Bottle Conditioning with Dry Yeast: a practical approach

Steven Pauwels – Boulevard Brewing Co.

Why use dry yeast (different yeast) for bottle conditioning?

- Yeast availability
- Aesthetics:
  - Low glass adherence
  - 'Flake' formation
- Primary fermentation yeast protection
- Alcohol tolerance
- Off-flavor

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Dry yeast re-hydrations steps

- 10 ml of rehydration solution for 1 gram of yeast
- Warm rehydration solution (30°C to 35°C)
- Low sucrose concentration optional (1 to 2 %^{m}/v)
- 15 min no mixing
- Followed by 15 min mixing

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Re-hydration step #1

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Craft Brewers Conference 2012
San Diego

Duvel

Duvel: Group's Main Brand

- Strong Golden Ale
- 8.5% ABV
- Bottle conditioned
- 2 months maturation in cellars

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Maredsous

Maredsous

- Abbye Beers
- 6 / 8 / 10 % ABV
- Bottle conditioned
- 2 months maturation in cellars

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Vedett

Vedett

- Premium Lager / Belgian White
- 5.2 % ABV
- Bottle conditioning for White
- Retro marketing

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Chouffe

La/Mac/N'Ice/Houblon Chouffe

- Ales from the Ardennes
- 8 – 10 % ABV
- Bottle conditioning
- Use of spices in some…

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Liefmans

Fruitesse / Oud Bruin

Cuvée Brut / Gouden Band

- Mixed fermentation, open fermentors
- 3.8 – 9 % ABV
- Maturation phase up to 12 months

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De Koninck

De Koninck

- Amber, Spéciale Belge
- 5 % ABV
- Closely linked to city of Antwerp
- Pouring in 2 steps

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Ommegang

Hennepin / Abbey Ale / Three

Phil's / Rare Vos / Witte / …

- Belgian Styles
- Bottle conditioning
- Brewed in Cooperstown

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Yeast dosing

Bottle conditioning

° Culture yeast strains (4)
- Top harvesting (4x1100 hl fermentors)
- Freshly propagated (4x80 hl propagators)
- Own culture, dried

° In-line proportional dosing
- Viability measurement
- Accurate dosing through flow/mass-flow/turbidity measurement

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Conditioning Parameters

Bottle conditioning

° Carbonation levels
- 3.5 – 5.0 g/l. start bottle conditioning
- 6.5 – 8.5 g/l. target (~ beer type)

° Yeast concentration
- 2.0 – 2.5 mio cells/ml.

° Sugar dosing
- 0.6 – 1.0 ° Plato

° Temperature/time
- 2 weeks warm cellars, 24°C

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Extra focus on…

° Clean yeast (tight sedimentation – clear pour)
- Yeast washing (demineralised water / sedimentation)
- Possibly proteolytic enzymes added to washing water

° Yeast flocculence
- Choice 1: top CCT harvested yeast
- Choice 2: propagation yeast
- Choice 3: dried own culture yeast

° Microbiology

° Viability >95%

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Bottle conditioning: gain or pain?…

- Origin: Dom Perignon, abbye Haut Villers
- Coincidence:
  - Incomplete attenuation
  - Poor beer filtration techniques = natural or farmer method
  - Duvel glass: marketing or necessity

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Bottle conditioning: gain or pain?…

Is bottle conditioning still an advantage towards shelf life?

Saccharomyces cerevisiae, limited viability (temperature, time, strain, ABV, …)

- Autolysis: release of enzymes:
  - proteolytic
  - ester degrading
  - …

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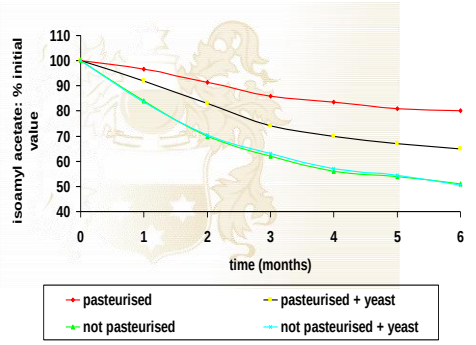
'ester hydrolysis vs. pasteurisation intensity'



