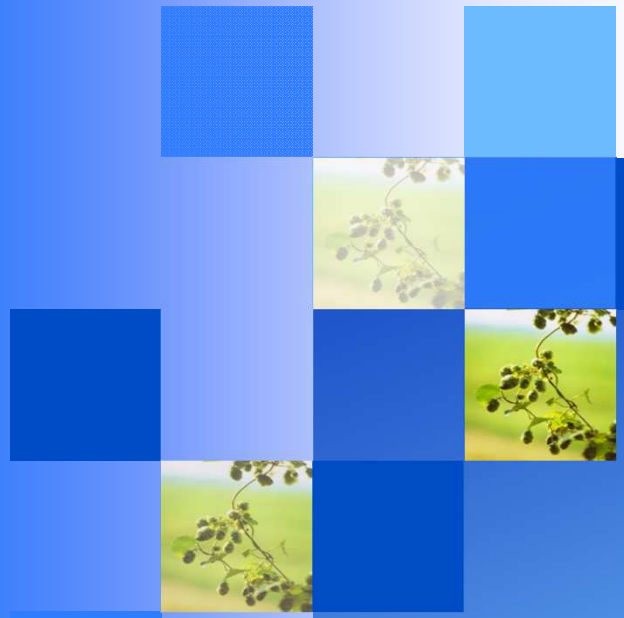
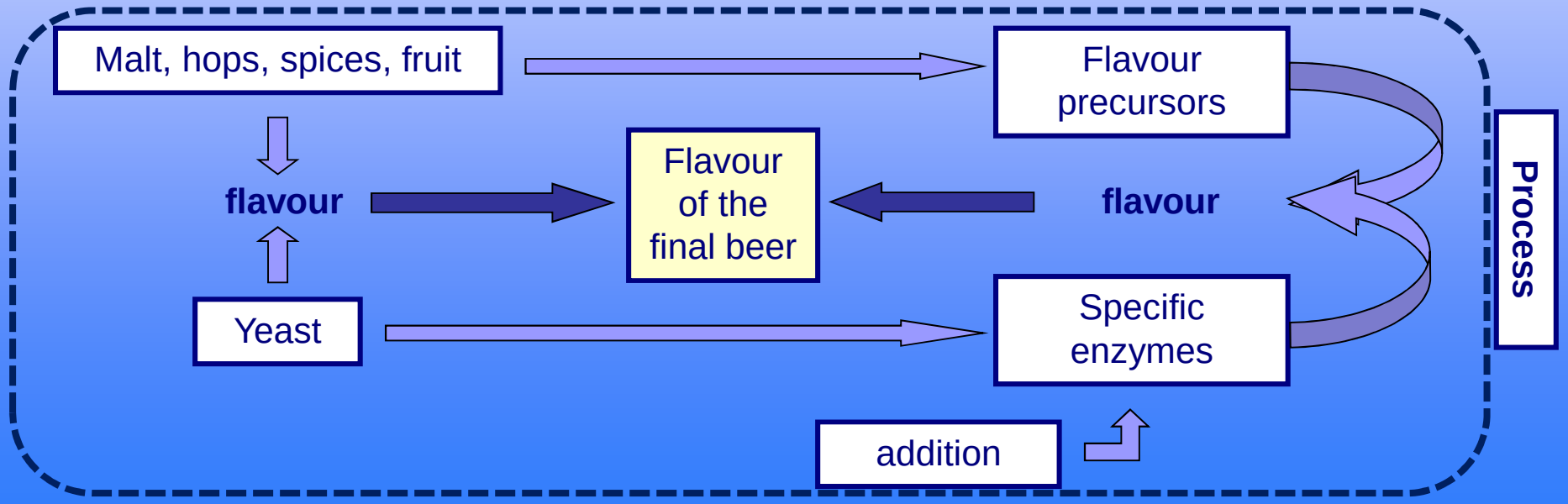


Use of beta-glucosidase activity for flavour enhancement in specialty beers

Luk Daenen
Chair De Clerck 2012

Flavour complexity

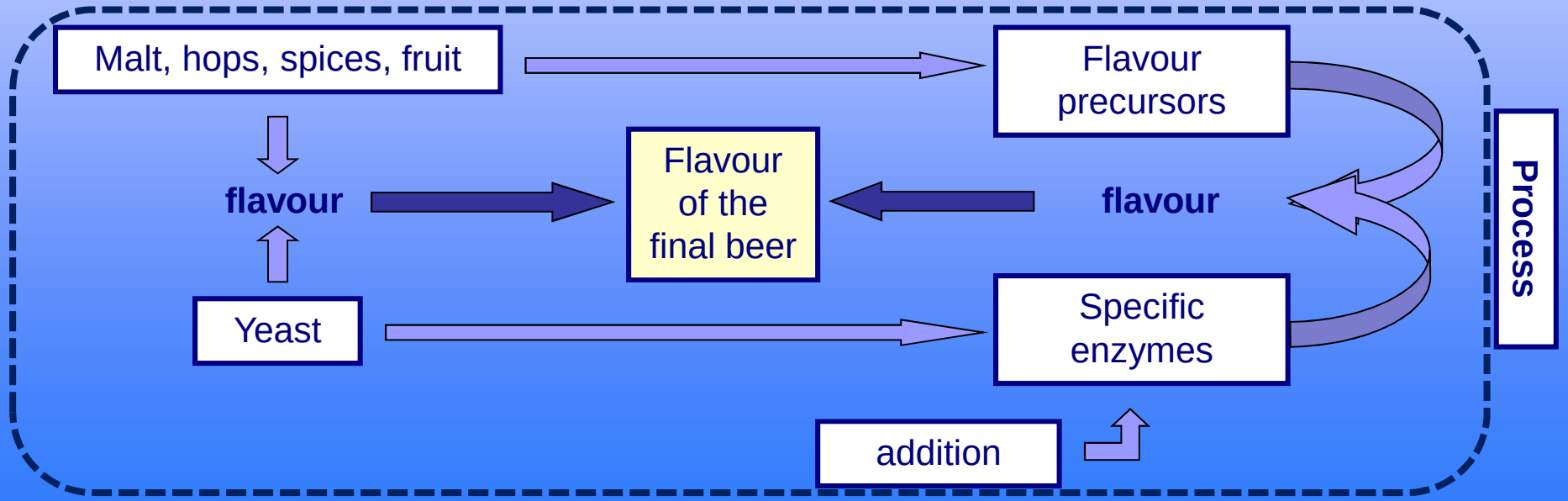


Flavour modification

Here, focus on **biological** approach (no flavour addition):

