November 2, 2005

# Enzymes in brewing


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**www.novozymes.com**


Rocky Mountain District

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Unlocking the magic of nature

# …continuing enzyme presentation in May 2005

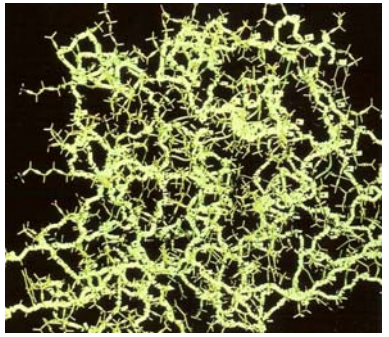
Coors Brewery Institute

## Practical Brewing Enzymology

Tobin L. Eppard

***Rocky Mountain District - MBAA***

May 11, 2005



# Today's presentation

- Recap of the nature of enzymes
- Malt enzymes vs. exogenous enzymes
- Benefits of using exogenous enzymes
  - optimization of "traditional brewing" resulting in
    - 1) More throughput,
    - 2) Use of non-malt raw materials,
    - 3) Reduction of off-flavors
  - production of new beer types
- New opportunities with exogenous enzymes
- Future collaboration between brewmasters and enzyme suppliers
  - Enzymes moving from" first aid kit" to "natural part of the process"
  - Enzymes moving from "part of a problem" to "part of a solution"

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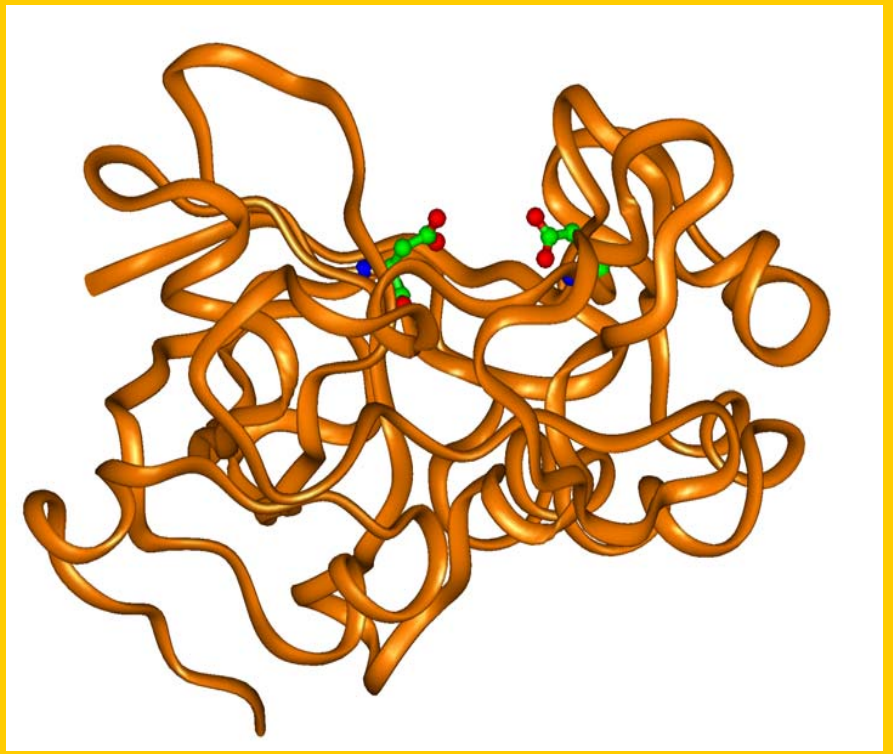
# What are enzymes?

- Enzyme are
  - *proteins* with the same characteristics as any other protein in nature including that it is totally degradable and digestible
  - proteins with an *active site*
  - true *catalysts*



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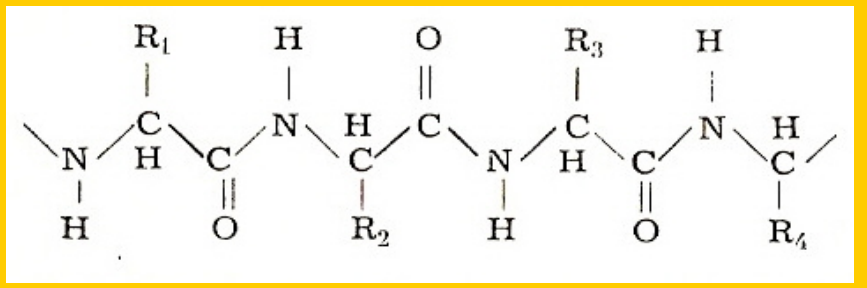
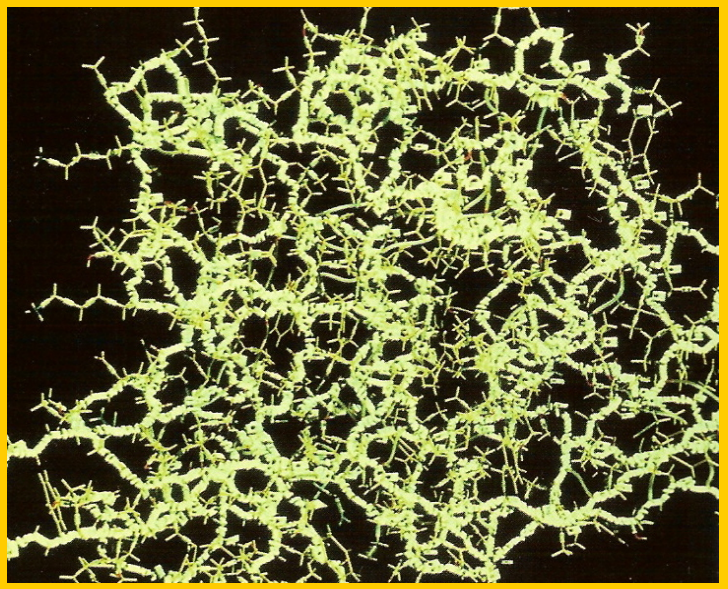
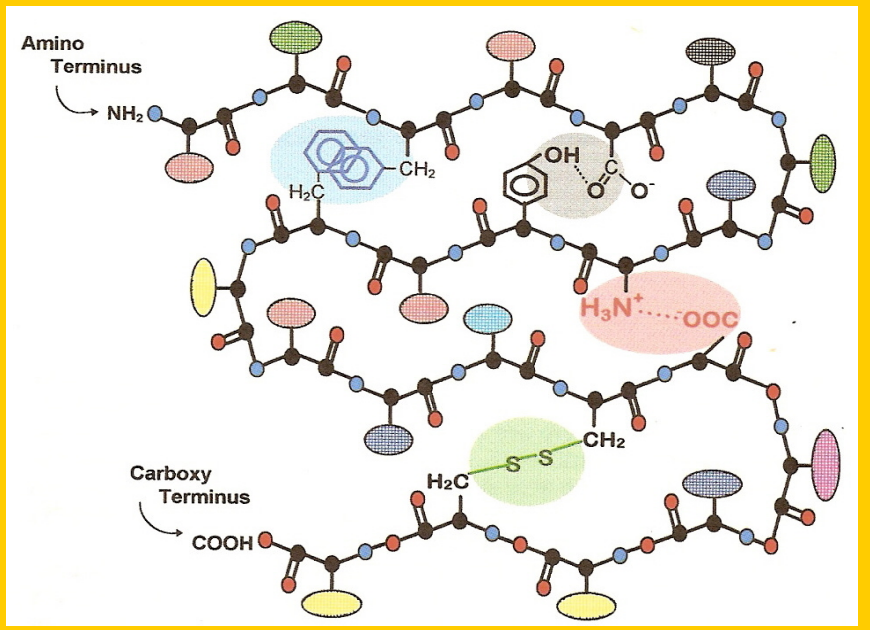
# Enzymes are proteins