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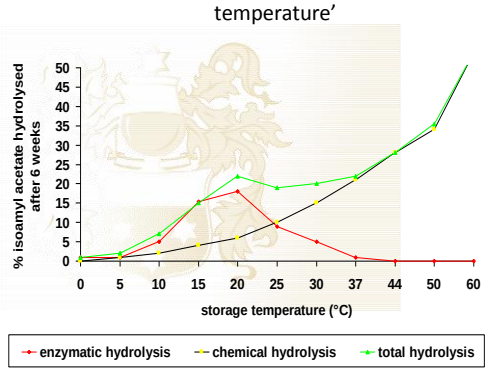
'effect of pasteurisation in function of storage temperature'



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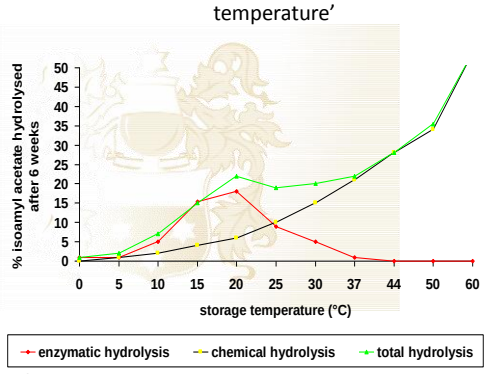

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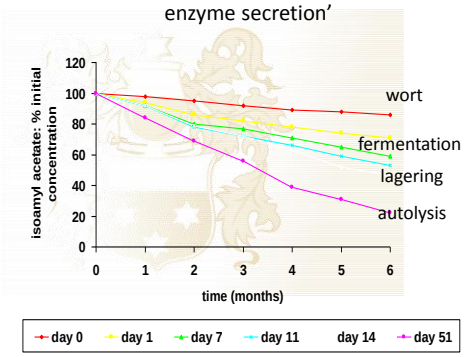
'screening of fermentation and lagering on enzyme secretion'



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'screening of fermentation and lagering on enzyme secretion'

- top fermented beer (O.G. 17.0 °P)
- *Saccharomyces cerevisiae cerevisiae* CMB212
- fermentation: 7 days, 24°C
  lagering: 7 days, 0°C
  induced autolysis: 36 days, 28°C
- samples were filtered and bottled on days:
  1 - 7 (fermentation)
  8 - 15 (lagering)
  30 and 51 (induced autolysis)
- corrections: pH, ethanol % (v/v), isoamyl acetate, isoamyl alcohols, acetic acid

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Consequences on Aroma Evolution

Some observations on Acetate- and Acyl-esters

- release of ester-hydrolysing enzymes during fermentation and lagering
- enzymes remain active in non-pasteurised, filtered beers
- optimal hydrolysis of isoamyl acetate at:
  15-20 °C
  pH 7, however also significantly active at pH 4
- possible esterolytic activity towards other beer esters

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Bottle conditioning: gain or pain?…

Is bottle conditioning still an advantage towards shelf life?

- Enzyme secretion
- Technologically unnecessary, even at high carbonation levels
- Microbiological risks
- Inconsistency (sulphury compounds, diacetyl, acetaldehyde, …)
- Colloidal stability
- Need for education consumers (80 pct of Duvel complaints are about the 'hazy' aspect)

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Bottle conditioning: gain or pain?…

Why do we bottle condition our beers… with Saccharomyces cerevisiae

- Slower oxydation processes because of oxygen uptake (?)
- Reducing power of the yeast (?)
- Because of… tradition (?) marketing (?) artisanal image (?)

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Bottle conditioning: gain or pain?…

Bottle conditioning with Saccharomyces cerevisiae: sometimes a good friend, sometimes your worst enemy

Imagine:

- Overseas transportation up to end consumer
- Temperature effects (even with all precautions possible)
- Manipulation of the container/bottles
- Very precise pouring ritual

Is having 2 million dead yeast cells/ml the ideal situation for these circumstances?

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Bottle conditioning: gain or pain?…

What would be the ideal situation then…?

