Maintaining Ideal Yeast Health: Nutrients Yeast Need
“What makes a great beer?

Fermentation. You put the yeast in, and then the fermentation takes about a week. A lot happens in that week. The trick is to give the yeast exactly what it wants. It’s a mould and it wants to eat, and while it eats, it makes alcohol and gas.”

Interview with Eric Molson; Globe and Mail, May 29, 2009
General concept

Wort nutrients

Yeast

Beer
Importance of Yeast to Beer Characteristics

- Around 950 compounds are thought to give beer its character.
- Of these, around 400 are produced by yeast!
WORT

(sugars, amino acids, etc.)

More yeast!

Yeast autolytic compounds

Phenols

Sulphur compounds

Aldehydes & ketones

Esters

Acids (organic & fatty)

Alcohols (ethanol & fusel oils)

CO₂

Glycerol
Elemental requirements for growth of brewing yeast

**Macroelements**

- H - cell water, organic material
- O - respiration, cell water, organics
- C - organic material
- N - proteins, nucleic acids, coenzymes
- S - proteins, coenzymes
- P - nucleic acids, phospholipids
- K - cell electrolyte, enzyme cofactor
- Mg - enzyme cofactor, cell division

**Microelements**

- Ca - flocculation
- Mn - enzyme cofactor
- Fe - cytochromes
- Zn - alcohol dehydrogenase
- Co - vitamin B12, coenzymes
- Cu, Ni, Mo - specialised enzymes
Yeast nutrients in wort

- **Carbohydrates** *(fermentable sugars)*
- **Nitrogen sources** *(mainly amino acids)*
- **Inorganic sources** *(P, S, Mg, Zn, other minerals)*
- **Oxygen** *(need around 25% saturation of wort)*
- **Yeast “foods”** *(vitamins, fatty acids, sterols)*

*NOTE: The level and availability of such nutrients is very important in governing fermentation performance of brewing yeast*
Wort composition influences:

- Rate of attenuation
- Degree of attenuation
- Amount of yeast produced
- Flocculation
- Beer characteristics
Carbon sources for brewing yeast

- **Hexose sugars**: glucose, fructose
- **Pentose sugars**: no (but possibly xylulose)
- **Disaccharides**: maltose, sucrose, melibiose
- **Trisaccharides**: maltotriose (slowly)
- **Tetrasaccharides**: maltotetraose (possibly some strains)
- **Oligosaccharides**: maltodextrins (unfermented)
- **Polysaccharides**: starch (unfermented)
- **Others**: ethanol, acetate, glycerol (only respired)
Nitrogen sources for brewing yeast

- Peptides
- Amino acids
- Ammonium salts (little present in wort)
Inorganic sources for brewing yeasts

Phosphorus, sulphur and potassium generally sufficient in wort

Metal cations may occasionally be deficient (and ratios of metal ions may be sub-optimal)

Essential bulk ions: K, Mg

Essential trace ions: Zn, Ca, Fe, Mn

Toxic trace ions: Pb, Cd, Cr, Hg, Cu etc.
Zinc

- Faster fermentation
- Better flocculation
- Stimulates uptake of maltose and maltotriose
- Stimulation of protein synthesis and yeast growth
- Protection of enzymes
- Stabilisation of protein and membrane system
Zinc Concentration in Wort

- During wort preparation approx. 95% of zinc from malt are lost with spent grain
- In modern stainless steel breweries the zinc concentration is not sufficient (<0.2 mg/L)

↓

Slow or stuck fermentation

LALLEMAND
Zinc wort content is almost zero after 1 hour.

Zn is entirely accumulated into cells which redistribute the metal among growing yeast.

LAGER strain-25 °C

Lager brewing yeast strain – lab conditions

G. Walker, ASBC 2007, Victoria, Canada
Zn & fermentation performance

Specific gravity

- Low zinc (0.05 ppm) delayed sugar utilization
- High zinc (10 ppm) slightly delayed sugar utilization – but same after 14 days fermentation
- 0.4 ppm-1.0 ppm Zn resulted in fastest fermentation rates

G. Walker, ASBC 2007, Victoria, Canada
Magnesium

- Absolutely essential (growth, fermentation)
- Cell Mg correlates with viability/vitality
- Required for glycolysis (stimulates fermentation)
- Maintains structural integrity of yeast (stress-protectant)
- Ca/Mg ratio is important – (a major consideration for commercial yeast food formulations)
Vitamin sources for brewing yeasts

Wort generally sufficient in folic acid, thiamine, riboflavin, pantothenic acid, niacin, inositol and biotin

