YEAST METABOLISM AND ITS INFLUENCE IN BEER

Magda Costa
INGREDIENTS OF BEER

Ingredients of beer
Yeast
Fermentation
Different flavours
INGREDIENTS OF BEER

Water

Malt

Hops

Yeast

Big interactions

Ingredients of beer

Yeast

Fermentation

Different flavours
Yeast

Description

- Eukaryotic
- Unicellular organisms
- Fungus kingdom
- Mostly reproduce asexually by mitosis
**YEAST**

Types for beer production

- **Saccharomyces cerevisiae**:  
  - ‘Ale’ brewing yeast.  
  - 10 to 25 °C for fermentation.  
  - Top-fermenting.

- **Saccharomyces pastorianus**:  
  - ‘Lager’ brewing yeast.  
  - 7 to 15°C for fermentation.  
  - Bottom-fermenting.
YEAST

Internal organisation

Yeast

Genes

Translated to

Proteins

Organised into

Pathways

Producing

Metabolites

Ingredients of beer  Yeast  Fermentation  Different flavours
YEAST

Pathways organisation

Substrate → Enzyme 1 → Product 1 → ... → Enzyme n-1 → Enzyme n → Final product = Metabolite

Ingredients of beer

Yeast

Fermentation

Different flavours
YEAST

Enzymes function
QUIZ 1

You have 30 seconds to answer two questions:

1. How many are the main ingredients of beer?
   - 2.
   - 3.
   - 4.
   - 5.

2. How are called the two main types of brewing yeast?
   - Saccharomyces cerevisiae and Saccharomyces pastorianus.
   - Candida albicans and Candida blankii.
   - Brettanomyces bruxellensis and Brettanomyces anomalus.

The answers to all the questions are at the end of the course.
FERMENTATION

Carbohydrates preferences

Carbohydrates in wort (%)

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Percent composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>10–15</td>
</tr>
<tr>
<td>Fructose</td>
<td>1–2</td>
</tr>
<tr>
<td>Sucrose</td>
<td>1–2</td>
</tr>
<tr>
<td>Maltose</td>
<td>50–60</td>
</tr>
<tr>
<td>Maltotriose</td>
<td>15–20</td>
</tr>
<tr>
<td>Dextrins</td>
<td>20–30</td>
</tr>
</tbody>
</table>

Saccharomyces cerevisiae can ferment:
- Glucose
- Fructose
- Sucrose
- Maltose
- Maltotriose

Saccharomyces pastorianus can ferment:
- Glucose
- Fructose
- Sucrose
- Maltose
- Maltotriose
- Melibiose

Ingredients of beer
Yeast
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Different flavours
FERMENTATION

Carbohydrates transporters

**Facilitated transport**

Spontaneous passive transport: uses no ATP to move the molecules from one side to the other of the biological membrane. Follows concentration gradient.

Glucose/Fructose

*Hexose transporter (HXT1 & HXT2 & HXT3 & HXT4 & HXT6 & HXT7)*

Facilitated transport of glucose and fructose into the cells by hexose transporters. Some have high-glucose affinity to glucose, others low-glucose affinity.

Ingredients of beer  Yeast  Fermentation  Different flavours
FERMENTATION

Carbohydrates transporters

Active transport of molecules through the biological membrane from a region of lower concentration to high concentration. Since it is against the concentration gradient, the organisms need to spend energy: molecules of ATP or use the electrochemical gradient.

Maltose

- Maltose permease (MAL11 & MAL31)
- Maltase (MAL12 & MAL32)

α-D-glucopyranose
β-D-glucopyranose

Active transport of maltose into the cells by permeases, followed by metabolization of the molecule.