- build up of amino acids and folded in……

- Secondary Structure
- Tertiary Structure
- Quaternary Structure



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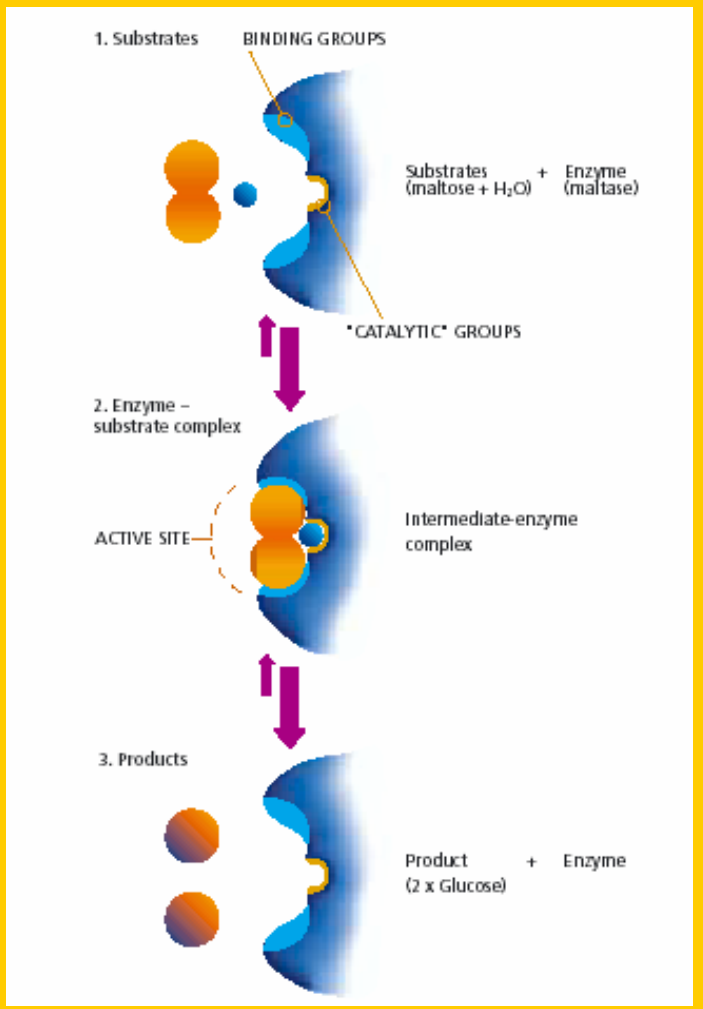
# Enzymes are proteins with an active site

The enzyme captures one or more substrates of a biochemical reaction and brings them into intimate contact as an intermediate-enzyme complex at an active site containing binding groups and catalytic groups.

A Simple enzyme-catalyzed reaction can be described as follows:

$$E + S \rightleftarrows ES \rightarrow E + P$$

where E, S and P represents the enzyme, substrate and product respectively and ES represents a reversible enzyme-substrate product

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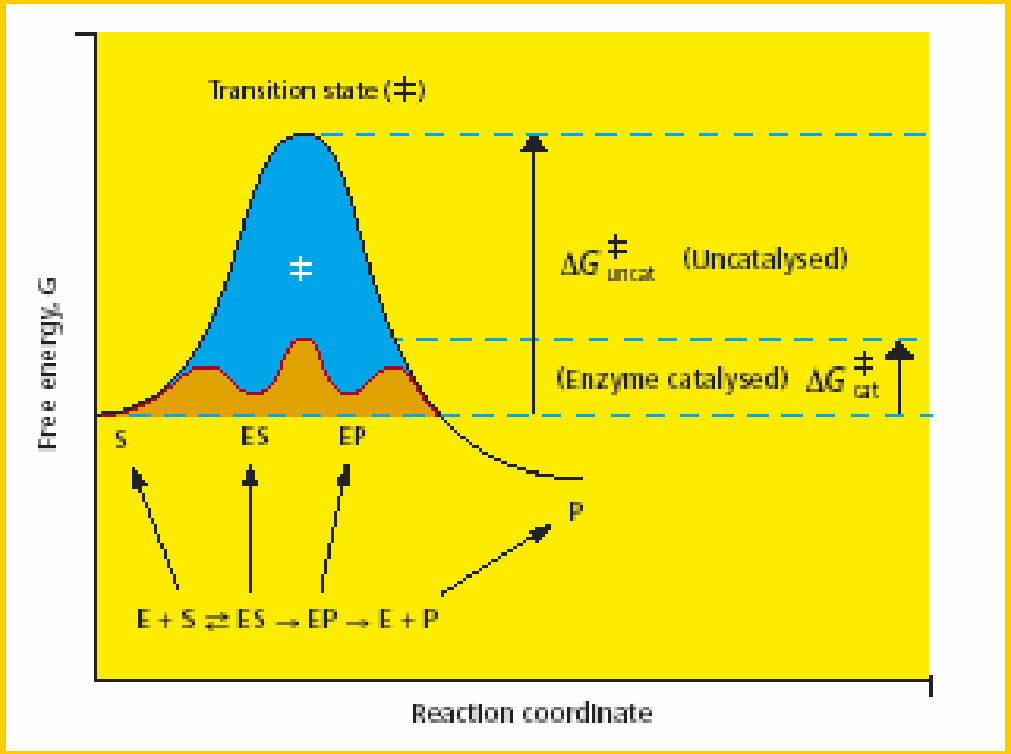
# Enzymes are true catalysts

A catalyst significantly speeds up a chemical process that would otherwise occur very slowly, or not at all at the given conditions

The catalyst works by lowering the activation energy (ΔG) needed for the reaction to take place

The catalytic enzymes remain unchanged after the reaction

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# Enzymes are everywhere

- Enzymes are found in every living organism: man, animals, plants or microorganisms such as bacteria or fungi.
- Enzymes are the tools of nature. They cut and paste products such as nutrients. Enzymes speed up all vital biological processes.
- The enzymes in the stomach, for instance, ensure that food is cut into tiny particles that can be converted into energy in the body. Wherever one substance needs to be transformed into another, nature uses enzymes to speed up the process.

***When you eat bread α-amylases in the salvia will already start to break down the bread starch to sugar in your mouth***

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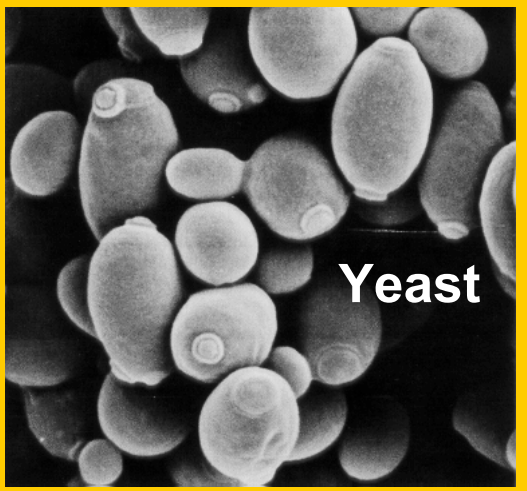
# Enzymes in brewing

**Malt and yeast can provide the necessary enzymes to**

- Convert starch into fermentable sugars
- Provide free amino nitrogen (FAN)
- Facilitate mash and beer filtration by hydrolysis of  e.g. beta-glucan
- Convert fermentable sugar to alcohol and $CO_2$
- Catalyze formation of flavour substances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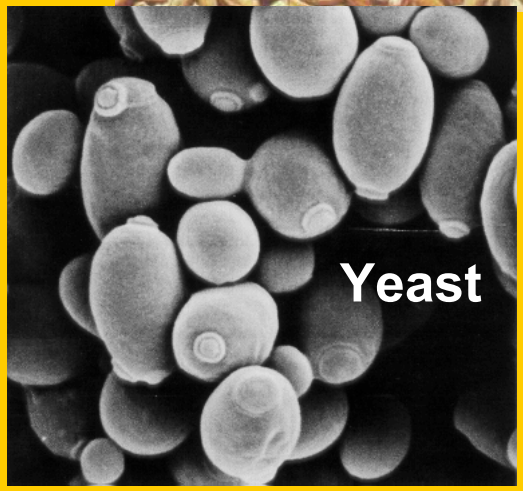
*..... So - You **can** brew a pretty good beer without exogenious enzymes*


But not without enzymes !

