DSM Food Specialties

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``Exploring Brewing Enzymes``

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Contents :

1) About Enzymes in General.

2) Brewing Enzymes - Endogenous & Exogenous.

3) Exogenous Enzymes - Application & Advantages
   - Recently launched products
   - Future trend & development

4) Summary & Way Forward.
About Enzymes in General

Enzymes are:

• proteins with catalytic activity or biological catalyst
• natural products, present in all living organisms
• keys in nature to build and degrade organic material.

A well known enzyme: amylase from saliva, important for a good «pre-digestion» of starch containing foodstuff

• specific: catalyse one type of reaction
• dependent on temperature and pH conditions
• inactivated by extreme conditions of temperature
3-D structure of an Enzyme
How do Enzymes work?

Enzyme

Modified substrate

Enzyme

Enzyme

Enzyme

Modified substrate
one enzyme = one substrate theory
Brewing Enzymes:

- **Endogenous** enzymes in malt -
  # produced during **barley germination**.
  # released from the **aleurone** layer of the barley grain.
  # done during the **malting process**.
German Purity Law for brewing :-
``Reinheitsgebot``

- ``Only barley (malted), hops and water could be used in the brewing of beer``
- Established in 1516
- This year 2016, is 500\textsuperscript{th} year jubilee
Barley to Malt

- Spraying the barley
- Empty Germination Vessel
- Germination Box
Barley to Malt

Before Steeping

After Steeping

After germination
Malting Process: KILNING

Kilning

- Green malt is transferred to kilns for warm air drying to arrest the germination process and reduce the moisture content of the grain to 3-4%.
- The kilning process takes around 18 - 24 hours. At this stage the malt takes on its distinctive flavour and colour.
Malted barley
The key malt enzymes
Unmodified Barley endosperm

Modified Malt endosperm
# Malt vs Barley brewing :-

## Differences in physiochemical characteristics

<table>
<thead>
<tr>
<th>Enzymatic activities</th>
<th>MALT</th>
<th>BARLEY</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Amylase</td>
<td>β-Amylase</td>
<td>β-Amylase</td>
</tr>
<tr>
<td>β-Amylase</td>
<td>Limit Dextrinase</td>
<td>Peptidases</td>
</tr>
<tr>
<td>Peptidases</td>
<td>Endo-proteases</td>
<td></td>
</tr>
<tr>
<td>β-glucanase</td>
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| Friability Moisture           | Good 3-4%                                 | Poor 12-14%                  |
This is the **most important step in the Brewhouse** :- **Mashing Regime**

The enzymes present in the barley malt contain all that is required to:

1) Convert the starch to fermentable sugars – mainly maltose

   temp. range  \( \alpha \)-amylase  60C to 74C

   \( \beta \)-amylase  58C to 69C

2) Hydrolyse proteins into peptides and amino acids

   temp. range  carboxy & amino peptidases; endoproteases  <52C

3) Breakdown the hemi-cellulytic material which makes up the cell walls

   temp. range  \( \beta \)-glucanases  <45C
Typical temperatures for barley malt mash

- **Proteolysis**: 45 – 50 °C
- **Saccharification**: 60 – 65 °C
- **Liquefaction**: 70 – 75 °C
**Exogenous or Industrial** enzymes :-

- **Exogenous** enzymes are :-
  
  # external enzymes produced by any living organism.
  
  # added to the brewing process.
Exogenous enzymes are produced from different sources:

1) Fungal Sources
   • Trichoderma longibrachiatum
   • Aspergillus oryzae
   • Aspergillus niger

2) Bacterial Sources
   • Bacillus amyloliquefaciens

3) Plant Sources
   • Papaya

4) Animal Sources
   • Pancreatic enzymes
Food enzyme production flow diagram

Strain
Lyophilised
Freeze dried

Pre-culture 1
Preculture 2
Inoculum
Fermentation
Solid-liquid Separation

Ultrafiltration Concentration

Formulation Standardisation
Packaging

Plate Filtration 1

Plate Filtration 2

Liquid

Blending
Packaging
Standardization
Enzyme Fermentation

Feed composition
- Carbon source
- Nitrogen source
- Trace elements

Growth factors
- pH
- O2

Growth of desired microorganism producing desired enzyme
Use of *Exogenous* enzymes in brewing:

