000 L significantly reduces, or eliminates, the formation of diacetyl during fermentation, resulting in no diacetyl off-flavors in the final beer – this can be achieved within the minimum fermentation/maturation time.

Key benefits

- No Diacetyl off-flavor
- Shorten, or even by-pass rate-limiting warm maturation (diacetyl rest)
- Optimize vessel usage
- Increase beer volume – a reduction in fermentation time means an increase in throughput
- Maintain high quality index of finished beer
- Increase 'right first time' ensuring no re-work
- Reduce energy consumption

8.1 Core enzyme application

The working conditions for Maturex 2000 L are 10-45°C and pH 4.0-7.0.

Maturex 2000 L is dosed into the cold wort in the fermenting cellar at the beginning of the fermentation process.

- It is important that Maturex 2000 L is present in the wort at the same time as yeast, to maximize potential diacetyl prevention
- The recommended dosage is 1-2g/hl cold wort
- In some cases, a higher dosage may be required
  - The optimal dosage is reached when the diacetyl level is below the flavor threshold at the end of fermentation
- Maturex 2000 L interacts with the environment that it is working in, so the results are not only pH and temperature dependent, but also related to yeast strain, wort composition and the original gravity, so individual optimization might be needed

8.2 Background to application

Diacetyl formation during fermentation

Diacetyl is one of the two vicinal diketones (VDKs); diacetyl (2,3-butanedione) and 2,3-pentanedione. During fermentation their pre-cursors, α-acetolactate and α-acetohydroxy-butyrate, are excreted from the yeast cell and by extracellular spontaneous oxidative decarboxylation converted to diacetyl and 2,3-pentandione, respectively. Late in the fermentation and during the maturation process, diacetyl and 2,3-pentandione are then taken up by the yeast and reduced into the much less flavor-active compounds acetoin (3-hydroxy-2-butanone) and 3-hydroxy-2-pentanone. This can be seen in Fig. 8.2-1.

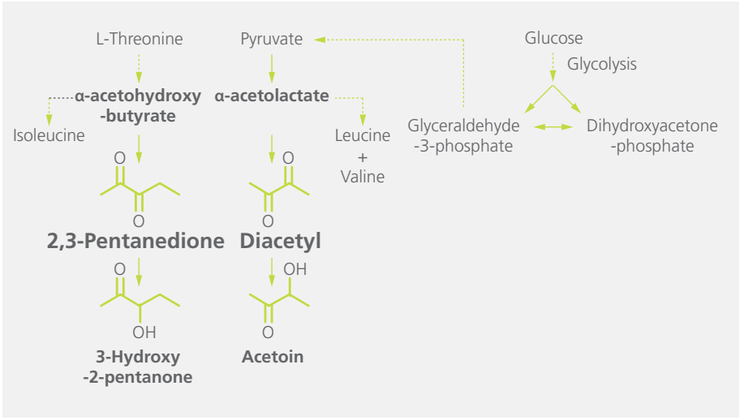


Fig. 8.2-1. Formation and reduction of diacetyl and 2,3-pentanedione during yeast fermentation of wort.

The flavor threshold for diacetyl is low (< 0.15 mg/l), while the flavor threshold for 2,3-pentanedione is 10 times higher: The formation of the two VDKs reaches similar levels at peak formation, so in practice 2,3-pentanedione is never an off-flavor problem, when the level of diacetyl is low.

The reduction of diacetyl and 2,3-pentanedione is accomplished by increasing the temperature to 14-20°C at the end of primary fermentation, or by an extended maturation period at a lower temperature. The introduction of a "diacetyl rest" at an evaluated fermentation temperature can decrease the extra time needed for avoiding diacetyl off-flavor, from weeks to 2-5 days. Depending on the adjunct ratio, wort concentration (Plato), yeast type, and physical environment, the rate of diacetyl reduction is variable in time and temperature requirements and not easily predicted. Therefore, the time needed to reduce diacetyl to an acceptable level below the flavor threshold can vary significantly.