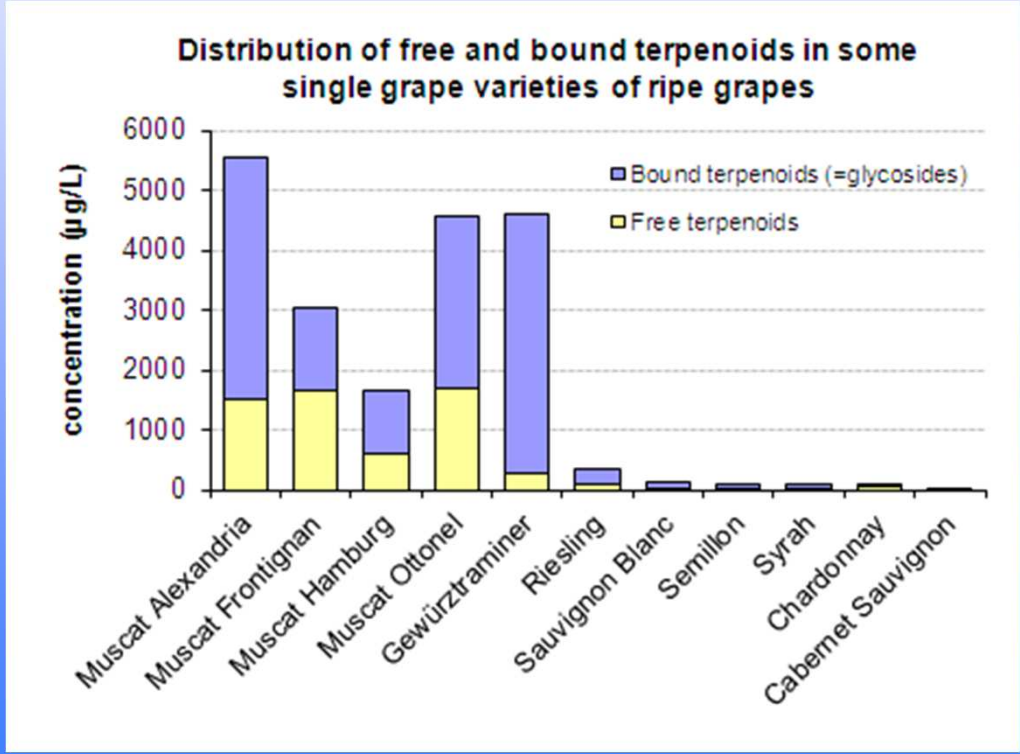
→ Use of plant parts/extracts, enzymes, microorganisms

- Preferred by consumers:
  - Natural product, traditional process, authenticity, health, environment
- Advantage for producer:
  - "clean label", often cheaper, development of complex and subtle flavours



Interest for wine-making

- Some grape varieties have a significant pool of glycosidically bound flavour compounds → **hidden flavour potential**
- Hydrolysis by yeast, enzymes,...



Gunata et al 1984

Interest for brewing industry

Hops

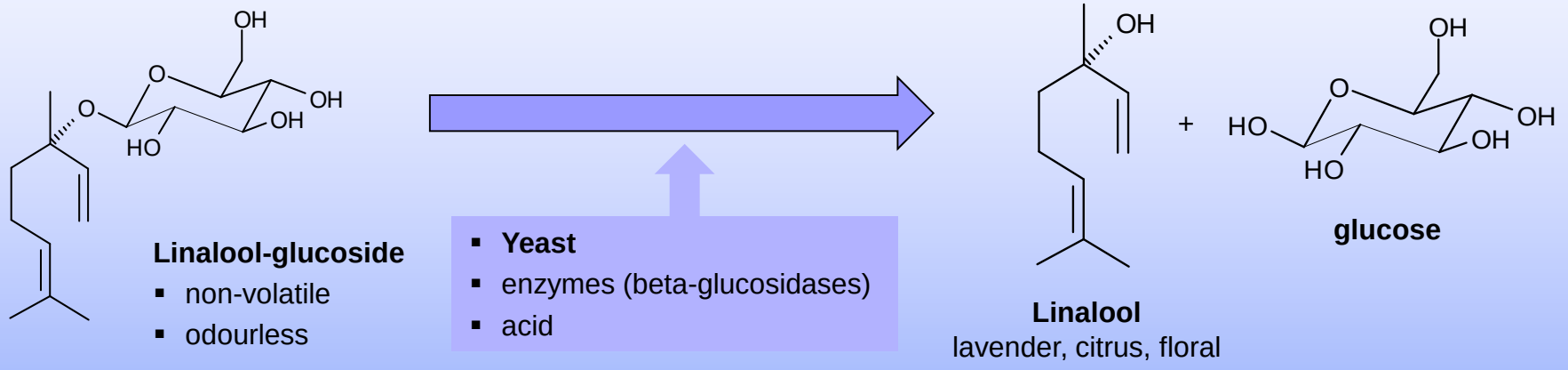
- Flavour precursors in “green” part of hops (flowers, leaves, ...)
- Enhance “hoppy” aroma by precursor hydrolysis
- Interest for **lager, ale, geuze, specialty beers, ...**

Cherry

- Flavour precursors in juice, kernel, skin
- Enhance cherry flavour by precursor hydrolysis
- Interest for **fruit beers**

Release of hidden flavour potential

Glycosidically bound flavour compounds = glycosides

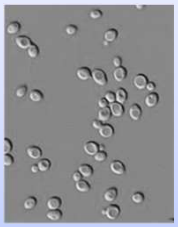


Treatment		Comment
Acid hydrolysis	Low pH (3,0 -3,5)	Separate proces; Sour beers
Enzymatic hydrolysis	Eg. Enzymes from *Aspergillus niger*	Side activities possible: ▪ HCD activity → 4-VG ▪ Anthocyanidin breakdown (loss of colour) ▪ Esterase activity → isoamylacetate ↓
Yeast biotransformation	*Saccharomyces* sp.	Mostly low or moderate activity
	Brettanomyces sp.	High activity; but also other Brett flavours (!)