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# Why use exogenous enzymes when malt enzymes are available ??

- To do the job faster
- To do the job more effectively
- To do the job that malt enzymes are not able to do

***Because industrial enzymes can be different from malt enzymes***

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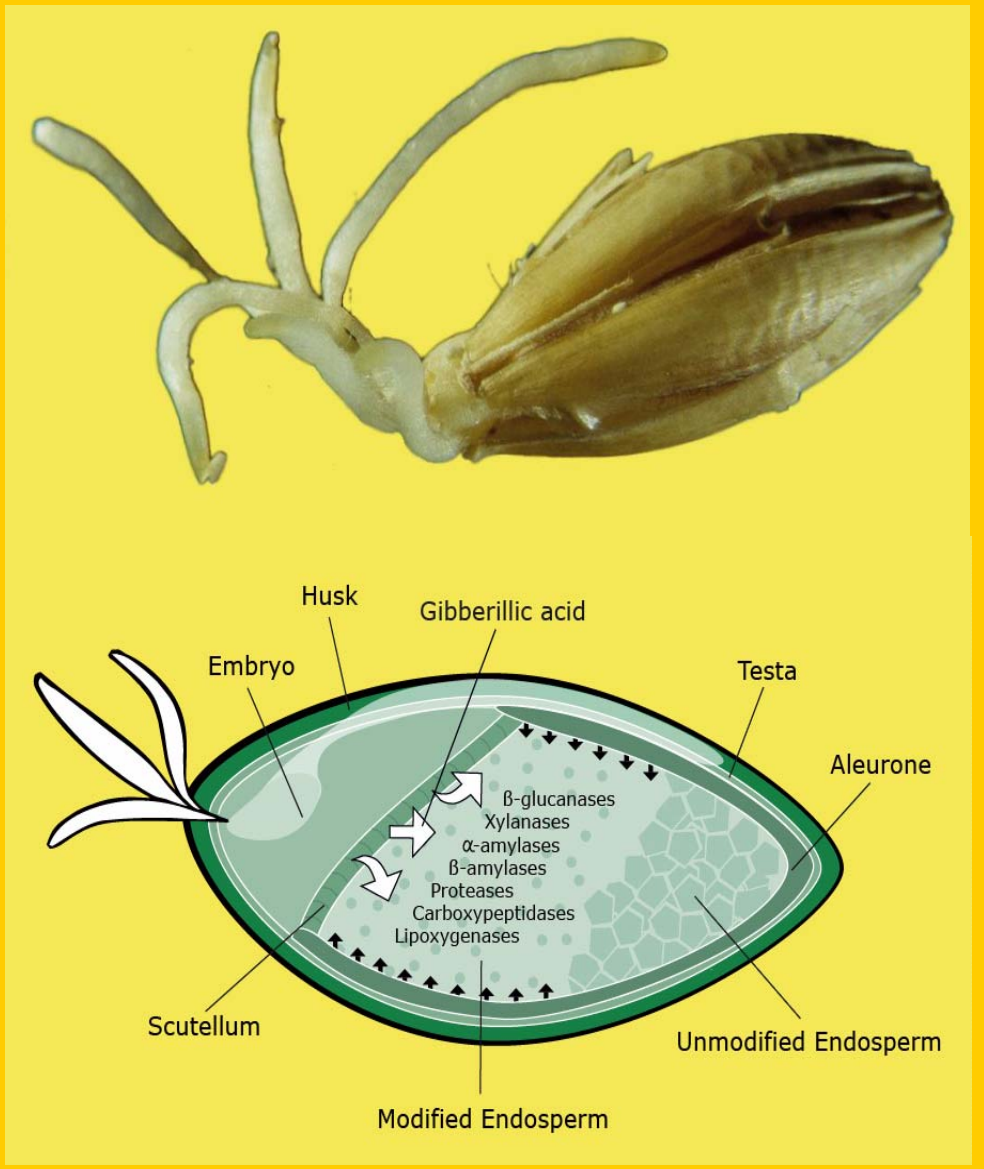
# Malt enzymes

- During germination of e.g. barley a lot of active enzymes are produced

- Most of these enzymes will remain in the final malt, and will act during mashing to form the wort components



# Malt enzymes

| Enzyme | Optimum mashing pH | Optimum mashing temperature | Inactivation temperature |
|---|---|---|---|
| β-glucanase | 4.5-5-0 | 40-50°C | 40 - 55 |
| β-glucan solubilase | 4.6-4.9 | 62°C | 73 |
| Endo-peptidase | 5.0-5.2 | 50-60°C | 70 |
| Carboxy-peptidase | 5.2 | 50-60°C | 70 |
| Amino/ dipeptidase | 7.2-8.2 | 40-45°C | 55 |
| Limit dextrinase | 5.1 | 55-60°C | 65 |
| β-amylase | 5.4-5.6 | 60-65°C | 70 |
| α-amylase | 5.6-5.8 | 70-75°C | 80 |

| Enzyme | Optimum mashing pH | Optimum mashing temperature | Inactivation temperature |
|---|---|---|---|
| Xylanase | 5.0 | - | - |
| Pullanase | 5.0-5.2 | 40°C | 70°C |
| Arabinosidase | 4.6-4.7 | 40°C | 60°C |
| Cellulase | 4.5-5.0 | 20°C | 20°C |
| Lipase | 6.8 | 35-40°C | 60°C |
| Peroxidase | - | 40-50°C | 65°C |
| Polyphenol-oxidase | - | 60-65°C | 80°C |
| Lipoxygenase | 6.5 | 40°C | 70°C |

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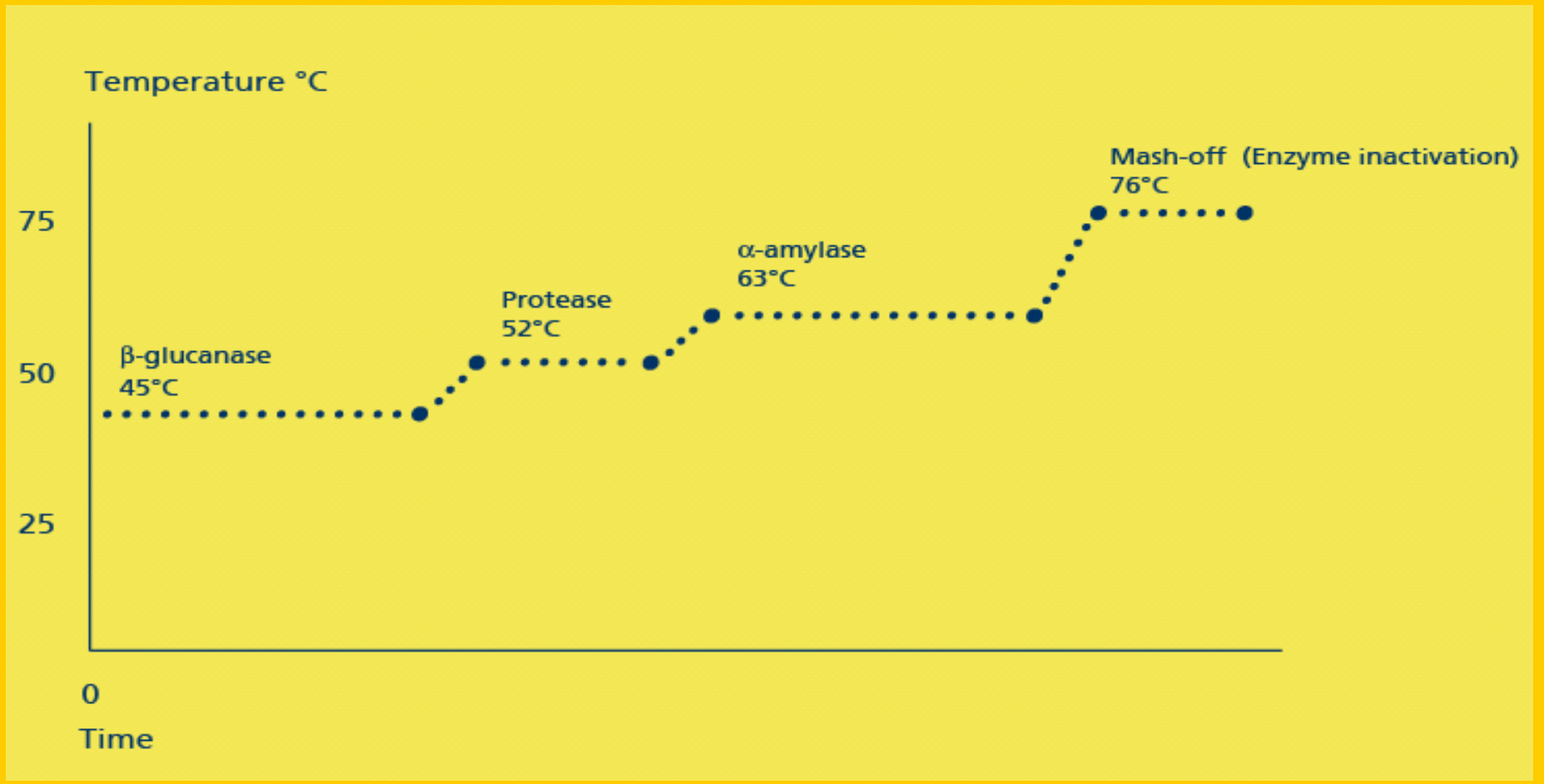
# Mashing programme for Pilsner-type beer