- Current research on new yeast strain through cross-breeding and selection
- We aim for:
  - Extended viability
  - Ethanol tolerancy
  - High production of SO2
  - Same flavour characteristics as initial strain

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Bottle conditioning: gain or pain?…

First results, in a nutshell…

- >90 % viable cells after 4 months at 30 C (vs control 0%)
- Thiobarbituric acid test: 20% of (staling) aldehydes vs control
- SO2 around 6ppm (yeast used for main fermentation as well) (vs control1,5 ppm)
- Lower esterolytic and proteolytic activity
- Consistently lower in acetaldehyde, diacetyl…

Currently further and deeper research, first industrial tests autumn 2012

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Re-hydration after 30 minutes

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Yeast can

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Dosing into bottling tank

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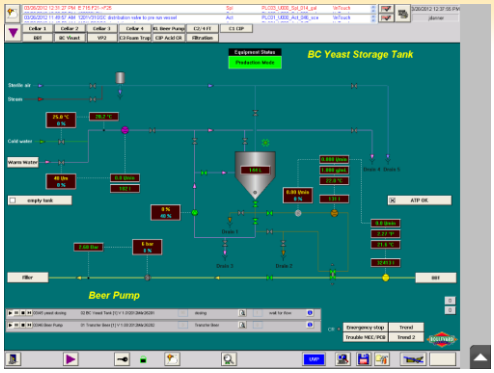

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Automated re-hydration and in-line dosing

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In-line Sugar Dosing

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Bottle conditioning with dry yeast

Advantages

- Production flexibility
- Minimal labor
- Minimal equipment
- Predictable cell count
  (min $7x10^3$ viable cells/gram)
- Low operating cost

Disadvantages

- Viability
- Fermentation speed
- Batch differences
- Limited choice
- Contamination
  (max $2x10^3$ bacteria/gram)

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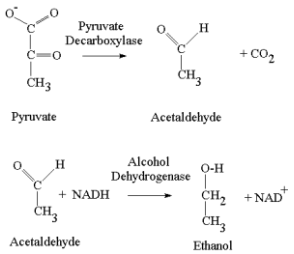
Acetaldehyde: a sign of 'unhappy' yeast?



- Threshold: 10 ppm
- Typical concentration:
  1 to 24 ppm (0.1 to 2.4 FU)
- Apple-skin like, green-leave like, fruity, grassy
- Influences:
  - Less vital/viable → yeast growth ↘
  - Oxidation of ethanol
  - Yeast flocculation
  - Under-aeration
  - Temperature

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Choosing the right dry yeast

- Alcohol tolerance*
  - Ale yeast up to 8 ABV
  - Champagne yeast for 8 ABV to 11 ABV
- POF+ / off-flavor
- Speed of bottle-conditioning
- Attenuation
- Firm sediment in bottle
- No flake formation
- Good glass adherence

* Practical use of dried yeast in the brewing industry - Steven van den Berg and Anita Van Landschoot - *Cerevisia* 28 (3) 2003

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Conclusion

- Dry yeast has been used in a bottle conditioning application at Boulevard Brewing Co. for the last ten years
- Dry yeast is not a perfect solution
- Use of dry yeast helps to run the bottle conditioning process independent of availability of liquid yeast

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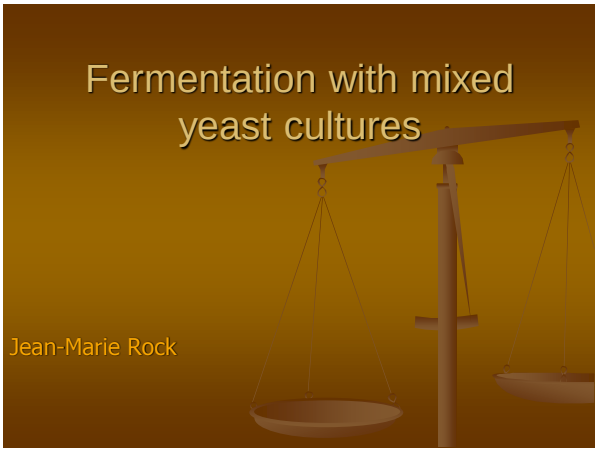


Aromas related to the use of mixed yeast cultures like *S. cerevisiae* and *Brettanomyces* sp. are:

- 1. Volatile phenols:
  smoky, pharmaceutical, ranzid…..
- 2. "Currant wine", "fruit jelly", fruity (peach, apricot,…) en floral (rose,…) aromas

What about the knowledge today?