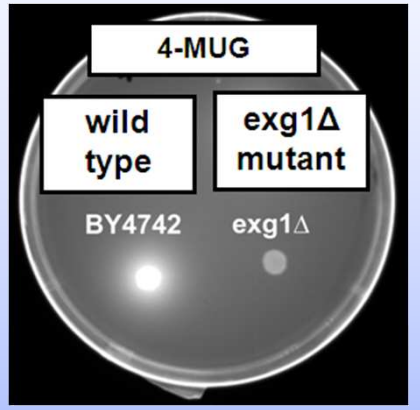
→ Different treatments lead to different flavour profiles

Selection of yeast strains

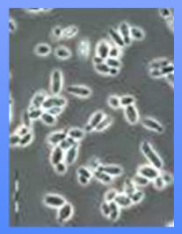
Saccharomyces sp.



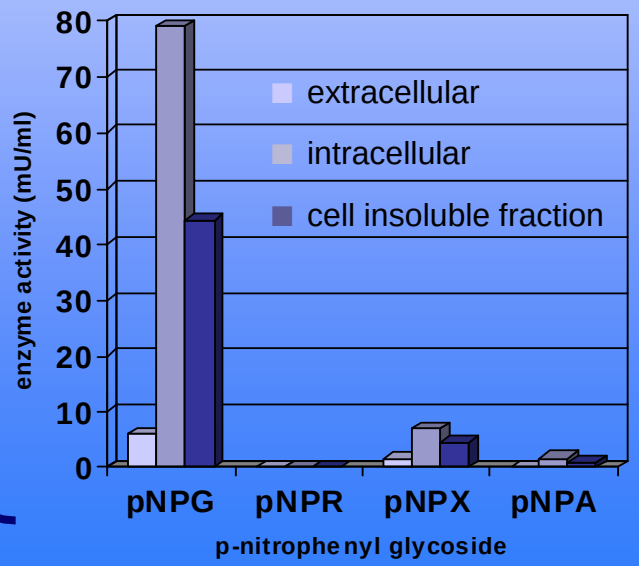
- **Mostly <u>no</u> β-glucosidase**
- **All have exo-1,3- β-glucanse activity** →
  - → Low to moderate activity
- **Some commercial wine strains with inducible β-glucosidase**



Brettanomyces sp. →



- **Some with <u>high</u> β-glucosidase**
- **Mainly cell associated** (low extracellular)
- **Also minor glycosidase activities**



Isolation of hop glycosides

Hop pellets

(Saaz)

Supercritical CO_2 extraction

Essential oils

Soft resins

spent hops / hop residue / hop solids

1) ethanol / water
2) ethanol evaporation

extraction

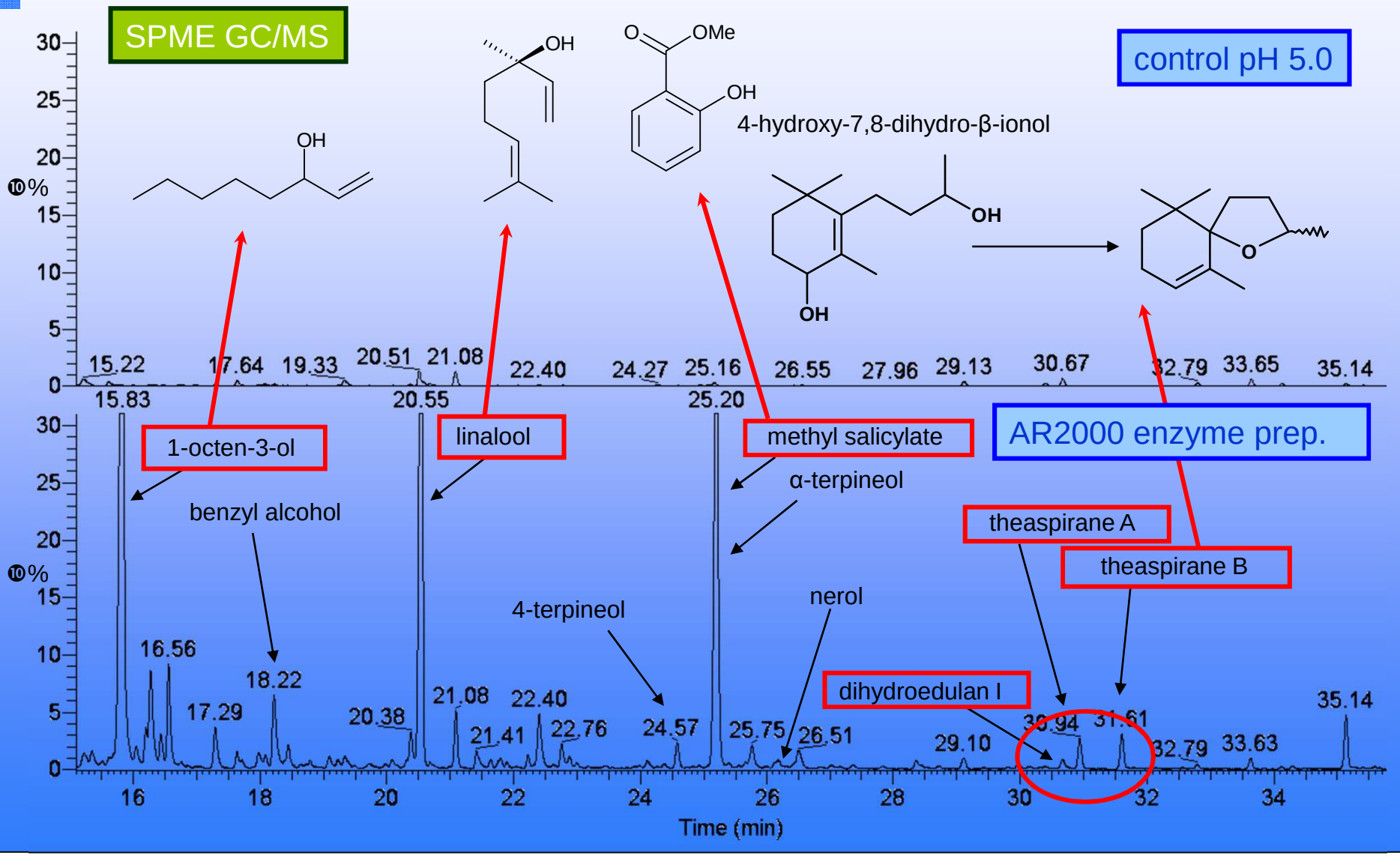
watery extract

<u>Solid Phase Extraction (XAD2)</u>
1) wash with water
2) elution with ethanol
3) evaporation
4) PVPP → remove polyphenols