**Advantages** :

1) Reduce production cost and have process flexibility by adopting high % of adjunct in the brewhouse grist recipe (rice, corn, barley, sorghum etc)

Feasible recipe (proven successful):

- # 60% rice
- # 30-100% barley brewing
- # 100% sorghum
• Brewers Compass™ is a natural mix of enzyme activities, complementing and working synergistically with barley enzymes
• Brewers Compass™ added at mash-in
• Total mash profile time can be reduced as the percentage of malt in the grist increases
• Dose rate dependent on mash cycle time available, raw material quality (malt and barley) and economics

### Dose rate recommendations

<table>
<thead>
<tr>
<th>Barley (%)</th>
<th>Malt (%)</th>
<th>Dose rate (kg/T grains)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0</td>
<td>2.0 - 4.0</td>
</tr>
<tr>
<td>80</td>
<td>20</td>
<td>1.3 - 3.0</td>
</tr>
<tr>
<td>60</td>
<td>40</td>
<td>0.5 - 1.0</td>
</tr>
<tr>
<td>40</td>
<td>60</td>
<td>0.3 - 0.6</td>
</tr>
<tr>
<td>30</td>
<td>70</td>
<td>0.2 - 0.4</td>
</tr>
</tbody>
</table>
2) Increase Brewhouse efficiency and yield :-

# could smoothen process variation whenever there is raw materials quality fluctuation .

# more robust in term of thermal stability , pH tolerance and hence better performance than malt enzymes.
3) Could improve wort filtration and finished beer filtration by adding thermal stable beta glucanase + xylanase at the mashing stage.

Resulting:
- # better brewhouse and cellar yield
- # reduced cost of production
- # reduced filter aids usage and reduced waste disposal
- # improved production efficiency
- # reduced carbon footprint
Green Technology

4) Safe the environment by reducing the carbon footprint in the brewing process.

Since, # lesser malt are used
# better brewhouse yield
# better plant efficiency
# lesser energy used
Summary of LCA results kg CO$_2$ equivalent

CO2-eq emission per Hl beer (from field to BBT)

- 100% malt, conventional stab
- 100% malt, PSEP stab
- 100% barley, conventional stab
- 100% barley, PSEP stab

Avg efficiency
State of art eff.
LCA Output summary, 100% malt, stabilised with Brewers Clarex

**Barley**
- Malt, 16 kg, 10.4 kg CO₂ eq.
- Grist, 16 kg, 10.7 kg CO₂ eq.
- Mash, 56 kg, 10.7 kg CO₂ eq.
- Wort, 79.2 kg, 5.0 kg CO₂ eq.
- Feed, 13.7 kg, -6.1 kg CO₂ eq.

**Others**
- Yeast, COD, etc, 1.4 kg CO₂ eq.
- Others, 0.116 kg CO₂ eq.
- Others, 0.013 kg CO₂ eq.

**Beer stabilizer**
- Brewers Clarex, 0.013 kg CO₂ eq.
- Electricity, 7.4 MJ, 1.4 kg CO₂ eq.
- Heat, 34.4 MJ, 2.44 kg CO₂ eq.
- Heat, 86.1 MJ, 6.1 kg CO₂ eq.
- Electricity lower for PSEP compared to SHG + PVPP

**Energy**
- Electricity, 22.9 MJ, 4.3 kg CO₂ eq.
- Heat, 86.1 MJ, 6.1 kg CO₂ eq.

**Heat**
- Beer, 1 Hl, 16.8 kg CO₂ eq.

**Others**
- Feed, 13.7 kg, -5.7 kg CO₂ eq.
- Electricity, 7.4 MJ, -0.4 kg CO₂ eq.

**Beer**
- Malt, 16 kg, 10 kg CO₂ eq.
- Grist, 16 kg, 10.4 kg CO₂ eq.
- Mash, 56 kg, 10.7 kg CO₂ eq.
- Wort, 79.2 kg, 5.0 kg CO₂ eq.