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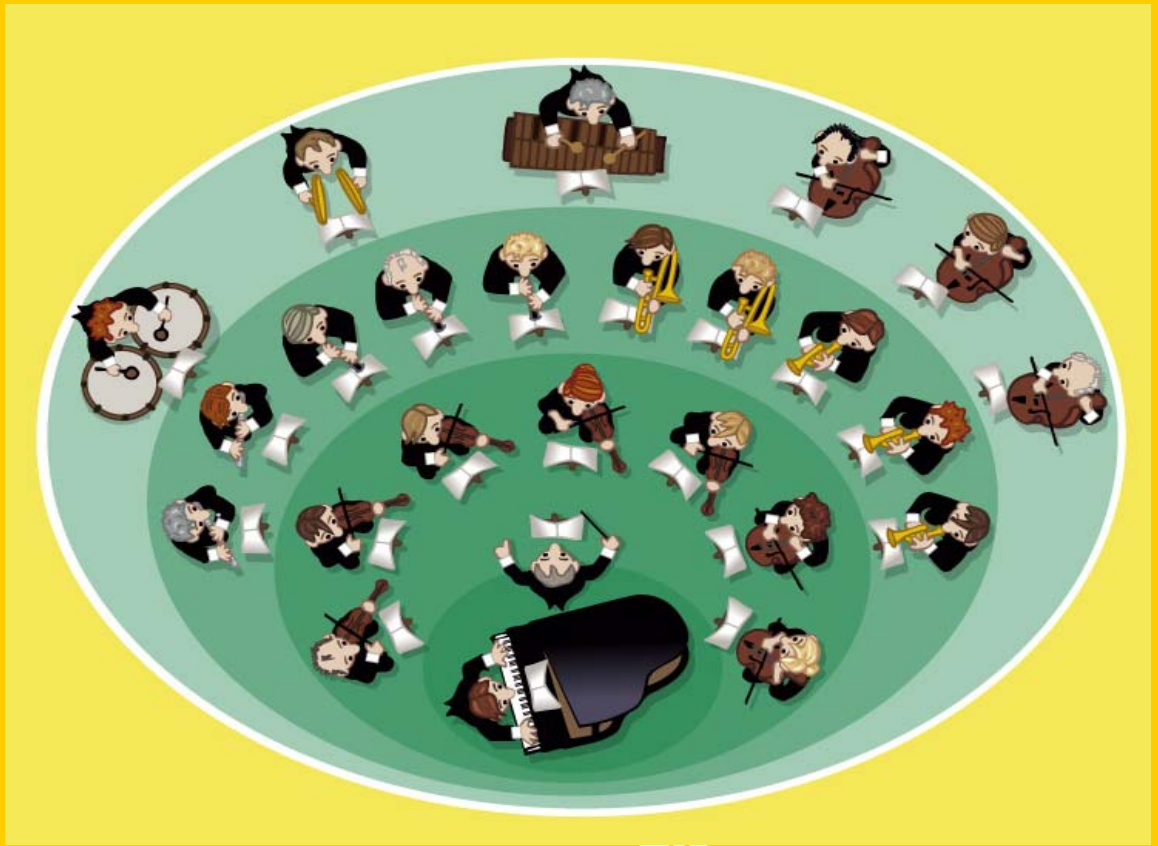
With exogenous enzymes you simply get

More freedom to act
More instruments to play on



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# Benefits of exogenous enzymes

- Supplement malt enzymes
- Add enzymes that are superior to malt enzymes
- Add enzymes with new properties

- Capacity increase
- Cost reduction
- Innovation
- Quality

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# Benefits of exogenous enzymes

- Optimization of “traditional brewing”
  - More throughput
  - Use of non-malt raw materials
  - Reduction of off-flavors

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# Best malt – Best equipment – Best enzyme

- Industrial tests were carried out in a modern European brewery making all-malt brews with well modifeid malt of international stardard using a traditional mashing profile

- A total of 29 test brews with **Viscoflow** compared with the normal production

  The average results were the following
  - 7% more brew house capacity (= 3 brews more per week)
  - 40% longer filtration runs giving 40% less kieselguhr consumption
  - 0.5% less "beer lost“
  - More clear wort resulting in faster fermentation = 7% increased capacity
  - Better clarity and taste of the final beer resulting in higher "Quality Index"

- ***No change in raw materials, equipment, procedures etc***

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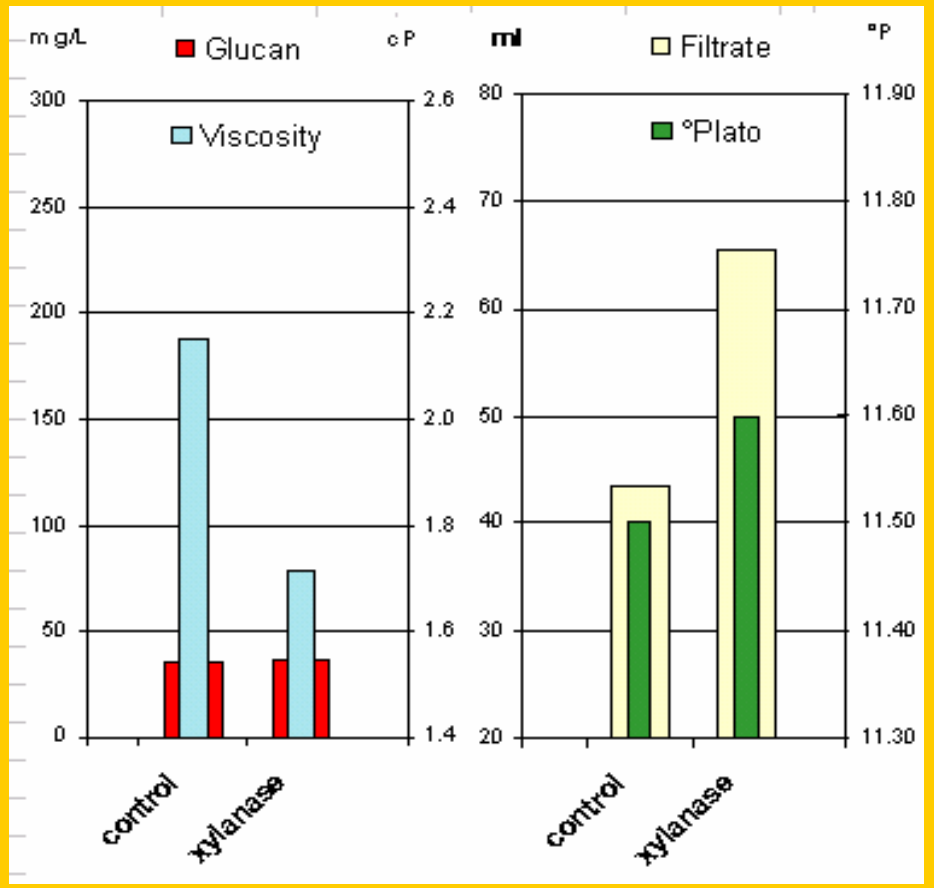
# Viscoflow with the right xylanase




