A novel glucoamylase for highly attenuated beer production

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2. Novozymes A/S, Bagsvaerd, Denmark 2880
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Agenda

• Beer Attenuation – Low Carbohydrate/Light Beer Category

• Malt and Solid Adjunct Starch Structure and Enzymes that breakdown starch

• Introducing a novel enzyme in development and evaluation (NS 26262)

• Laboratory Brewing Trials (evaluating NS 26262 against a Control – a blend containing both glucoamylase and Pullulanase enzymes)
  • Reduced Mashing Time Trials and impact on Sugar Profile (both Malt/Adjunct and 100% Malt brews)
  • Impact on Non-Fermentable Sugar profile with reduced mashing time
  • Impact on Filtration
  • Iso-Thermal Brewing

• Summary
Beer Attenuation:

• Low/Ultra Low Carbohydrate and Light Style Beers
  ➢ Beer Brands with Different levels of Carbohydrate and Alcohol Content

• “Light Beers” - Dominant Style of Beer in the USA (51%)

• Significant Growth Category in several countries around the World

• Appealing to younger drinkers and those seeking a healthy alternative to traditional full strength beers (which are higher in carbohydrates)

• The need to achieve a specific level of Attenuation is by selecting the appropriate brewing conditions

• For High to Very High Attenuated beers it is necessary to use the appropriate enzyme or a combination of enzymes at selected dosing levels and the appropriate brewing conditions (Time and Temperature)
Structure of Starch: Amylose and Amylopectin

Amylose:
- $\alpha$-1,4 glycosidic bond (~100%)
- 10–30% typical composition

Amylopectin:
- $\alpha$-1,4 glycosidic bond (94–95%)
- $\alpha$-1,6 glycosidic bond (5–6%)
- 70–90% typical composition
ENZYMES FOR CONTROL OF ATTENUATION

Fungal alpha-amylase
✓ Hydrolyzes 1,4-alpha linkages in dextrins
✓ Mainly maltotriose and branched dextrins

Pullulanase
✓ Debranching enzyme
✓ Hydrolyzes 1,6-alpha linkages of branched dextrins
✓ Linear carbohydrates

Amyloglucosidase
✓ Hydrolyzes 1,4-alpha and 1,6-alpha linkages of dextrins from non-reducing end to release glucose
# Novozymes’ Commercial Enzymes for Attenuation

<table>
<thead>
<tr>
<th>Novozyme Commercial Brewing Attenuation Enzymes</th>
<th>Function and Activity</th>
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</thead>
<tbody>
<tr>
<td><strong>Attenuzyme® Pro</strong></td>
<td>A high performing blend of glucoamylase and pullulanase that makes it possible to hit high attenuation targets in short reaction times, taking advantage of the synergy between these two enzyme activities during the hydrolysis of amylopectin and amylose.</td>
</tr>
<tr>
<td><strong>Attenuzyme® Core</strong></td>
<td>A glucoamylase for producing highly fermentable glucose based worts.</td>
</tr>
<tr>
<td><strong>AMG® 300 L BrewQ</strong></td>
<td>A classic glucoamylase for producing highly fermentable glucose based worts.</td>
</tr>
<tr>
<td><strong>Novozym® 26062</strong></td>
<td>A pullulanase that accelerates attenuation and can be applied for a moderate increase in the attenuation of maltose based wort.</td>
</tr>
<tr>
<td><strong>Fungamyl® BrewQ</strong></td>
<td>A maltogenic alpha amylase used to breakdown of starches, facilitating a higher alcohol output.</td>
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Novozymes Enzyme - NS 26262:

Origin:

• A novel PE Glucoamylase enzyme, a variant from Penicillium oxalicum glucoamylase and expressed in Aspergillus niger is currently being developed and evaluated by Novozymes.

Action:

• The novel enzyme has been shown (in laboratory and pilot plant studies) to have superior glucoamylase activity allowing for a much faster saccharification process. Unlike most glucoamylases, this amyloligocamylase (AMG) is able to hydrolyse both (1,4) and (1,6) alpha-D-glucoside linkages.
• The new glucoamylase appears to provided higher levels of attenuation than other best-in-class commercially available attenuation solutions in the market.
• The higher-level activity of this new enzymes could potentially allow for the production of the lowest Carbohydrate beers.
• Has a higher level thermostability (78°C+) which permits simultaneous lautering and saccharification thereby reducing the brewing processing time frame.
Fermentable Sugar Profile for Reduced Mashing Time for Malt/Adjunct Brews

(A) Standard malt/adjunct mashing profile

(B) Reduced Time malt/adjunct mashing profile

Time reduction of 45%

(A) Standard malt/adjunct mashing

(B) Time reduced malt/adjunct mashing

Same sugar profile

Attenuzyme® Pro  NS 26262
Fermentable Sugar Profile for Reduced Mashing Time for 100% Malt Brews

- Time reduction of 50%
- Glucose yield increase of 1%

(A) Standard malt mashing
(B) Time reduced malt mashing
Non-Fermentable Sugar Profile for Reduced Mashing Time

(A) Reduced Time malt/adjunct mashing profile

(B) Non-Fermentable Profile

(B) Non-Fermentables Profile
**Filtration Performance:**

- Filtration volume increase of 10% within the first 10 minutes
- Final volume increase of 2%

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**Time reduced malt/adjunct mashing**

- Filtration volume increase of 40% within the first 10 minutes
- Final volume increase of 2%
Isothermal Mashing Trials:

- Time reduction of +60%
- Glucose yield increase of 3%

(A) Standard 100% malt Mashing Profile
(B) Isothermal Mashing Profile

(A) Sugar Profile - Standard Mash
(B) Sugar Profile - Isothermal Mash

- DP1
- Fermentables
- Calc RDF

% of DP1, Fermentables, Calc RDF for Attenuzyme® Pro and NS 26262.
The experimental Enzyme NS 26262 has been shown to

• First single component enzyme containing both glucoamylase with pullulanase activity
• Degrades starch and dextrins to a maximum yield of fermentable glucose and acts faster than any other AMG’s on the market
• The ability to reduce mashing times whilst still meeting the required Sugar Profile
• Faster Lautering Capability with slightly increase final volume
• The Novel enzyme is Thermostable (78°C+) which allows it to work under Lautering and makes it less dependent on the saccharification rest
• Its thermostable properties also allow for process simplification such as protein rest followed by isothermal mashing (significantly reducing mashing time)
• Can be applied with a broad range of raw materials