- *Saccharomyces cerevisiae*
  - Ethanol tolerance - Ethanol toxicity
  - Temperature sensitivity
  - Ester production
  - Flocculation

What about the knowledge today?

- The brewer needs to take care about the following parameters:
- Yeast strain: ethanol tolerance, temperature sensitivity, ester production, flocculation characteristic.
- Yeast dosage rate.
- Wort composition: FAN, fatty acid content, O2 dosing rate.
- Temperature applied during fermentation.
- To expect following results:

- Higher alcohols content
  - Isoamyl alcohol : from 60 to 140 mg/L or 0,7 to 3,0 FU
  - Phenyl ethanol : from 40 to 80 mg/L or 0,1 to 0,8 FU
  - …
- Ethyl esters content
  - Ethyl acetate : from 3 to 120 mg/L or 0,1 to 4,0 FU
  - Isoamyl acetate : from 0,5 to 6,6 mg/L or 0,4 to 5,5 FU
  - …
- Acyl esters content (fatty esters)
- Carbonyl bindings (aldehydes and ketones)
- Beer aging: evolution of aromatic profile

What about the knowledge today?

- *Brettanomyces* sp. *en Dekkera* sp.

Research really limited and not in accordance with the increasing use of this strains.

What about the knowledge today?

- *Brettanomyces* sp. *en Dekkera* sp. are able to produce:
  - Lactic acid production:
    - about 600 mg/L after 30 days in fermentable wort
    - global production after 1 year : about 1000 mg/L
  - Acetic acid production:
    - about 275 mg/L after 30 days in fermentable wort
    - global production after 1 year : about 800 mg/L
  - Dextrins fermentation:
    - about 9.0 g/L after 30 days in fermentable wort
    - global utilization after 1 year : about 25 g/L

What about the knowledge today?

- *Brettanomyces* sp. *en Dekkera* sp.
  - Enzyme activity:
    - Alcohol dehydrogenase = growth on sugar extract:

Very slow grow(alcohol dehydrogenase activity on pH 6,0 is reduced by half comparing with the one of *S. cerevisiae*);

- Aldehyde dehydrogenase = capacity to grow on ethanol:

more efficient comparing with the one of *S. cerevisiae* as result a higher aldehyde concentration including acetaldehyde and other typical aldehydes.

What about the knowledge today?

- *Brettanomyces* sp. *en Dekkera* sp.
  - Enzyme activity:
    - Esterases = hydrolysis of fruity aromas as for example isoamyl acetate with production of isoamyl alcohol and acetate.

This is a typical activity of *Brettanomyces* sp. and *Dekkera* sp.

- Development of Brett-flavour:

"Bretty" and "Mousy" aromas = flavours described as "harsh, mawkish and old beer flavour"

Aromas components in Orval beer

	Orval 6 months		Orval 4 years	
	mg/L	FU	mg/L	FU
n-propanol	33.10	0.041	96.67	0.12
isobutanol	45.75	0.23	49.81	0.25
Isoamyl alcohol.	222.67	3.18	222.56	3.18
Ethyl acetate	20.79	0.69	15.14	0.50
Isoamyl acetate	0.398	0.33	0.057	0.047
Ethyl hexanoate	0.0953	0.45	0.0931	0.44
Ethyl octanoate	Not determined		Not determined	
acetaldehyde	14.28	0.57	13.36	0.53
isovaleraldehyde	Not determined		Not determined	

What about the knowledge today?