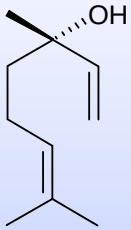
purification

hop glycoside extract

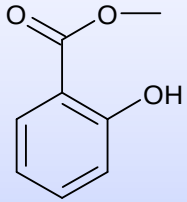
Aglycones from hop glycosides



YPD fermentation with added hop glycosides

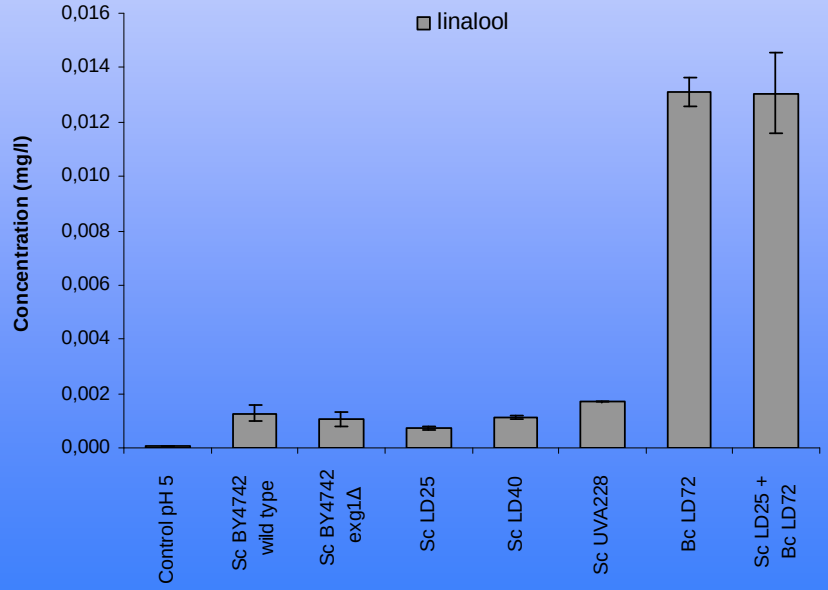


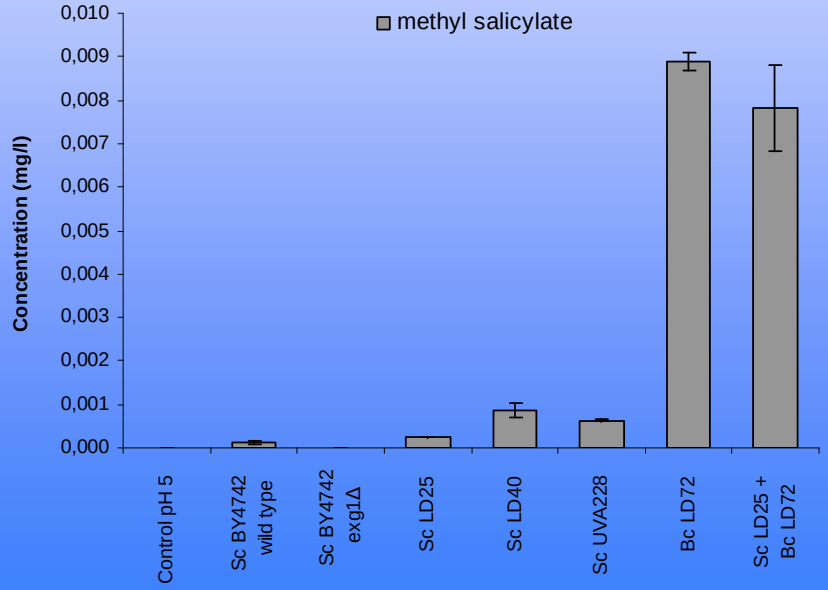
Linalool:
- citrus, floral, aniseed
- important contributor to hop aroma



Methyl salicylate:
- wintergreen, minty, spicy

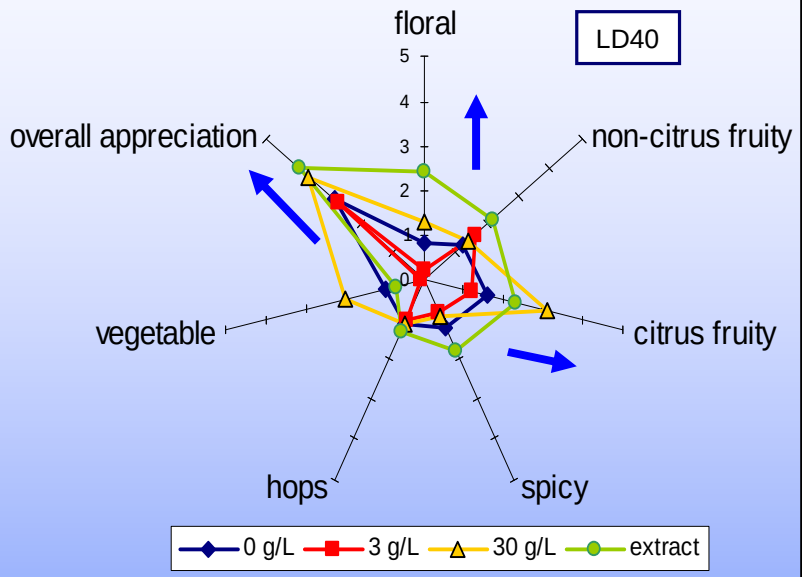
Comr	Mi	Control at pH 5.0 without yeast	LD72	vity