**Total CO₂ eq.**
- Barley: 6.5 kg CO₂ eq.
- Beer: 16.8 kg CO₂ eq.
- Energy: 30.2 kg CO₂ eq.
- Heat: 92.2 kg CO₂ eq.
- Others: 1.4 kg CO₂ eq.
- Total: 152.3 kg CO₂ eq.
LCA Output summary, 100% barley brewed with Brewers Compass, stabilised with SHG + PVPP

- Yeast, COD, etc: 1.4 kg CO₂ eq.
- Feed: 13.7 kg, -6.1 kg CO₂ eq.
- Enzyme complex: 0.05 kg, 0.3 kg CO₂ eq.
- Grist: 17.6 kg, 6.6 kg CO₂ eq.
- Malt: 16 kg, 10 kg CO₂ eq.
- Barley: 17.6 kg, 6.2 kg CO₂ eq.
- Wort: 79.2 kg, 1.4 kg CO₂ eq.
- Mash: 56 kg, 7.1 kg CO₂ eq.
- Beer: 1 Hl, 14.3 kg CO₂ eq. (-15%)
LCA Output summary, 100 % barley brewed with Brewers Compass, stabilised with Brewers Clarex

- Barley: 17.6 kg, 6.2 kg CO₂ eq.
- Malt, convt: 16 kg, 10 kg CO₂ eq.
- Mash: 56 kg, 7.1 kg CO₂ eq.
- Grist: 17.6 kg, 6.6 kg CO₂ eq.
- Wort: 79.2 kg, 1.4 kg CO₂ eq.
- Feed: 13.7 kg, -6.1 kg CO₂ eq.
- Yeast, COD, etc: 1.4 kg CO₂ eq.
- Enzyme complex: 0.05 kg, 0.3 kg CO₂ eq.

- Beer, 1 Hl: 13.2 kg CO₂ eq. (-21%)
- Beer stabilizer: 0.013 kg CO₂ eq.
- Electricity: 22.9 MJ, 4.3 kg CO₂ eq.
- Heat: 86.1 MJ, 6.1 kg CO₂ eq.
- Electricity lower for PSEP compared to SHG + PVPP
- Others: 0.016 kg CO₂ eq.

Electricity lower for PSEP compared to SHG + PVPP.
Producing Specialty beers :-

5) Option of producing Specialty beers such as Dry beers or Low Calorie beers may need some specific enzymatic activities.

Such as glucoamylase

Dry beer & Low Calorie beers :-

• with a low carbohydrate level (dextrins).
• Could be achieved by a controlled hydrolysis of dextrins by glucoamylase
• This is not naturally present in the malt.
COMPARATIVE ACTION OF VARIOUS AMYLASES ON STARCH

Values in %

Malt amylases

- 31
- 60
- 18
- 7

Bacterial amylase

- 8
- 68
- 10
- 3

Fungal amylase

- 21
- 26
- 50

Gluco-amylase

- 7
- 7

Legend:
- Glucose
- Maltose
- Maltotriose
- Dextrins

DSM
BRIGHT SCIENCE. BRIGHTER LIVING.
**Problems / Challenges**

- Liquefaction and saccharification problems.
- Lazy fermentation or insufficient limit-attenuation.
- Colloidal hazes and filtration problems related to β-glucans.
- Chill hazes.

**Enzyme type**

- Alpha Amylase
- Glucoamylase
- Beta glucanse
- Proline Specific Endo Protease

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**Unlimited. DSM**
Potential benefits of Using Exogenous Enzymes in Brewing

→ 80 % tested malts are significantly improvable.