- Greatly reduces wort viscosity
- Promotes faster wort separation
- Together with β-glucanases, helps release more extract

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# Quantification of benefits

**Economical Model for SBO enzymes**

***SBO = Smooth Brewing Operations***

| **Parameters (to be filled in by the brewery)** | **Unit** | | **Comments** |
|---|---|---|---|
| Enzyme Price | USD/kg | 15 | |
| Enzyme Dosis | g/ton malt | 150 | |
| Malt Price | USD/ton | 320 | |
| Original gravity of cold wort | % Pl | 12 | |
| Brewhouse extract yield | % | 76 | of total kg raw material (malt and adjunct) |
| Adjunct ratio | % | 0 | of total kg total raw material (malt and adjunct) |
| Total beer sales volume | hl/year | 1000000 | |
| No. of brews per day | brews/day | 8 | |
| Total no. of operators employed in the brewhouse | | 10 | |
| Total no. of operators employed in filtration | | 3 | |
| average wage for one operator (man hour cost) | USD/year | 32000 | |
| Average length of filterrun | hours | 6 | |
| Kieselguhr comsumption | kg/hl beer | 0,12 | total per hl sales beer |
| Kieselguhr price | USD/kg | 0,82 | |
| Cost of disposal of kieselguhr sludge | USD/l | 0,1 | |
| Water consumption for start-up/shut down of filter | hl/hl beer | 0,25 | total per hl sales beer |
| Fresh water price | USD/hl | 0,03 | |
| Waste water price | USD/hl | 0,1 | |
| Beer loss in filtration | % of volume | 1,5 | approximately 25 % of the total loss after wort cooler |
| Prodcution cost of filtered beer | USD/hl beer | 8,3 | |

**Extract (Brewhouse Yield)**

**Increase in brewhouse extract yield means less malt consumption**

| | | |
|---|---|---|
| Extract yield without SBO enzyme | % | 76 |
| Extract yield with SBO enzyme | % | 76,5 |
| Increase in extract yield | % | 0,5 |
| Saving in malt each % extract yield is increased | kg/hl*% | 0,215 |
| Cost saving KX | USD/hl | 0,0344 |

**Lautering**

**Reduction of brew seqeunce (if lautering is bottleneck)** ⇨ **more brew per day can be prodcued and man-hours can be reduced**

| | | |
|---|---|---|
| No. of brews per day without SBO enzyme | | 8 |
| No. of brews per day with SBO enzyme | | 8,56 |
| Increase in in no. of brews | | 0,56 |
| Cost saving KL | USD/hl | 0,0224 |

**Filtration**

**The filtration time before filter blocking can be increased** ⇨ **less filterruns to produce same amount of beer**
**reduction of man-hours, beer loss, kieselguhr and water consumption**

| | | |
|---|---|---|
| Filterrun length without SBO enzyme | hours | 6 |
| Filterrun length with SBO enzyme | hours | 10,8 |
| Increase in filterrun length | hours | 4,8 |
| Cost saving KF | USD/hl sales beer | 0,2361 |

**Economical Calculations**

| | | | | |
|---|---|---|---|---|
| Enzyme Treatment Costs | USD/ton malt | | 2,25 | |
| Beer/ton malt | hl/ton malt | | 60,43 | |
| **Enzyme treatment costs PE** | USD/hl beer | | **0,0372** | |
| KX | USD/hl beer | 0,0344 | | |
| KL | USD/hl beer | 0,0224 | | |
| KF | USD/hl beer | 0,2361 | | |
| **KX+KL+KF** | **USD/hl beer** | **0,2929** | | |
| **Benefit per hl beer = (KX+KL+KF)-PE** | **USD/hl beer** | | **0,2557** | positive result means cost savings by enzyme addition |
| **Total Benefit** | **USD/year** | | **255701** | negative result means enzyme cause extra costs |

Process figures and “today’s prices

Increased extract yield

Shorter mashing filtration time

Longer beer filtration cycles

**Total savings**

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## Economical Calculations

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| **Benefit per hl beer = (KX+KL+KF)-PE** | **USD/hl beer** | | **0,2557** | positive result means cost savings by enzyme addition |
| **Total Benefit** | **USD/year** | | **255701** | negative result means enzyme cause extra costs |

# High % adjunct or/and undermodified malt

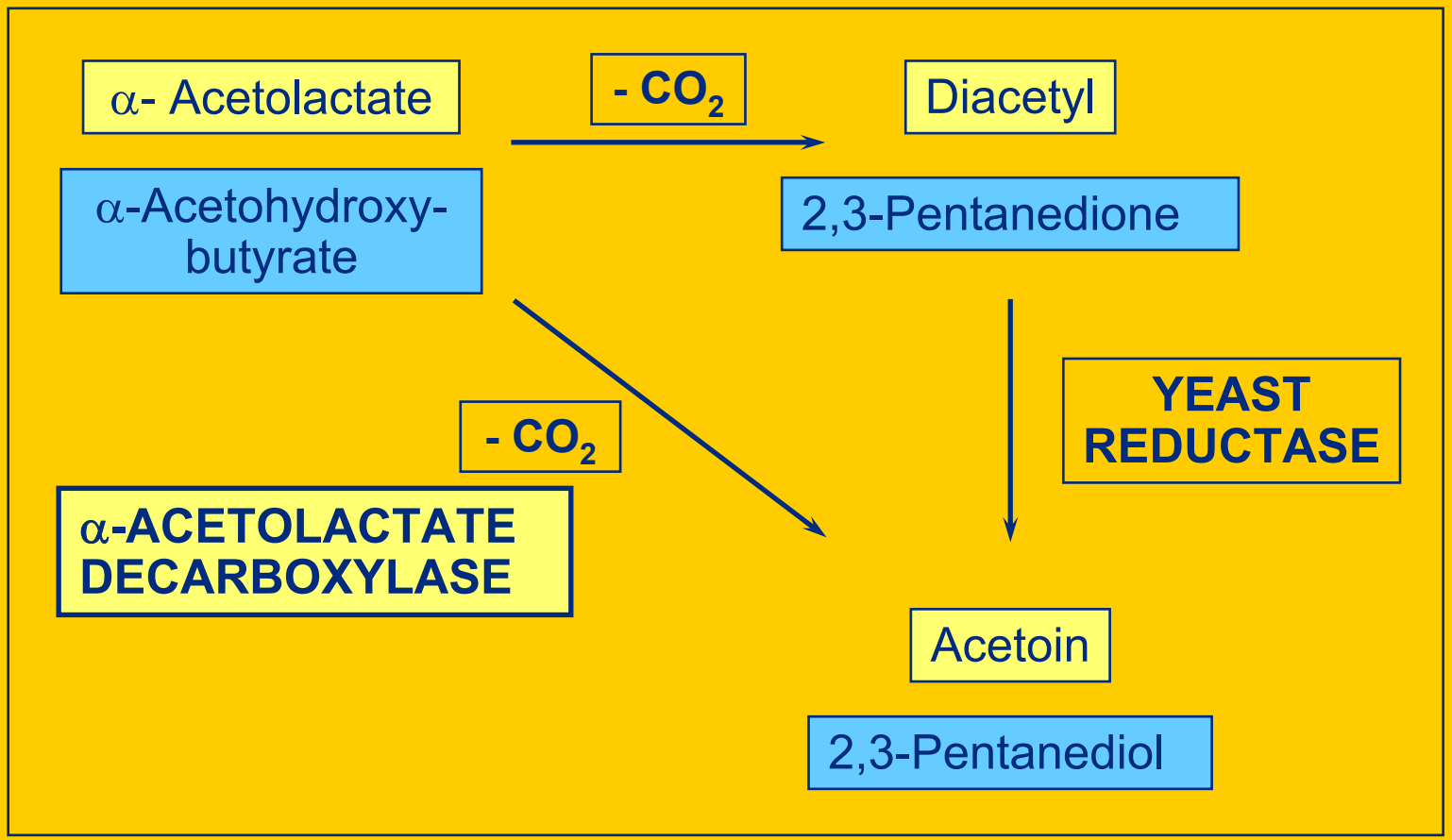
- Use of industrial enzymes in combination with undermodified malt or/and high ratios of adjunct gives additional savings on the cost of raw materials
- For these purposes β-glucanases and xylanases should be supplied with proteases. Special mixed products are made for this purpose