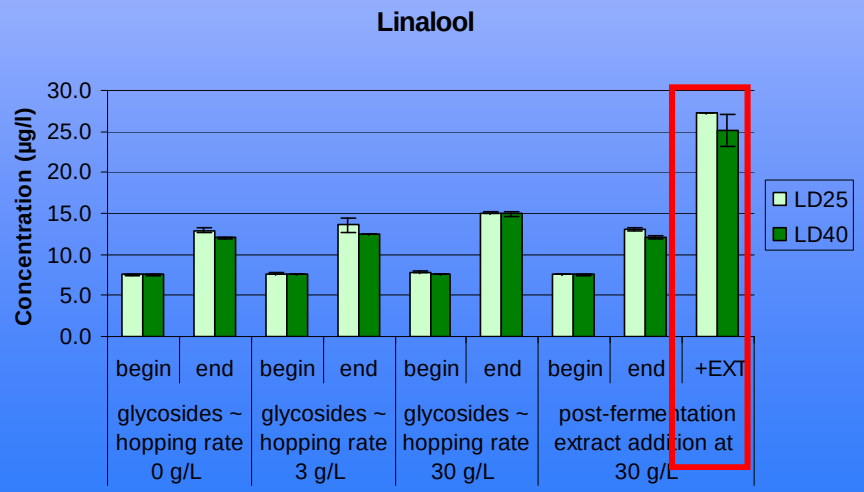


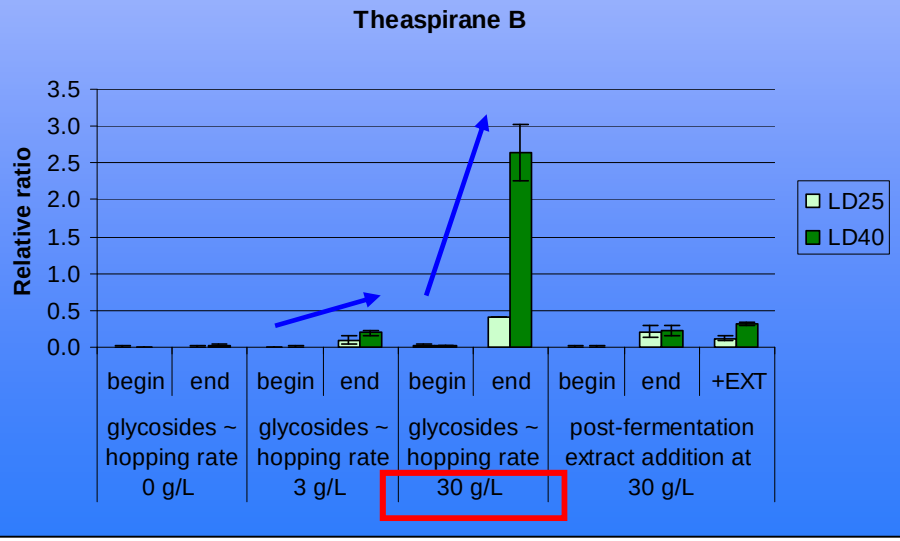


Wort fermentation with *S. cerevisiae* brewing strains

- Wort + Saaz hop glycosides, or post-fermentation addition of a pre-hydrolyzed extract
- Fermentation with *S. cerevisiae* LD25 and LD40 (low and high exo-1,3-β-glucanase)
- **Results**:
  - Linalool: significant ↑ in extract; similar for 2 yeasts
  - Dihydroedulan & theaspiranes: clear ≠ for 2 yeasts
  - Sensory: significant increase; other compounds seem to be involved