Biotin, however, may occasionally be deficient

Can supplement wort with “yeast foods”
### Typical levels of vitamins in wort

<table>
<thead>
<tr>
<th>VITAMIN</th>
<th>LEVEL IN WORT /100ml</th>
<th>METABOLIC ROLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin</td>
<td>0.5µg</td>
<td>Carboxylations</td>
</tr>
<tr>
<td>Thiamin (B1)</td>
<td>60µg</td>
<td>Decarboxylations</td>
</tr>
<tr>
<td>Pantothenate (B5)</td>
<td>~50µg</td>
<td>Acetylations, CoA</td>
</tr>
<tr>
<td>Nicotinic acid (B3)</td>
<td>1000µg</td>
<td>Coenzymes</td>
</tr>
<tr>
<td>Riboflavin (B2)</td>
<td>50µg</td>
<td>Coenzymes</td>
</tr>
<tr>
<td>Pyridoxine (B6)</td>
<td>85µg</td>
<td>Amino acid metabolism</td>
</tr>
<tr>
<td>Inositol</td>
<td>10mg</td>
<td>Membranes</td>
</tr>
</tbody>
</table>
Yeast transport strategies for wort nutrients

Yeast cell

WORT NUTRIENTS

YEAST METABOLITES (beer)

LALLEMAND
Translocation of wort nutrients into brewing yeast

**CELLULAR BARRIERS**
- capsule, cell wall, periplasm, plasma membrane, organelles

**PHYSICO-CHEMICAL BARRIERS**
- chelation, adsorption, molecular size, binding (e.g. nutrient unavailability in wort particles)
PHYSICO-CHEMICAL BARRIERS

- Zinc chelates with phytic acid, proteins & polyphenols
- These complex compounds are very stable
- Only ionic zinc is available to yeast
- Regular AAS is a destructive method, which measures total zinc not available zinc
- Ion-exchange separation separates complex and ionic zinc before AAS
Total and complex zinc concentration of industrial wort samples

Consequences of low assimilable nitrogen wort

Low available nitrogen, especially in very high gravity wort, below a threshold of around 150mg/L, may lead to “stuck” fermentations.
Amino acid uptake by brewing yeast

- **GROUP A (fast)**
  - Glu, Asp, Asn, Gln, Ser, Thre, Lys, Arg

- **GROUP B (intermediate)**
  - Val, Met, Leu, Isoleu, His

- **GROUP C (slow)**
  - Gly, Phe, Tyr, Try, Ala, NH$_3$

- **GROUP D (little or no)**
  - Pro

NOTE: A variety of transport permeases exist for (active) amino acid uptake by brewing yeast
Yeast growth vs. Diacetyl Levels

Valine/Isoleucine synthesis required
α-acetolactate produced

Yeast growth g/L
Diacetyl (mg/L)

Days Fermentation

Degrees Plato
Yeast growth g/L
Diacetyl (mg/L)
Mechanisms for yeast food enhanced reduction of diacetyl levels?

α-acetolactate
By-product of valine and isoleucine synthesis by yeast

Oxidative Decarboxylation (Slow)

Yeast reductase - fast

Diacetyl
Requires functioning cell membrane to allow for uptake of diacetyl prior to reduction

Acetoin
Provision of usable nitrogen source plus other nutrients results in a more vital cell that is enhancing diacetyl uptake and reduction?

Amino acids supplied by yeast foods
May reduce requirement for synthesis of valine/isoleucine (and thus α-acetolactate and diacetyl production)?
Yeast nutrition potential problems for fermentation

- **Spectrum/availability of wort sugars**
  - e.g. High glucose worts may slow fermentation

- **Insufficient FAN**
  - Stuck fermentation, change in flavour profile

- **Metal ion availability**
  - e.g. Low Zn, excess Ca, insufficient Mg, toxic Cu

- **Vitamin deficiency**
  (e.g. pantothenate - sulphur off-notes)

- **Stressed yeast - impaired nutrient uptake**
  (ethanol, pH, CO₂, temp., starvation, osmotic pressure....)
Yeast Foods
Yeast “foods”

Multifunctional roles

• Alleviate CO$_2$ inhibitory effects
• Extra sources of assimilable nitrogen (hydrolysed protein)
• Extra sources of vitamins
• Extra sources of metal ions (to increase bioavailability)
Commercial Yeast Foods
Available Components

• Amino acids (FAN) - as protein hydrolysate or added as individual amino acids
• Low molecular weight peptides
• NH₄⁺
• Growth factors (vitamins)
• Protein (non-utilisable) – active in CO₂ nucleation?
• Sulphur/Phosphorous
• Zn/Cu/Mn/Ca (enzymatic and structural roles)
• Yeast ‘hulls’ (broken cell walls) as sterol/FA source, nucleation site
• Ergesterol – expensive
Typical Yeast Food Effects*