8.3 Action of the enzyme

Maturex 2000 L is an acetolactate decarboxylase (ALDC). It reduces the formation of the vicinal diketones (VDK's) diacetyl and 2, 3-pentanedione, by converting their precursor, α-acetol-actate and α-acetohydroxy-butyrate directly into acetoin and 3-hydroxy-2-pentanone, respectively. The action of Maturex 2000 L is shown in Fig. 8.3-1 and 8.3-3, but for simplicity, only the formation and reduction of diacetyl is shown.

Maturex 2000 L competes with the spontaneous decarboxylation of α-aceto-lactate to diacetyl. But this reaction is slow when compared with the action of Maturex 2000 L transforming the precursor directly to acetoin, so at sufficiently high dosages of Maturex 2000 L, no diacetyl will be formed at all.

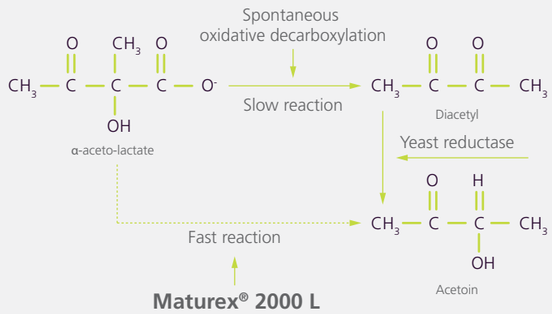


Fig. 8.3-1. Action of Novozymes Maturex® 2000 L during fermentation

The formation of diacetyl does not need to be completely suppressed, but diacetyl/VDK should be under the flavor threshold at the end of fermentation to guarantee the shortest maturation time possible. Fig. 8.3-2 shows the effect of Maturex 2000 L addition on the formation of diacetyl (DA) and 2,3-pentanedione (2,3-P) in a fermenting, all-malt wort.

Minor amounts of diacetyl are still formed in the Maturex 2000 L treated wort, but taken up again by the yeast, so the diacetyl level is under the flavor threshold at the end of fermentation.

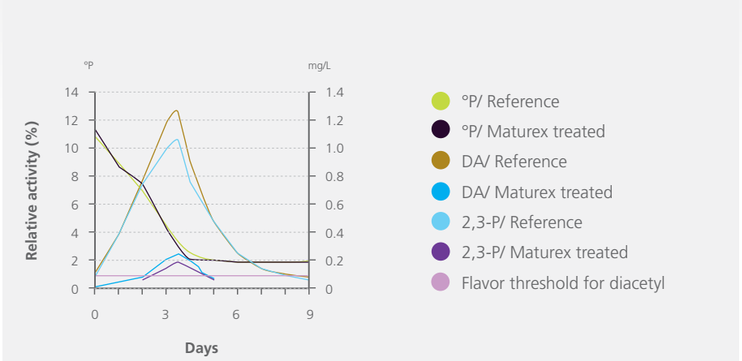


Fig. 8.3-2. Comparison of diacetyl and 2,3-pentandione formation and removal in fermenting wort with and without addition of Novozymes Maturex® 2000 L.

Important note: Maturex 2000 L does not reduce or eliminate diacetyl or 2,3-pentanedione already formed in beer – Maturex is only effective on the precursor to these compounds, and only when they are excreted from the yeast cells and present in the fermenting beer. This is demonstrated in Fig.8.3-3.