# High % adjunct or/and undermodified malt

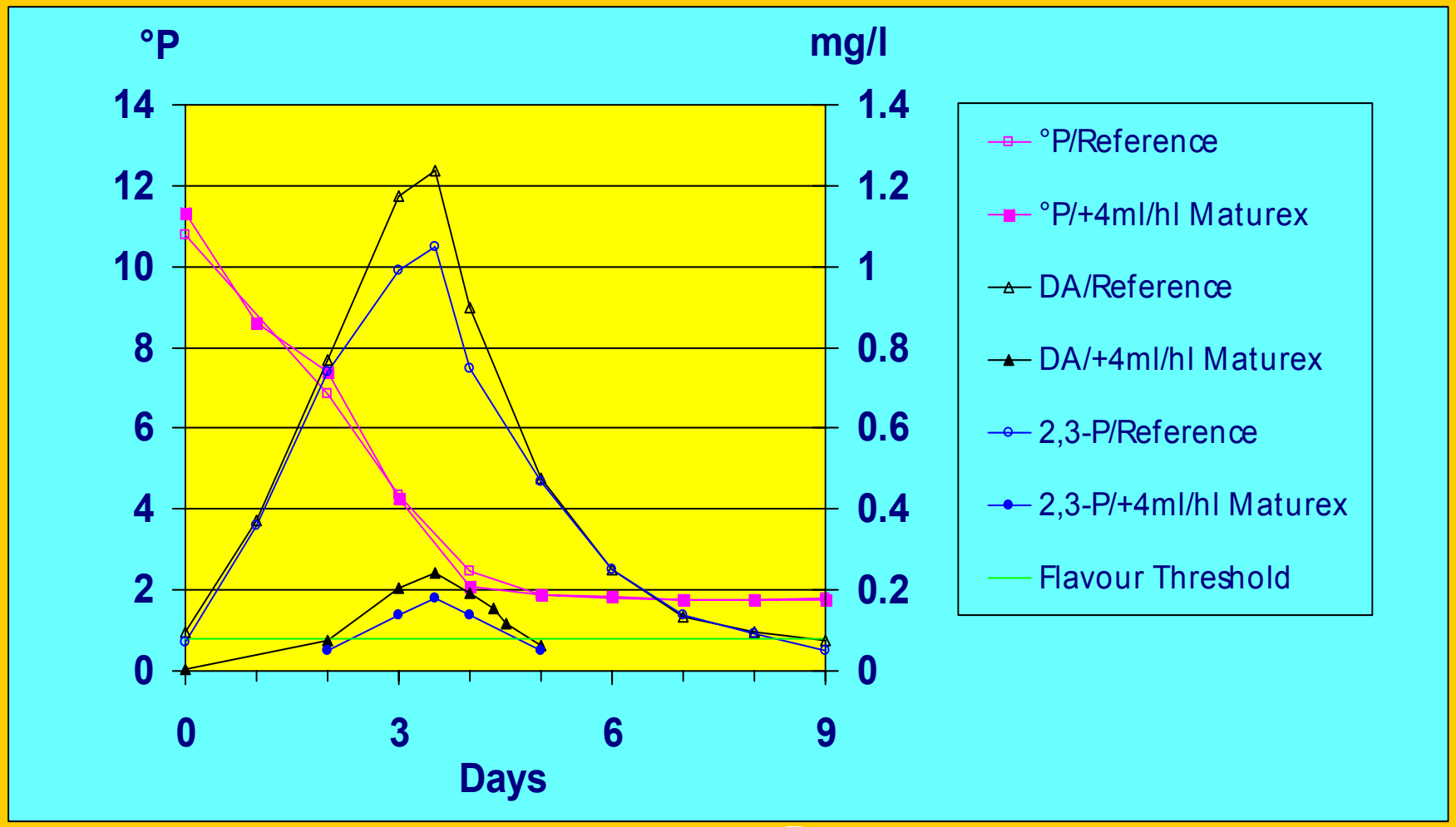
| Mother Beer type | Recipe | | Recommended enzyme products |
|---|---|---|---|
| Wort Type | ton/brew | ppm | |
| 16% Plato | 7,2 ton local malt | 1000 | Termamyl 120 L (for rice) |
| | 3,2 ton Imported malt | 100 | Cemerix 2XL |
| | 1,84 ton rice | 150 | Viscoflow MG |
| | 1,0 ton sugar | | |
| 15% Plato | 7.0 ton local malt | 200 | Termamyl 120 L |
| | 3,0 ton imported malt | 250 | Ceremix 2XL |
| | 1,9 ton local barley | 150 | Viscoflow MG |
| 17% Plato | 8,1 ton local malt | 200 | Termamyl 120 L |
| | 3,5 ton imported malt | 100 | Ceremix 2XL |
| | 2,61 ton sugar | 150 | Viscoflow MG |
| 16% Plato | 6,0 ton local malt | 150 | Termamyl 120 L |
| | 4,5 local barley | 650 | Ceremix 2XL |
| | | 200 | Viscoflow MG |
| 15% Plato | 6,2 ton local malt | 150 | Termamyl 120 L |
| | 2,6 ton imported malt | 100 | Ceremix 2XL |
| | 2,7 ton caramel malt | 150 | Viscoflow MG |
| 16% Plato | 3,9 ton local malt | 1000 | Termamyl 120 L (for rice) |
| | 3,9 ton imported malt | 150 | Viscoflow MG |
| | 3,0 ton rice | 225 | AMG 300 L |

# Direct off-flavor control

## – No formation of diacetyl



*Diacetyl is below threshold level at the end of fermentation….*

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# Indirect off-flavor control – Improved flavor stability

- High mashing-in temperature >60˚C
- **Low T2N formation**
- No malt β-glucanase
- High release of β-glucan
- **Termostable Viscoflow**

| **Mash-in Temperature** | **Enzyme treatment** | 23°P time | 27°P time | 23°P Fk index | 27°P Fk Index |
|---|---|---|---|---|---|
| 52°C/125.6°F | Control No enzyme | 11’ | 13’ | 0.22 | 0.50 |
| **52°C/125.6°F** | **Viscoflow** | **8’** | **7’** | **0.16** | **0.26** |
| | | | | | |
| 65°C/149°F | Control No enzyme | 17’ | 14’ | 0.34 | 0.52 |
| **65°C/149°F** | **Viscoflow** | **9’** | **9’** | **0.19** | **0.34** |