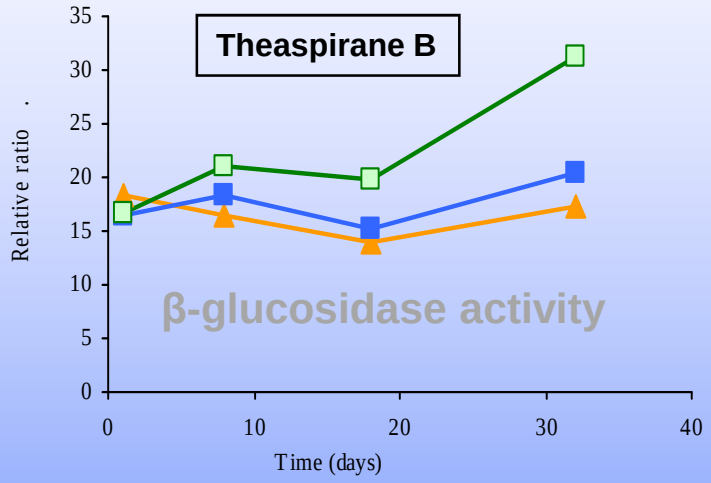


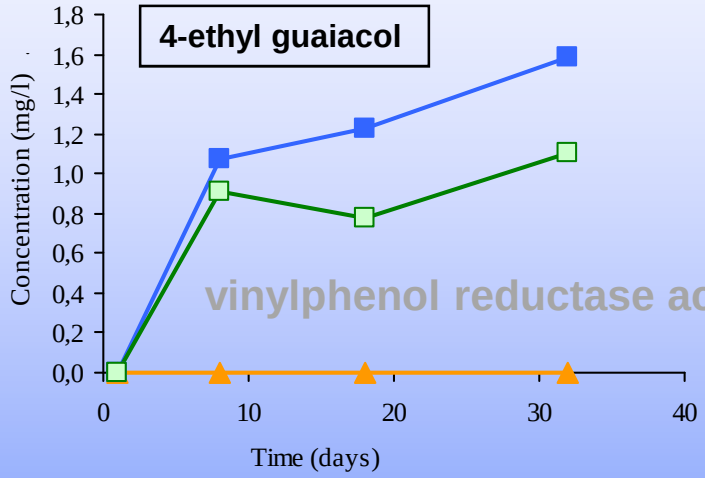


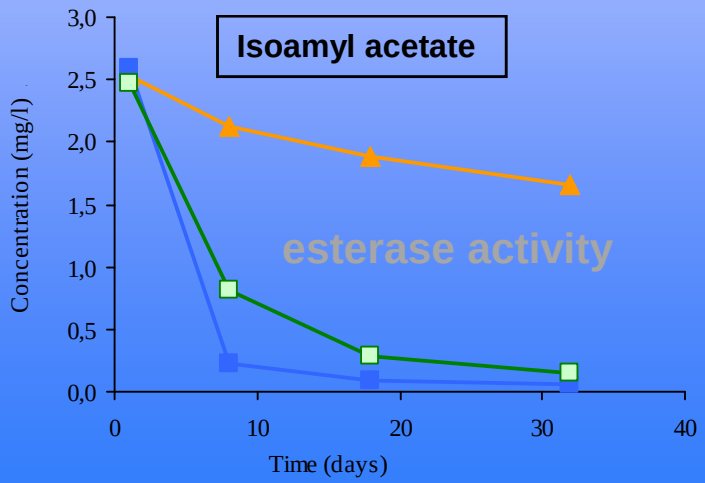


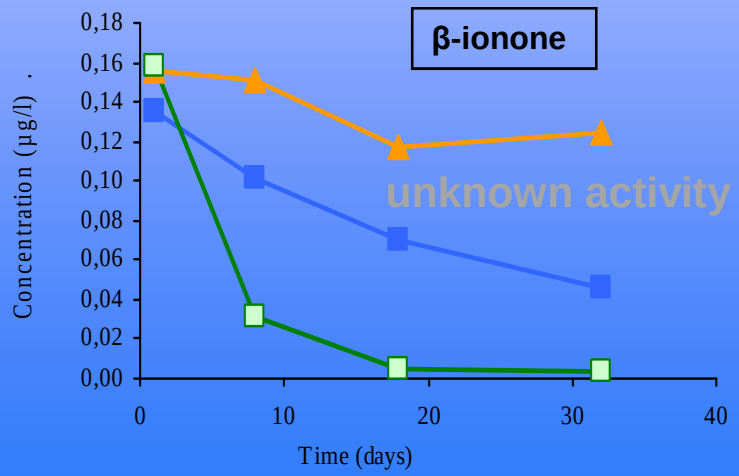
Refermentation of a dry-hopped beer by different yeasts

▲ *S. cerevisiae* ■ *S. cerevisiae + B. bruxellensis* □ *S. cerevisiae + B. custersii*









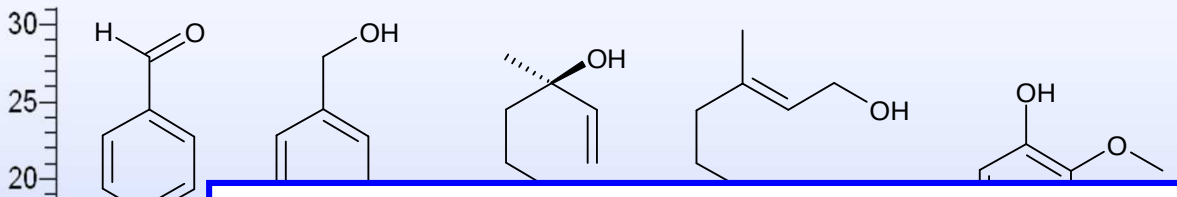
‘Kriek’-beer

- Traditionally:
  - intact sour cherries are added to wooden casks filled with young lambic
  - leaving it for 5 to 6 months
  - the sugar from the sour cherries triggers a second fermentation
- Currently:
  - also cherry pulp, cherry juice and cherry stones
  - fruit extracts and essences
  - flavours

Aglycones from sour cherry glycosides

SPME GC/MS

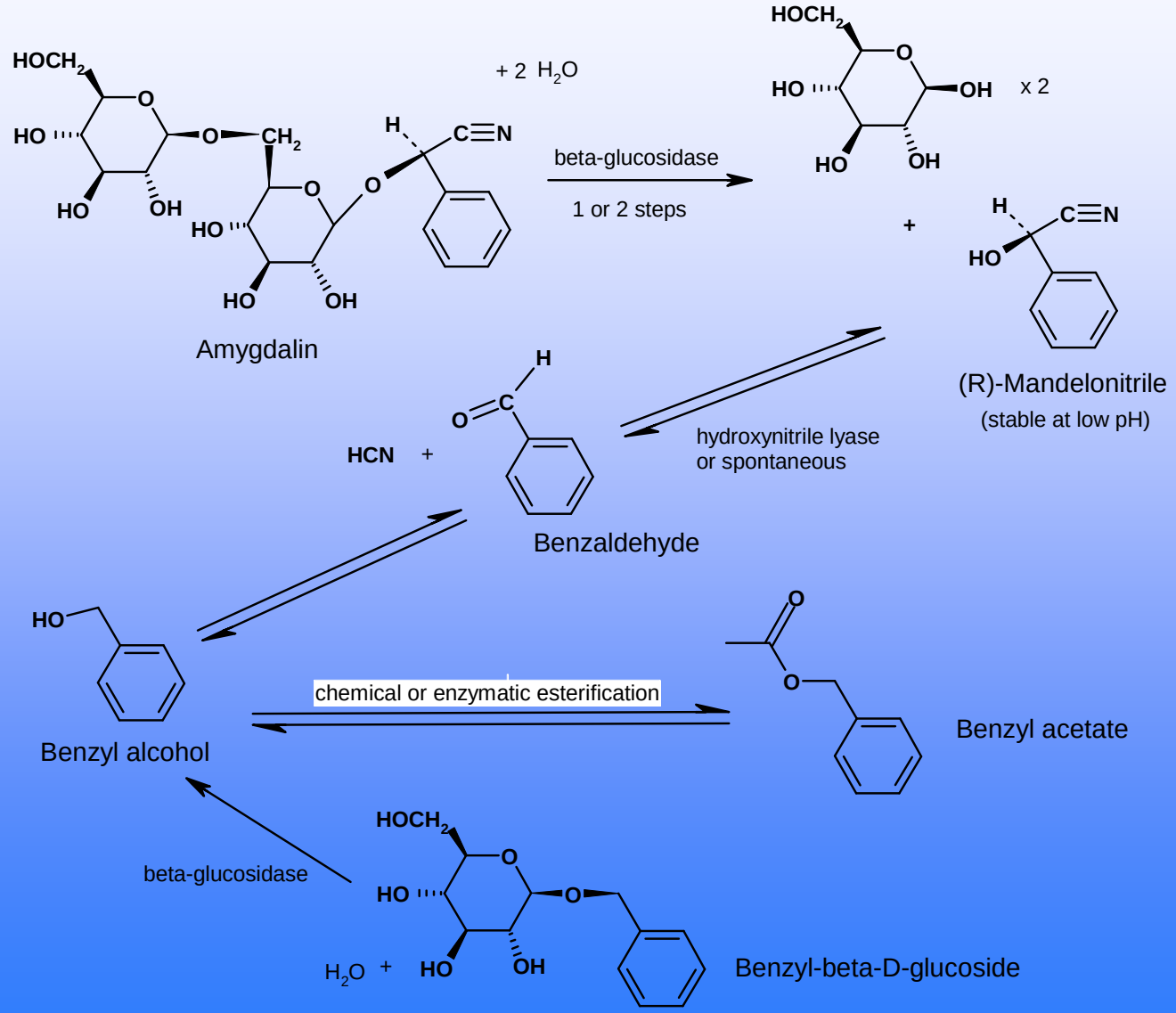
Control pH 5.0



Schmid and Grosch (1986): benzaldehyde, linalool and eugenol are some of the important contributors to cherry aroma