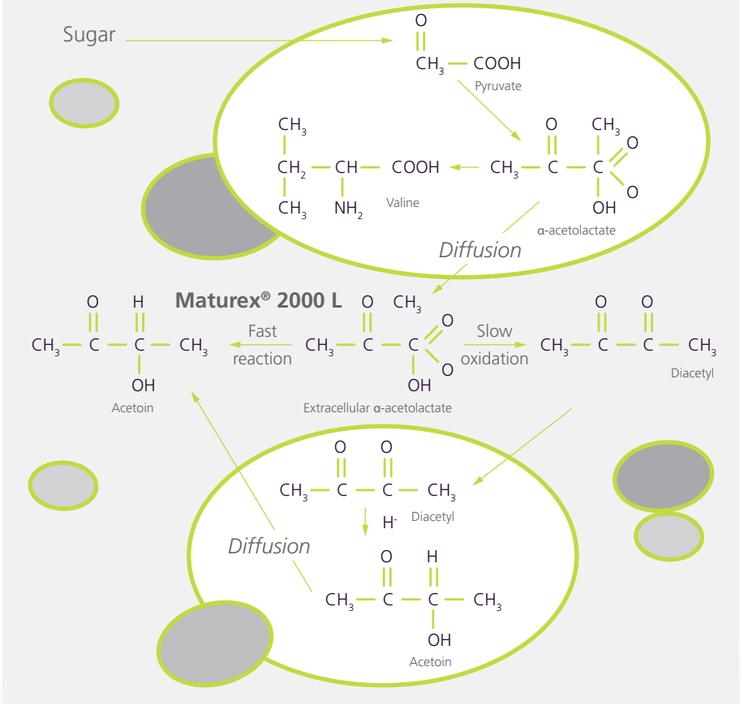


Fig. 8.3-3. Generation and reduction of diacetyl within the yeast cell and in the extracellular medium in the presence of Novozymes Maturex® 2000 L

8.4 pH and temperature curves

Fig. 8.4-1 and 8.4-2 show the influence of temperature and pH on the activity of Maturex 2000 L.

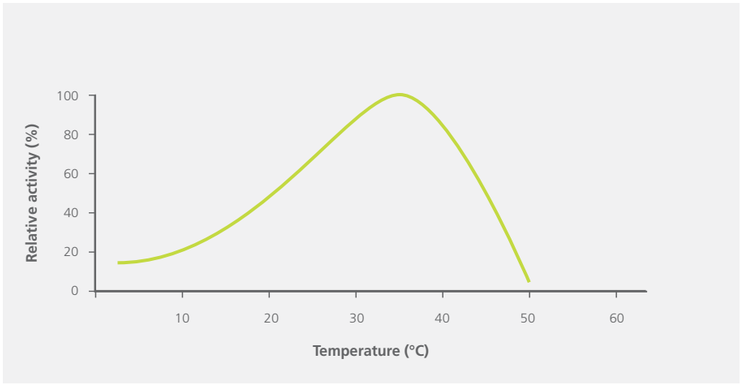


Fig. 8.4-1. Influence of temperature on the activity of Novozymes Maturex® 2000 L

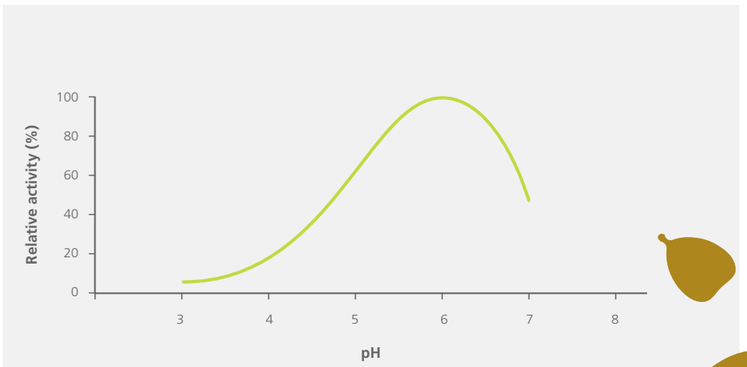


Fig. 8.4-2. Influence of pH on the activity of Novozymes Maturex® 2000 L

8.5 Practical applications

Maturex 2000 L is a unique enzyme specially designed for the brewing industry, making it possible to re-think fermentation profiles of pilsner type beer, or any beer where the diacetyl flavor is unwanted.

Maturex has been used for many years for quality and cost saving reasons. It is used year round or during special periods with tight capacity, for example, during peak season to ensure the possibility of extra sales, or in increasing markets experiencing a lack of fermentation capacity. Maturex 2000 L can also be used, if the yeast produces extra diacetyl as a result of stress. This could be due to low FAN.