Ingredients of beer  Yeast  Fermentation  Different flavours
Carbohydrates transporters

**Invertase (SUC2)**

**Breaking down of sucrose molecule outside of the cells by invertase.** After that, glucose and fructose enter the cell by the transporters previous seen (hexose transporters).
FERMENTATION

Glycolysis & Fermentation

Fermentation with the Amoeba Sisters

Ingredients of beer  Yeast  Fermentation  Different flavours
FERMENTATION

Glycolysis

Beginning

Glucose
Fructose

Converted into

2 x Pyruvate

Several enzymatic reactions

End

2 x ATP

2 x NADH

In more detail:

Ingredients of beer
Yeast
Fermentation
Different flavours
FERMENTATION

Glycolysis part 1

1. Glucose → β-D-glucopyranose 6-phosphate
   - Hexokinase (HXK1 & HXK2)
   - ATP → ADP + H^+ + β-D-fructofuranose 6-phosphate
   - Phosphoglucone isomerase (PGI1)

2. β-D-fructofuranose 6-phosphate → β-D-fructose 1,6-bisphosphate
   - Hexokinase (HXK1 & HXK2)
   - ATP → ADP + H^+ + β-D-fructose 1,6-bisphosphate aldolase (FBA1)

3. β-D-fructose 1,6-bisphosphate → D-glyceraldehyde 3-phosphate
   - Fructose-1,6-bisphosphate aldolase (FBA1)

4. D-glyceraldehyde 3-phosphate → Glycerone phosphate

Glycolysis part 2

- Glycerol production

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FERMENTATION

Glycolysis part 2

D-glyceraldehyde 3-phosphate

NAD\textsuperscript{+} + Phosphate

NADH + H\textsuperscript{+}

\[
\text{Glyceraldehyde-3-phosphate dehydrogenase (TDH1 & TDH2 & TDH3)}
\]

3-phospho-D-glyceroyl-phosphate

ADP + H\textsuperscript{+}

\[
\text{3-phosphoglycerate kinase (PGK1)}
\]

3-phospho-D-glycerate

ATP

\[
\text{Glycerate phosphomutase (GPM1)}
\]

2-phospho-D-glycerate

H\textsubscript{2}O

\[
\text{Enolase (ENO1 & ENO2)}
\]

Phosphoenolpyruvate

ADP + H\textsuperscript{+}

\[
\text{Pyruvate kinase (PYK2 & CDC19)}
\]

Pyruvate

Ingredients of beer  Yeast  Fermentation  Different flavours
In more detail:

- **Pyruvate** → **Acetaldehyde** → **Ethanol**
  - **Enzymatic reaction**

- **Carbon dioxide**
  - **Produced from**

- **ATP**
  - **Produced from**

**Ingredients of beer**

- **Yeast**
  - **Fermentation**
  - **Different flavours**
Fermentation

Pyruvate \[ \rightarrow \] Ethanol

Pyruvate decarboxylase (PDC1 & PDC5 & PDC6)

Alcohol dehydrogenase (ADH1 & ADH2 & ADH3 & ADH4 & ADH5)

[Diagram showing the fermentation process with pyruvate, acetaldehyde, and ethanol]
FERMENTATION

Ethanol properties

Its production profile usually follows the growth curve.

Helps with the perception of body and mouthfulness.

Most desirable metabolite

Produced as a defence mechanism against other organisms in high concentrations of carbohydrates.

Brewing yeast can usually only tolerate until 10-11 % (V/V).

Ingredients of beer

Yeast

Fermentation

Different flavours
QUIZ 2

You have 30 seconds to answer two questions:

3. Glucose and fructose are transported to the cytoplasm by:
   - Active transport.
   - Facilitated transport.

4. How much ethanol can yeast usually tolerate?
   - 10 - 11 %(V/V).
   - 5 – 6 %(V/V).
   - 13 – 15 %(V/V).

The answers to all the questions are at the end of the course.
DIFFERENT FLAVOURS

Glycerol production

In more detail:

Glycolysis

Glycerone phosphate

Converted into

Glycerol

Several enzymatic reactions

Ingredients of beer  Yeast  Fermentation  Different flavours
DIFFERENT FLAVOURS

Glycerol production

NADH + H⁺ → Glycerone phosphate → Glycerol-3-phosphate phosphatase (GPD1 & GPD2) → Glycerol-3-phosphate dehydrogenase (GPP1 & GPP2) → Glycerol

- Glycerol production
- Ingredients of beer
- Yeast
- Fermentation
- Different flavours
DIFFERENT FLAVOURS

Glycerol properties

Top-fermented beers: 1.3 – 1.7 g/L
Bottom-fermented beer: 1.3 – 1.7 g/L

Higher glycerol concentration results in less ethanol production

Odourless and tasteless

Helps with the perception of body and mouthfulness

Ingredients of beer  Yeast  Fermentation  Different flavours
DIFFERENT FLAVOURS

Diacetyl production

In more detail:

Glycolysis

Pyruvate  Converted into  Diacetyl  Converted into  Acetoin

Enzymatic and spontaneous reactions

Enzymatic reaction

Ingredients of beer  Yeast  Fermentation  Different flavours
DIFFERENT FLAVOURS

Diacetyl production

Pyruvate $\rightarrow \alpha$-acetolactate $\rightarrow$ Diacetyl $\rightarrow$ Acetoin

$\text{Butanediol dehydrogenase (BDH1)}$

$\text{NAD}^+ \rightarrow \text{NADH} + H^+$

Ingredients of beer  Yeast  Fermentation  Different flavours
DIFFERENT FLAVOURS

Diacetyl properties

Mostly an undesirable flavour, but desired in Czech beer

Buttery, butterscotch-like flavour

Diacetyl

Depends on fermentation temperature, aeration level, bacterial contamination, and the yeast strain

Beer maturation helps to get rid of this flavour, for this the temperature needs to be between 18 and 21 °C

Ingredients of beer  Yeast  Fermentation  Different flavours
DIFFERENT FLAVOURS

DMS production

In more detail:

Dimethyl sulfide (DMS) → Converted into → Dimethyl sulfoxide (DMSO)

Enzymatic reaction
DIFFERENT FLAVOURS

DMS production

DMSO

\[ \text{Methionine-sulfoxide reductase (MXR1 & MXR2)} \]

DMS

Ingredients of beer  Yeast  Fermentation  Different flavours
**DIFFERENT FLAVOURS**

DMS properties

- **Cooked maize-like flavour**
  - Can be produced by heat decomposition of S-methylmethionine, produced during barley germination; mostly lost by heat treatment.

- **Dimethyl sulfide (DMS)**
  - Yeast can reduce another precursor, DMSO, and the extension of the conversion depends on the yeast strain.

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**Ingredients of beer**
- Yeast
- Fermentation
- Different flavours
DIFFERENT FLAVOURS

Aldehydes & higher alcohols & esters production

Amino acids

Converted into

Keto acids

Converted into

Aldehydes

Converted into

Higher alcohols

Pyruvate

Converted into

Esters

In more detail:
DIFFERENT FLAVOURS

Aldehydes production

Amino acids

Pyruvate

Converted into

Keto acids

Enzymatic reaction

Enzymatic reaction

Converted into

Aldehydes

In more detail:
DIFFERENT FLAVOURS

Aldehydes production

For example:

L-leucine

2-oxoglutarate

L-glutamate

4-methyl-2-oxopentanoate

H⁺

Phenylpyruvate decarboxylase (THI3 & ARO10)

3-methylbutanal

L-valine

2-oxoglutarate

L-glutamate

3-methyl-2-oxobutanoate

CO₂

Pyruvate decarboxylase (PDC1 & PDC5 & PDC6)

Isobutanal

NADH

NAD⁺

Isobutanol

Branched-chain transaminase (BAT1 & BAT2)

Ingredients of beer Yeast Fermentation Different flavours
DIFFERENT FLAVOURS

Aldehydes properties

- Aging flavours
- Aldehydes
  - Can be produced in the degradation of amino acids
  - Can be produced during the brewing process due to the heat load applied