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# Benefits of exogenous enzymes

- Production of new beer types
  - Low carbohydrate beer
  - Happoshu
  - “Third category beer”

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# High attenuation – Low carbohydrate content

- Approximately 25% of the carbohydrate extract is present in the beer as non-fermentable short-chain dextrins
- When production of highly attenuated beer types is desired, an increased fermentability is targeted by further hydrolysis of residual dextrins
- Traditional brewing methods can't compensate for the lack of enzymes necessary for the purpose

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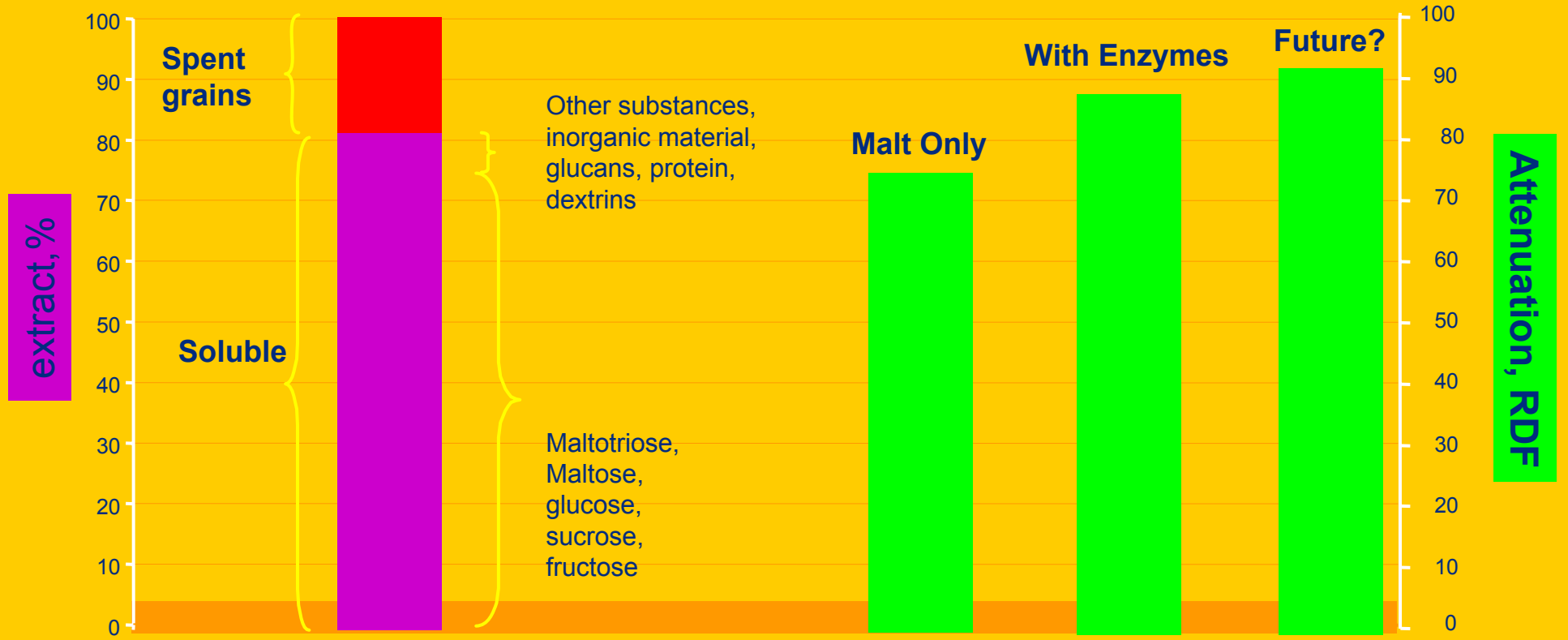
# Extract and Degree of Attenuation

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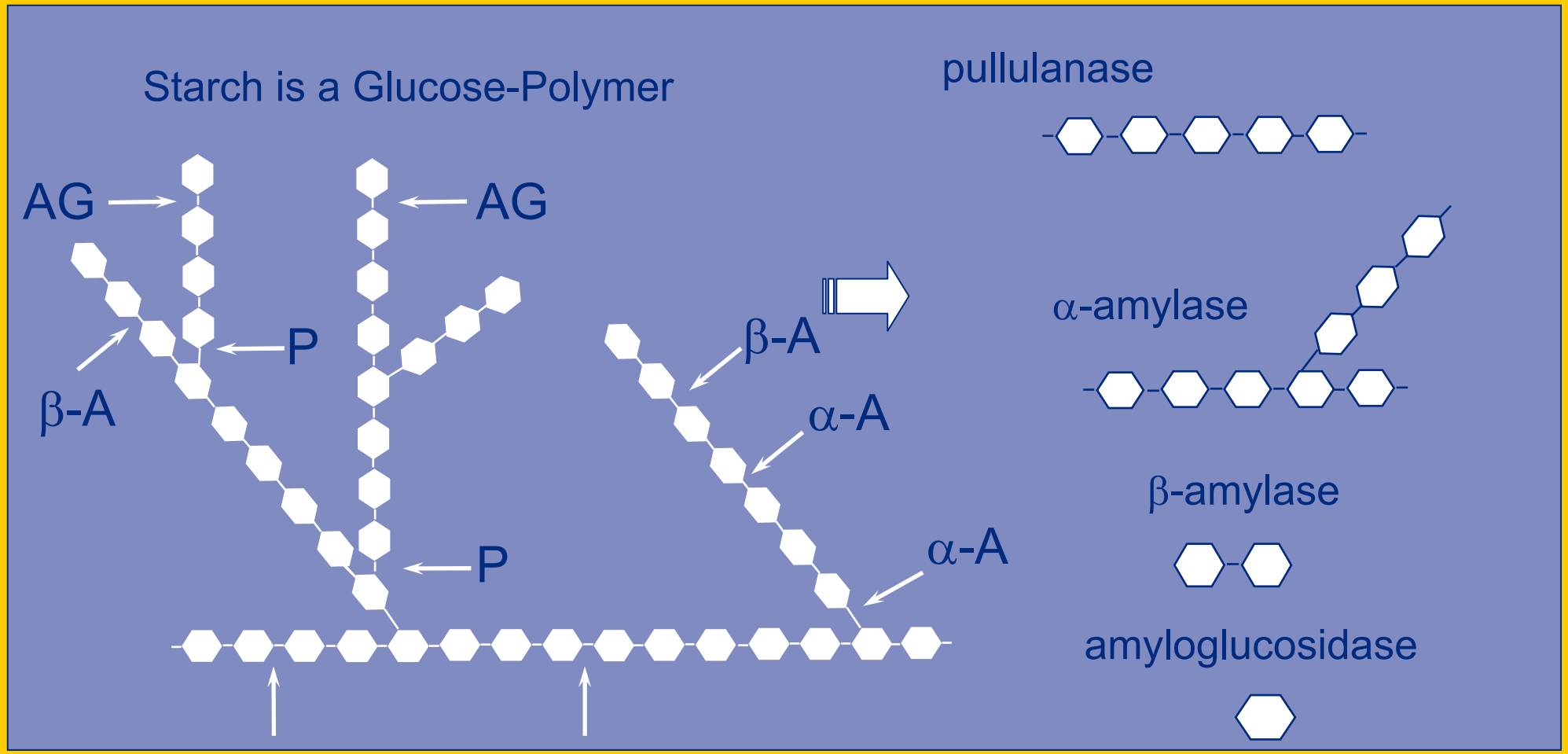
# Starch modifying enzymes

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# Enzymes for adjustment of attenuation