Maturex 2000 L is also used during the production of special beers, for example, using special yeast strains, cool fermentation or stopped fermentation.

In all cases addition of Maturex 2000 L will result in optimized productivity.

Monitoring the effect of Maturex 2000 L

Standard measurements for VDK and diacetyl, for example, ANALYTICA EBC 9.24.1 and 9.24.2 can be used to evaluate the effect of Maturex 2000 L. Throughout trials, it is recommended to follow the VDK or diacetyl development during fermentation by taking samples once or twice every day. Both methods can be used to measure the actual amount of VDK or diacetyl, as well as the "total VDK and diacetyl potential".

To measure the "total VDK and diacetyl potential", the wort or beer must be heat treated prior to analysis. Heat treatment at 60˚C for 90 minutes converts the precursor α-acetolactate and α-acetohydroxy-butyrate to diacetyl and 2,3-Pentandione, respectively.

Please note that Maturex 2000 L works on the precursor released into the fermenting wort. These precursor can be excreted by yeast, and also by some microorganisms lacking ALDC, such as Lactococcus lactic and Pediococcus damnosus. Some microorganisms, however, contain ALDC, and consequently diacetyl is formed inside the cells. In these cases, Maturex 2000 L cannot reduce or eliminate diacetyl formation.

8.6 Practical examples

1. Diacetyl rest – large scale trial

Using a standard fermentation temperature profile with a diacetyl rest at 14.5˚C, the addition of Maturex 2000 L resulted in achieving acceptable diacetyl values 4 days early – at day 7 instead of day 11. This is demonstrated in Fig. 8.6-1 and 8.6-2. In this case, the diacetyl rest was reduced from 4 to 2 days thereby saving energy.

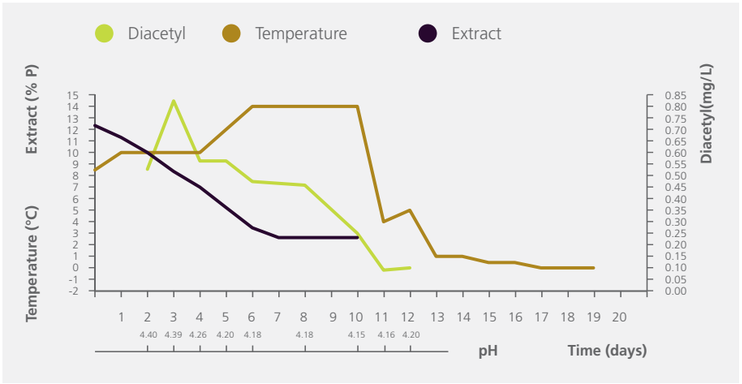


Fig. 8.6-1. Reference

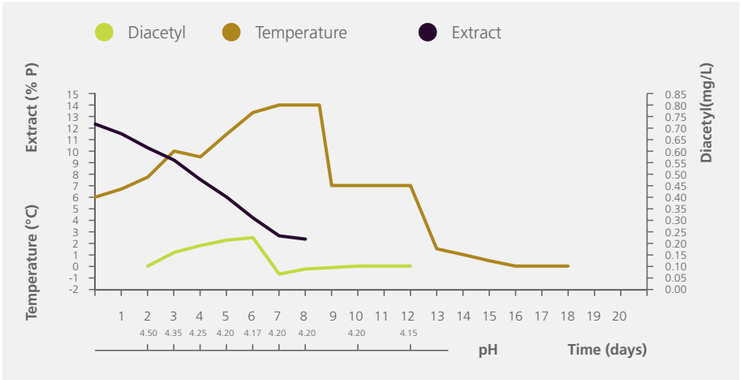


Fig. 8.6-2. Trial with Novozymes Maturex® 2000 L addition (2g/hl) and reduced diacetyl rest

2. Warm main fermentation – warm maturation – large scale trial

The initial fermentation temperature was 9°C and the maximal temperature 20°C. Using Maturex 2000 L dosed at 1g/hl, the level of acceptable diacetyl of 0.07 mg/l was reached when final attenuation was reached. This was after 84 hours of fermentation, which can be compared to 132 hours as experienced during the reference test without Maturex 2000 L. This is demonstrated in Fig. 8.6-3.