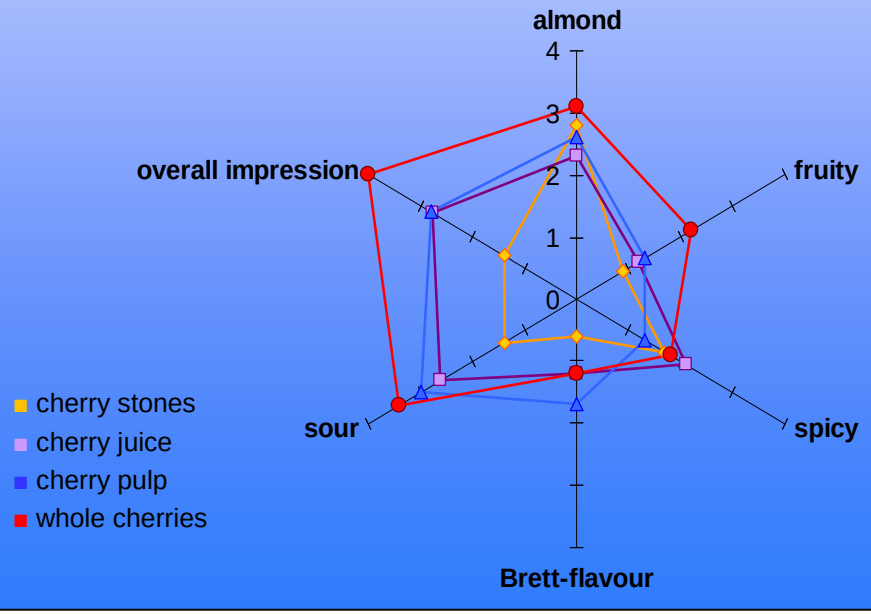
AR2000

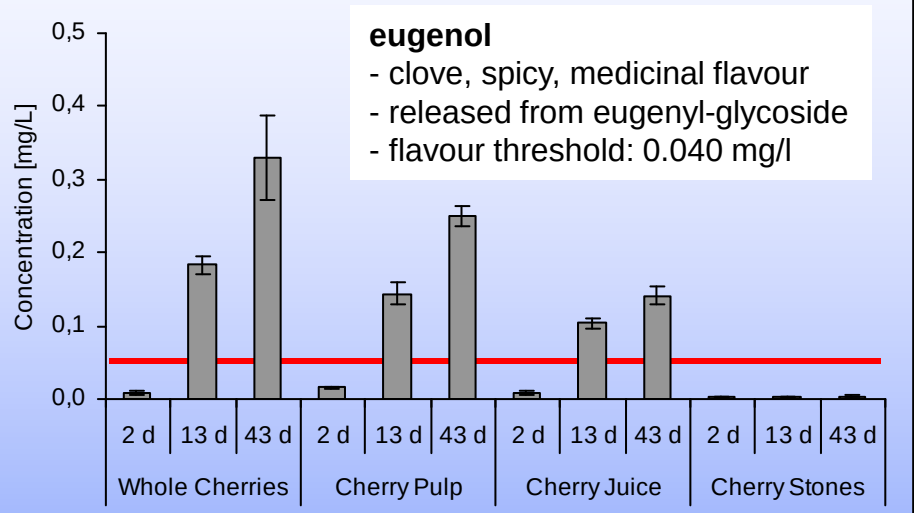
Cherry stones contain the cyanogenic glycoside amygdalin

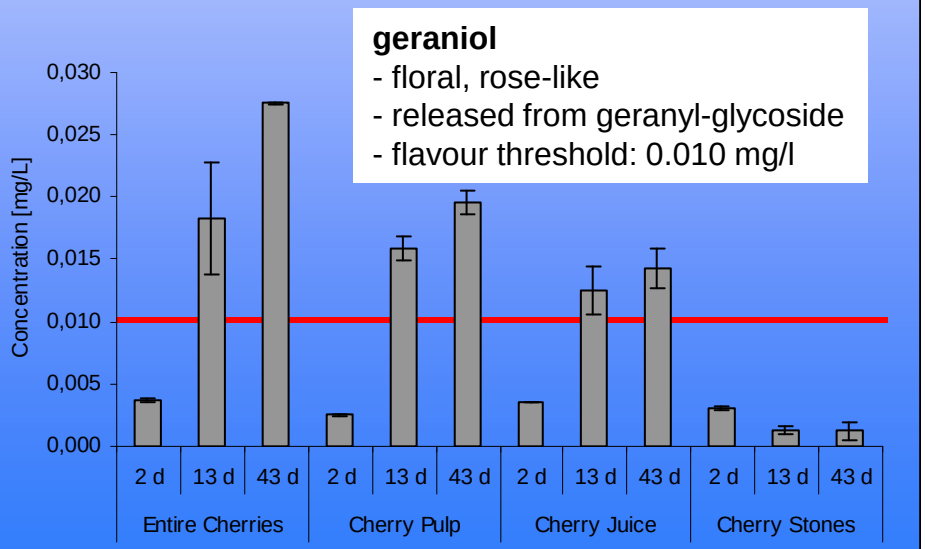


Development of cherry flavour

- Refermentation of a base beer, with addition of **200g sour cherries** per liter
- Yeast**:** ***Brettanomyces custersii*** strain, selected for its high beta-glucosidase activity
- **Results**:
  - Increase in benzyl compounds (especially benzaldehyde reduced to benzylalcohol)
  - also other compounds important for cherry flavour: geraniol, eugenol, linalool