→ Starch is often responsible for filtration delays and beer hazes.

→ Wort viscosity may be decreased by a maximum of 10 % to 15 %.

→ Wort filterability often improved by more than 30%.

→ Beer filtration sessions are longer (sometimes + 50 % to + 60 %), with savings in water, steam and filter aids.

→ Extract is significantly improved (average of + 1 %).

→ High brilliance of the wort, guarantee of a good fermentation.
Recently launched Exogenous Enzymes :-

1) **Brewers Clarex** for colloidal haze stabilization :-
   - a proline-specific endo-protease
   - hydrolyses **only** the haze –active proteins (proline) where bonding with the polyphenols occurs, hence preventing the complex formation of chilled haze.
Computer simulation of interaction between proteins and polyphenols :-

Proline in chain 1

Proline in chain 2
Computer simulation of colloidal haze formation :-}
Brewers Clarex stabilizes beer by specifically degrading haze sensitive proteins (because these are high in Proline content).

- **Haze sensitive proteins**
  - High in **Proline** content
  - These complex with polyphenols to form colloidal beer haze

- **DSM Brewers Clarex specific hydrolysis**
  - (only Proline rich proteins)

- **Hydrolyzed peptides**
  - Short fragments of Haze sensitive proteins
  - Other (foam positive) proteins are not touched.
Degraded protein will not form visible aggregates

Hydrolyzed peptides

Peptide-polyphenol aggregates are **too small to be visible**
Advantages of using Brewers Clarex:

- Removes the deep cooling step in stabilization, **significant maturation capacity increase**.
- Increases cold stabilization **temperature** (up to 7°C tested) **significant energy savings**.
- Does not have to be filtered out and no rinsing is needed, resulting **reduced beer loss** and **filtration cycle increased**.
- Is a liquid technology, **no production waste** as compared with other powder stabilizers.
- **Reduced carbon footprint** → a green technology.
- Is easy to apply, **low dosage** (2-3 g/hl), needs **no big CAPEX investment**.
- Has **no impact on beer** (taste or foam) quality.
2) Produced Gluten Free beer using Brewers Clarex
Euromonitor: Gluten-free audience is far beyond coeliacs :-

GLOBAL INSIGHT
Target Audience Stretches Beyond Coeliac Sufferers

- Coeliac disease
- Sensitive to gluten
- Gluten-free diet considered as healthier
- Feeling more energetic
- Weight management
Gluten-free touches close to 1 in 10 consumers in some European markets

Source: Mintel, 2015
A record number of gluten-free beers launched in 2015

Source: Mintel GNPD, January 2016
Making beer gluten free

- Regular beer contains gluten from barley
- Using gluten-free grains such as rice, sorghum greatly impacts the taste.
- With enzymes brewers can easily create gluten-free beers from malted barley
- Brewers Clarex® is the patented solution to make the same beer, same taste, gluten free!
Adding Brewers Clarex® in the brewing process

Add Brewers Clarex, 2-3 gr./hl before fermentation

Measure gluten content with R5 competitive ELISA

20-100 ppm gluten

< 10 ppm gluten

Gluten free (green) beer
**Future trends** on Acceptance of Exogenous Enzymes

Higher acceptance in food application due to:

- Clean, Sustainable process
- Less chemicals, lower temperatures...
- Pressure on raw material costs / increasing population
- Need to optimize the use of agro-resources
- Waste valorization
New Exogenous Enzymes Development - Future Trend

• Focus on :-
  # Eco /Environment friendly
  # Simplified production process
  # Reduced energy consumption
  # Minimum CAPEX
  # Improved yield and process efficiency
  # Minimize waste production, reuse by-product
  # Non GMO
Summary and Way Forward :-

• Considering the many challenges facing the world such as resources scarcity, continuous population growth, environment destruction, technology advances and on the other hand, the advantages of using Exogenous Enzymes, so are you still hesitating of using them?

--→ The Decision is Yours!
Thank you for your attention