Ingredients of beer | Yeast | Fermentation | Different flavours

34
DIFFERENT FLAVOURS

Higher alcohols production

In more detail:

Ingredients of beer | Yeast | Fermentation | Different flavours

Aldehydes + NADH → Converted into Higher alcohols

Enzymatic reaction

Aldehydes: Propanal, Butanal

NADH

Higher alcohols:
DIFFERENT FLAVOURS

Higher alcohols production

3-methybutanal

Alcohol dehydrogenase (ADH4 & ADH5)
Formaldehyde dehydrogenase (SFA1)

3-methylbutanol

NADH

NAD+

Isobutanal

Alcohol dehydrogenase (ADH4 & ADH5)
Formaldehyde dehydrogenase (SFA1)

Isobutanol

NADH

NAD+

For example:
DIFFERENT FLAVOURS

Higher alcohols properties

Considered undesirable in lagers, but desirable in ales

General “alcoholic” taste and warming sensation in the mouth and stomach
**DIFFERENT FLAVOURS**

**Esters production**

In more detail:

Higher alcohols + Acetyl CoA → Converted into Enzymatic reaction → Esters

Ingredients of beer  Yeast  Fermentation  Different flavours
**DIFFERENT FLAVOURS**

Esters production

Aliphatic alcohol

- Acetyl CoA
- Coenzyme A

**Alcohol acetyltransferase (ATF1 & ATF2)**

Acetyl ester

Ethyl ester

- Acetyl CoA
- Coenzyme A

**Ethanol O-acyltransferase (EEB1)**

**Ethanol hexanoyl transferase (EHT1)**

**Ingredients of beer**

**Yeast**

**Fermentation**

**Different flavours**
DIFFERENT FLAVOURS

Esters properties

Fruit-like flavours

Dependent on yeast characteristics, wort composition, and fermentation conditions

High carbohydrates concentrations results in higher esters and higher ethanol content

Higher temperatures results in higher concentration of esters

Ingredients of beer Yeast Fermentation Different flavours
DIFFERENT FLAVOURS

Yeast metabolism

Ingredients of beer

Yeast

Fermentation

Different flavours

Yeast metabolism

Carbohydrates

Sulfide

DMSO

DMS

α-acetolactate

Pyruvate

Keto Acids

Acetoacetate

Acetoin

Diacetyl

Aldehydes

Higher Alcohols

Esters

Glycerol

Ethanol

Acetaldehyde

Acetic acid

Acetyl CoA

Fatty acid CoA

Amino acids

Acetyl CoA

Fatty acid CoA

Esters

Ingredients of beer

Yeast

Fermentation

Different flavours

Yeasts metabolism

Carbohydrates

Sulfide

DMSO

DMS

α-acetolactate

Pyruvate

Keto Acids

Acetoacetate

Acetoin

Diacetyl

Aldehydes

Higher Alcohols

Esters

Glycerol

Ethanol

Acetaldehyde

Acetic acid

Acetyl CoA

Fatty acid CoA

Amino acids
QUIZ 3

You have 30 seconds to answer two questions:

5. High concentration of glycerol means:
   • high ethanol content.
   • low ethanol content.

6. Diacetyl has the very characteristic taste and smell of:
   • Old socks.
   • Cooked corn.
   • Butter.

The answers to all the questions are at the end of the course.
QUIZ 3

You have 30 seconds to answer two questions:

7. Aldehydes can produced by:
   • Degradation of amino acids.
   • Extra heat load during the brewing process.
   • All the above.

8. Esters provide which type of flavours?
   • Fruit-like flavours.
   • Aging-like flavours.
   • Cooked corn-like flavour.

The answers to all the questions are at the end of the course.
QUIZ ANSWERS

1. C
2. A
3. B
4. A
5. B
6. C
7. C
8. A
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