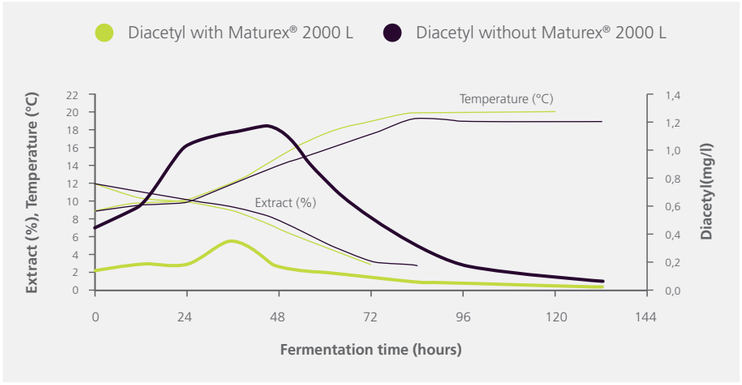


Fig. 8.6-3. The effect of Novozymes Maturex® 2000 L on the diacetyl content

3. Capacity increase by regular use of Maturex 2000 L

After implementation of regular Maturex 2000 L use, and with no change in the fermentation profile, it was possible to achieve a 30% output increase through the filters. This means that maturation time can be shortened by three days, requiring just one day instead of four. This is demonstrated in Fig.8.6-4 A and 8.6-4 B.

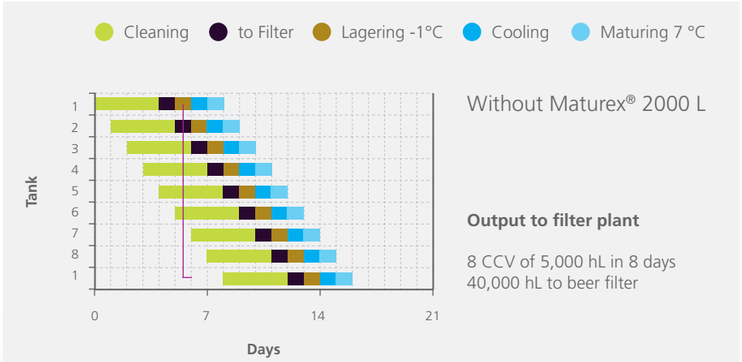


Fig. 8.6-4 A. Reference

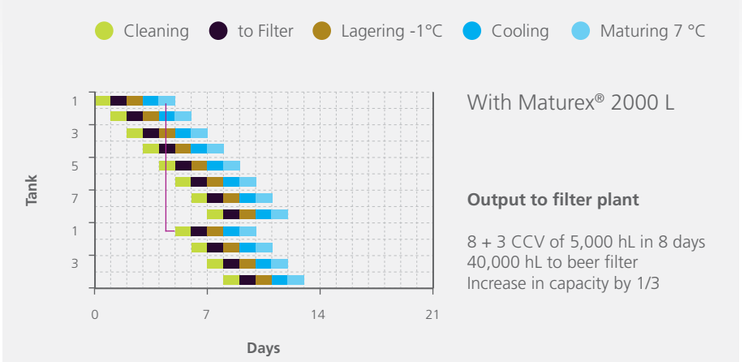


Fig. 8.6-4 B. Output with regular use of Novozymes Maturex® 2000 L

8.7 Enzyme data table

Novozymes Maturex® 2000 L	
Declared enzyme	Acetolactate decarboxylase (ALDC)
Catalyzes the following reaction:	(2S)-2-hydroxy-2-methyl-3-oxobutanoate <=> (3R)-3-hydroxybutan-2-one + CO_2
Declared activity	2000 ADU/g
E.C/ I.U.B. no:	4.1.1.5
Physical form	Liquid
Production method	Submerged fermentation of a genetically modified microorganism. The enzyme protein, which in itself is not genetically modified, is separated and purified from the production organism.

Table 8.7-1. Enzyme data

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