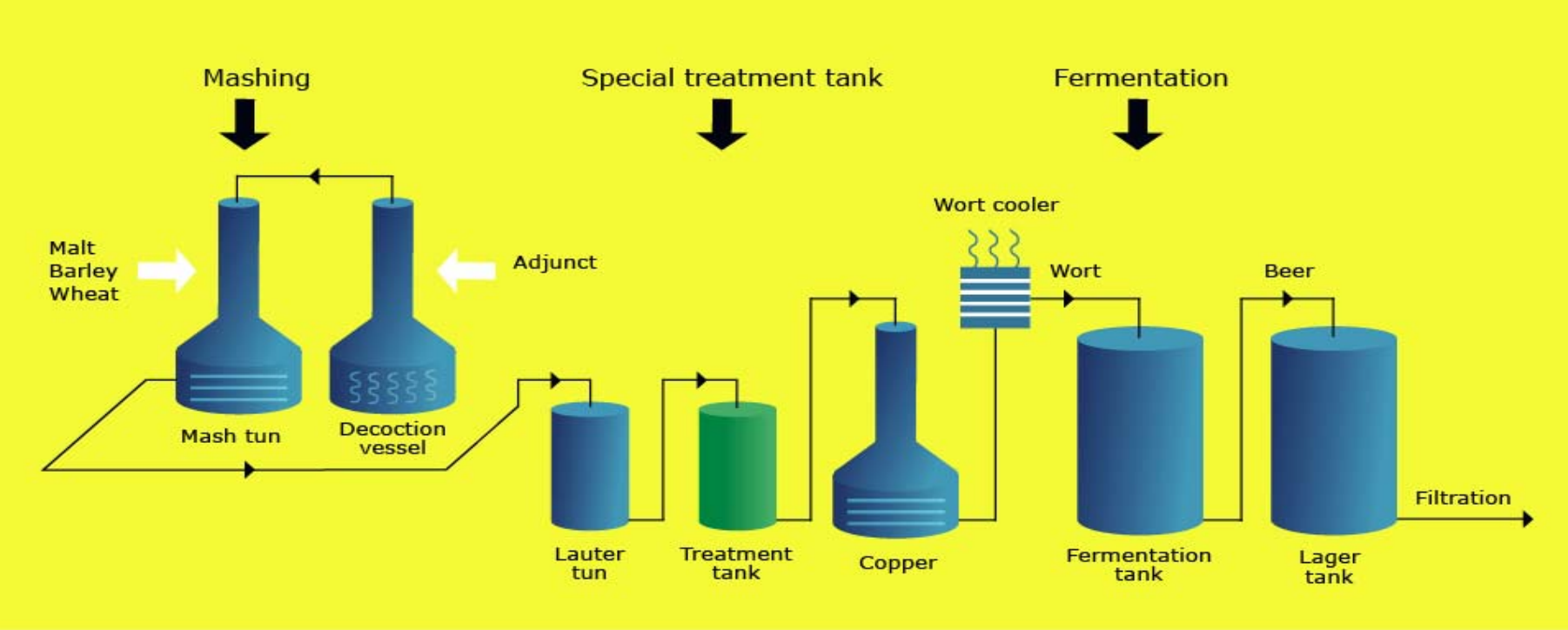
- Fungal alpha-amylase
  - Hydrolyzes 1,4-alpha linkages in dextrines
  - Mainly maltose and maltotriose and branched dextrins
- Pullulanase
  - Debranching enzyme
  - Hydrolyzes 1,6-alpha linkages of branched dextrins
  - Linear carbohydrates
- Amyloglucosidase
  - Hydrolyzes 1,4-alpha and 1,6-alpha linkages of dextrins from non-reducing end
  - Glucose

# Fermentability increase

- From small adjustments to super attenuated beer
- Enzymes in mash or to fermenter
  - Fungal alpha-amylases, e.g. Fungamyl 800L
  - Amyloglucosidases, e.g. AMG 300L
  - Pullulanase, e.g. Promozyme 400L
- Small adjustments
  - Fungamyl 800L in fermenter
- Highly attenuated beer
  - AMG and Promozyme or Fungamyl at mashing in
  - Promozyme at mashing-in + Fungamyl in fermenter
  - AMG in fermenter

# Fermentability increase

## Enzymes for attenuation control can be added at:




- **glucoamylase - pullulanase - α-amylase** ***alone or in combination***

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# New opportunities with exogenous enzymes

- New products
- New processes
- New raw materials
- **New mindset**
  - **by enzyme suppliers**
  - **by brewmasters**

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# New image of enzymes in the brewing industry

- ***Not*** **a "First aide Kit"**
- ***Not*** **a drug or medicine**
- ***Not*** **just for trouble shooting**

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# New image of enzymes in the brewing industry

- **Vitamins**
- **Natural processing aids**
- **Part of the solution**
- **A strategically choice**
- **For trouble prevention**

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# New opportunities with exogenous enzymes

- Common ideation sessions
- Partnership

***Still enzymes are the brewmaster's "helping hand", also in his/her new roles***

- *More for innovation*
- *Less for trouble shooting*

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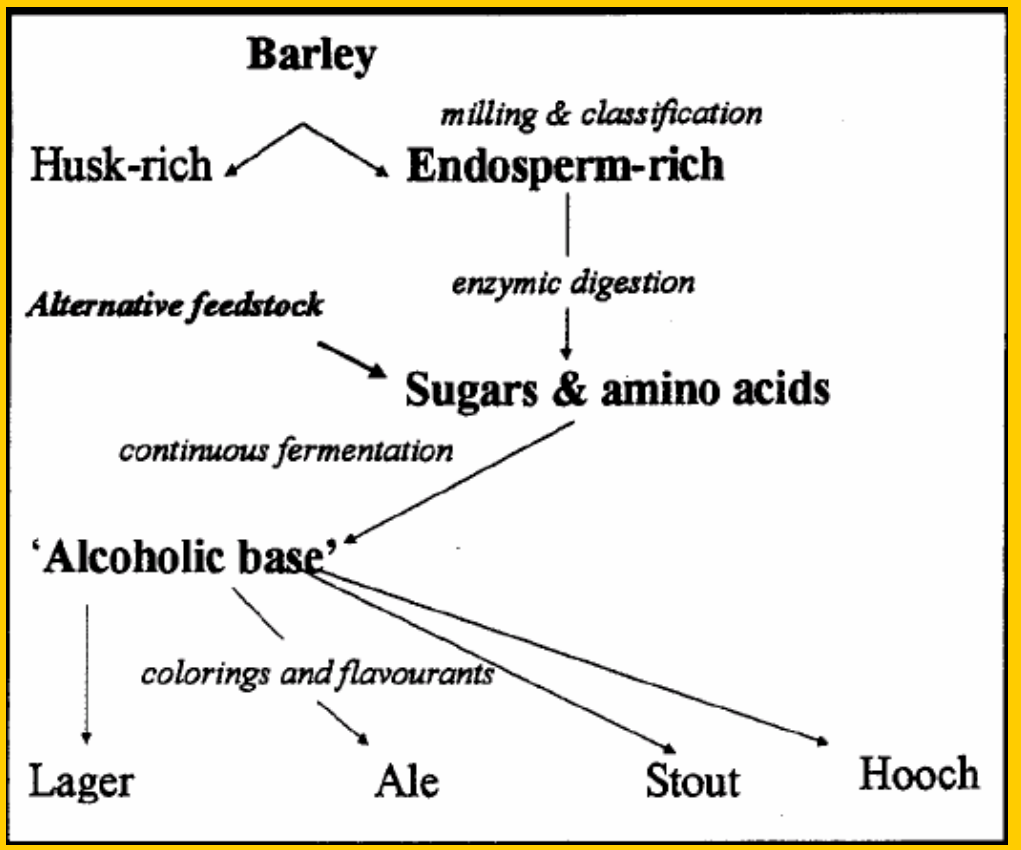
# Today’s brewmasters are not in the brew house

# New opportunities with exogenous enzymes

- Common ideation sessions
- Partnership

***Still enzymes are the brewmaster's "helping hand", also in his/her new roles***

- *More for innovation*
- *Less for trouble shooting*

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# Thank you for your attention - Cheers

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