"Bretty" and "Mousy" aromas:

- Bretty = "a matter of taste for connoisseurs"
  - ranzid: fatty acid → isovaleric acid
  - cloves, pharmaceutical, smoked meat
  - → volatile phenolic compounds
  - sweat smell, wet leather, goaty, caprylic, wet dog
  - → caprylic, caproic and capric acid
- Mousy = aroma always rejected
  - mouse- and rabbit urine, dry nuts
  - → 2-acetyl-1-pyrroline, ethyltetrahydropyridine, acetyl-tetrahydropyridines

NOWADAYS RESARCH

Bretty = really appreciated by beer hunters

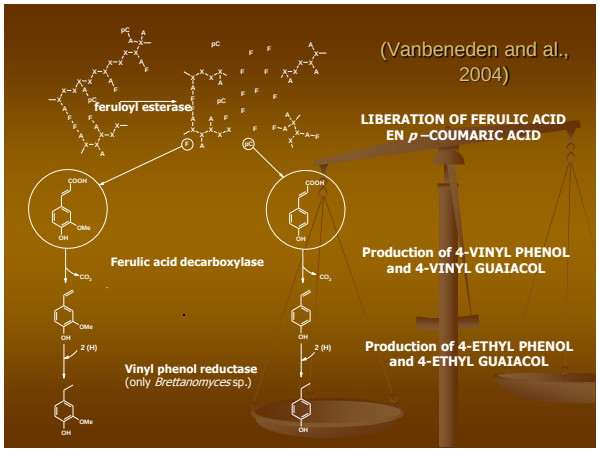
1. Volatile phenols

cloves, pharmaceutical, smoked meat

- 1. Volatile phenols
- :

4-ethyl guaiacol, 4-ethyl phenol,
4-vinyl guaiacol, 4-vinyl phenol:

Produced during yeast metabolism
of
Brettanomyces sp. *Dekkera* sp. *and also by Saccharomyces cerevisiae!!*



(Vanbeneden and al., 2004)

Aromas related to the use of mixed yeast cultures like S. cerevisiae en Brettanomyces sp. are:

2. "Currant wine", "fruit jelly", fruity (peach, apricot,…) en floral (rose,…) aromas

Origin: glucoside and glycoside from malt and hop.

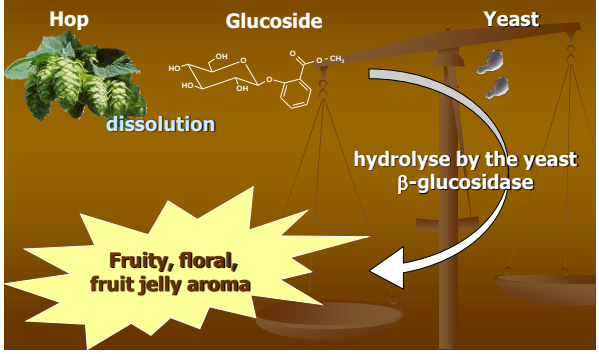
Tranformed by β-glucosidase activity of *Brettanomyces* sp.

- Glucoside and glycoside:

Origin of the aroma:

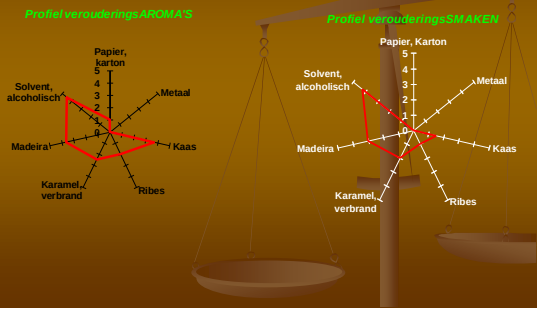
enzyme hydrolysis of the glycoside by the yeast with formation of a AGLYCON structure with aroma potential and a SUGAR residue.

example: aroma formation from a hop glucoside (o.a. Daenen et al, 2004)



Dry hopping and Brett

"Spider web" tasting Orval 4 years



Conclusion

- Added value by the use of a under control mixed yeasts strains
  - Exceptional beverage with wel marked taste and aroma-characteristic;
  - Natural way to achieve a specific flavour;
    - ➢➢ Bioflavouring
  - Longer reductase activity of the yeast (increased shelf-life).