- Improved alcohol yield (increased extract utilization)
- Reduction in fermentation time (12-24h)
- Enhanced yeast vitality – Protect against stress
- Enhanced diacetyl removal/consistent diacetyl specification
- Control of undesirable flavour compounds

* Depending on the yeast food composition
Nutrient Trial Results
Eastern European Brewery A

- Primary Fermentation Time (days)
  - Control: 6.8
  - Nutrient: 6.3

- VDK in specification at time (%)
  - Control: 71
  - Nutrient: 98

- Average Diacetyl (ppb)
  - Control: 34.9
  - Nutrient: 33.2
Nutrient Trial Results
Eastern European Brewery B

Primary Fermentation Rate (degP/Day)
- Control: 4
- Nutrient: 6

Final Diacetyl Level (ppb)
- Control: 3.7
- Nutrient: 3.3

Residual Extract
- Control: 160
- Nutrient: 148
Servomyces
Weihenstephan Trials

15L lab trials

- Sample 1: no zinc addition (O-wort)
- Sample 2: addition of 0.3 mg/L ZnCl₂
- Sample 3: addition of 8 mg/L inactive yeast
- Sample 4: addition of 8 mg/L SERVOMYCES
- Sample 5: addition of 2.3 mg/L SERVOMYCES
- Sample 6: addition of 8 mg/L inactive yeast + 0.3 mg/L ZnCl₂
- Initial yeast zinc pool: 4.1 mg/100 g

<table>
<thead>
<tr>
<th></th>
<th>0-wort</th>
<th>ZnCl₂</th>
<th>inactive yeast</th>
<th>SERVOMYCES High Addition</th>
<th>SERVOMYCES Low Addition</th>
<th>inactive yeast + ZnCl₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial wort [mg/L]</td>
<td>0.10</td>
<td>0.35</td>
<td>0.11</td>
<td>1.22</td>
<td>0.36</td>
<td>0.34</td>
</tr>
<tr>
<td>Crop yeast [mg/100 g]</td>
<td>2.67</td>
<td>4.15</td>
<td>2.63</td>
<td>11.61</td>
<td>5.13</td>
<td>3.11</td>
</tr>
</tbody>
</table>
Servomyces Weihenstephan Trials

Fermentation in 15L CCT at 10 °C

- ZnCl
- inactive yeast
- Servomyces - high addition
- 0-Wort
- Servomyces - low addition
- inactive Yeast + Zn Cl
Servomyces Trial Results
US Brewery

![Graph showing fermentation results with different Servo additions and control groups. The x-axis represents fermentation days, and the y-axis represents extract [Brix]. The graph compares Servo addition A, Servo addition B, No Servo A, and No Servo B. The extract levels decrease over time for all groups, with Servo additions showing a more rapid decrease compared to the control groups.](image-url)
Propagation

• With each cell division the internal zinc pool is divided between mother and daughter cell

• Fermentation 4-5 times biomass increase

• Propagation 15-20 times biomass increase
  => very low internal zinc pool

  ➢ Slower, less efficient propagation

  ➢ Fermentation problems in 1. generation beer
    ➢ Slower, stuck fermentation
    ➢ Longer diacetyl rest
    ➢ Less flocculent yeast
The Impact of Nutrients on High Gravity/Adjunct Brew

- 60% malt / 40% adjunct / 18°P / Gen 1

![Extract [% w/w] vs. time [day] graph with different lines for Control, A, B, D, C, and E. The graph shows the extract concentration over time for each condition.]
Serial Repitching

- Repeated stress for the yeast
- Decline in yeast activity
- Resistance to stress is strain dependent
- Contamination
- Genetic drift/petite mutation
The Impact of Yeast Generation on the Use of Nutrients
High Gravity/Adjunct Brew

Gen 1

Gen 6

Gen 8
Nucleation Sites

Effect of dissolved CO$_2$

- Inhibits growth and metabolism
- Decreases multiplication rate
- Reduces biomass yield
- Increases lag phase
- Inhibition of nutrient uptake
- Loss of cell division and bud formation
Nucleation Sites

- Particles working as nucleation sites reduce the solubility of CO$_2$
- Most nucleation in a fermenter occurred from particles that sedimented to its bottom
- Particle size is a likely determinant of the particle’s ability to be an effective CO$_2$ nucleator
- The more porous a particle is, the most efficient it is as a nucleation site
- Soy flour may be ruled out due to allergen issues
Nucleation Sites – Degassing Activity

released CO₂ after 2.5 hours
Why Nutrients?

- Sufficient supply of nutrients to the yeast
- Predictable, fast and consistent fermentations without the formation of undesired flavours
- Higher alcohol yields
- High gravity brewing and increased amounts of adjuncts can result in slow or stuck fermentation
- Insufficient nutrient supply will also result in formation of undesired flavours