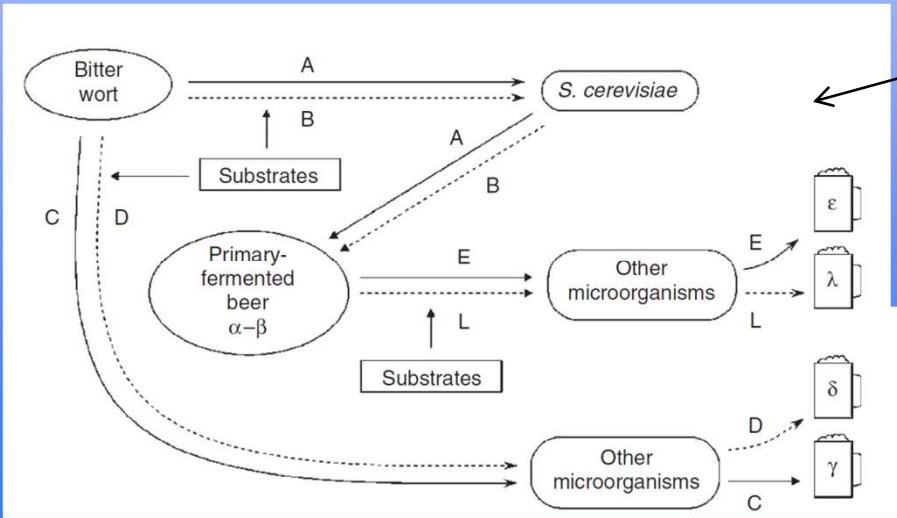




Summary

Treatment		Comment
Acid hydrolysis	Low pH (3,0 -3,5)	Separate proces; sour beers
Enzymatic hydrolysis	Eg. Enzymes from *Aspergillus niger*	Some have side activities: HCD activity → 4-VG Anthocyanidin breakdown (risk of color loss) Esterase activity → isoamylacetate ↓
Yeast biotransformation	***Saccharomyces* sp.**	Mostly low or moderate activity
	***Brettanomyces* sp.**	High activity; but also other Brett flavours

→ Different treatments lead to different flavour profiles

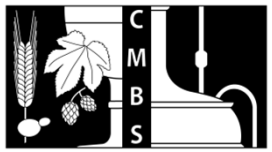


Glycoside containing substrates:

- **Hop** flowers, pellets or hop solids
- **Cherries** (whole, juice, pulp and/ or kernels)
- Other fruits, spices, ...

~ Thank you for your attention ~

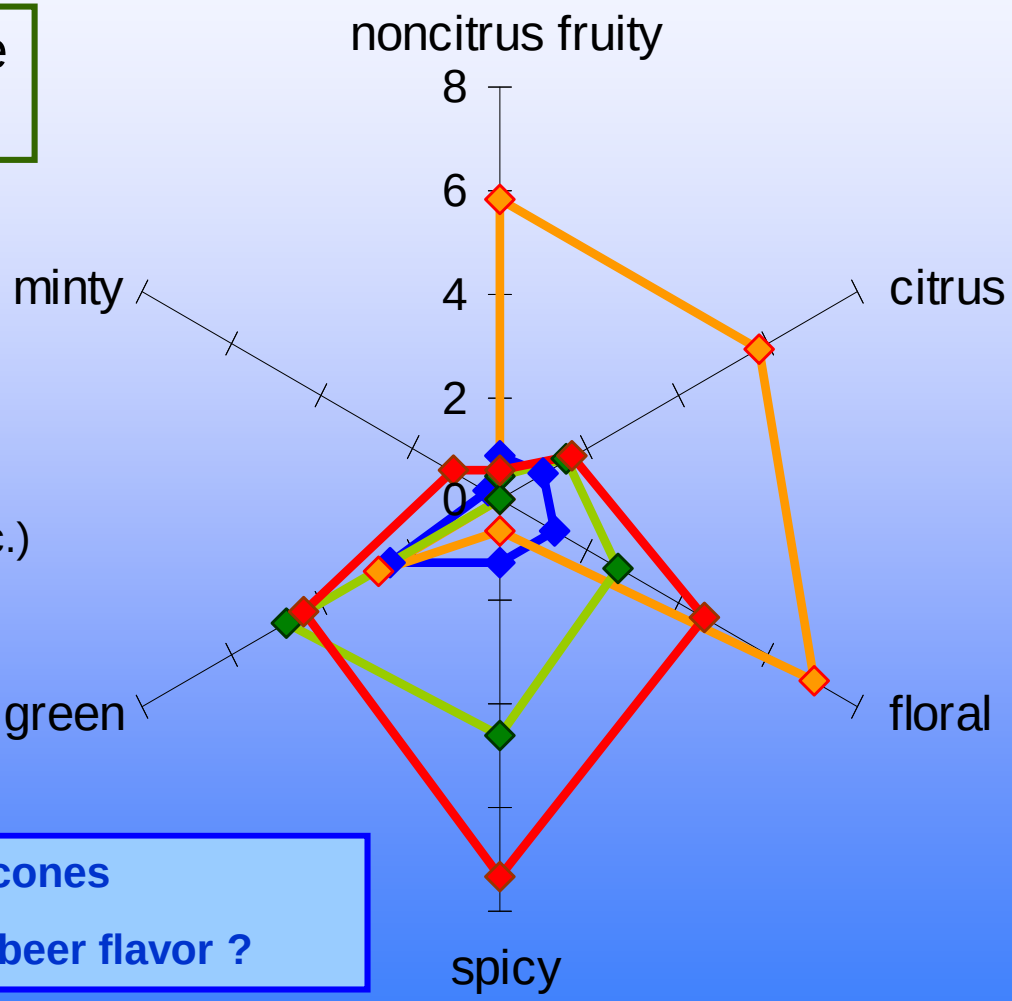
Back-up slides



Hop glycoside flavour potential

concentrated hop glycoside extract in buffered medium

- control at pH 5.0
- acid hydrolysis at pH 3.0
- enzyme hydrolysis (almond β-gluc.)
- enzyme hydrolysis (AR2000)



- Release of sensory active aglycones

- Possible impact on the overall beer flavor ?

Brettanomyces LD72 identification

- 1st identification → physiological tests
- 2nd → PCR fingerprinting (primers M13 and OPA09)
  - *Brettanomyces custersii* CMBS LD72
  - *Dekkera anomala* CBS 8139

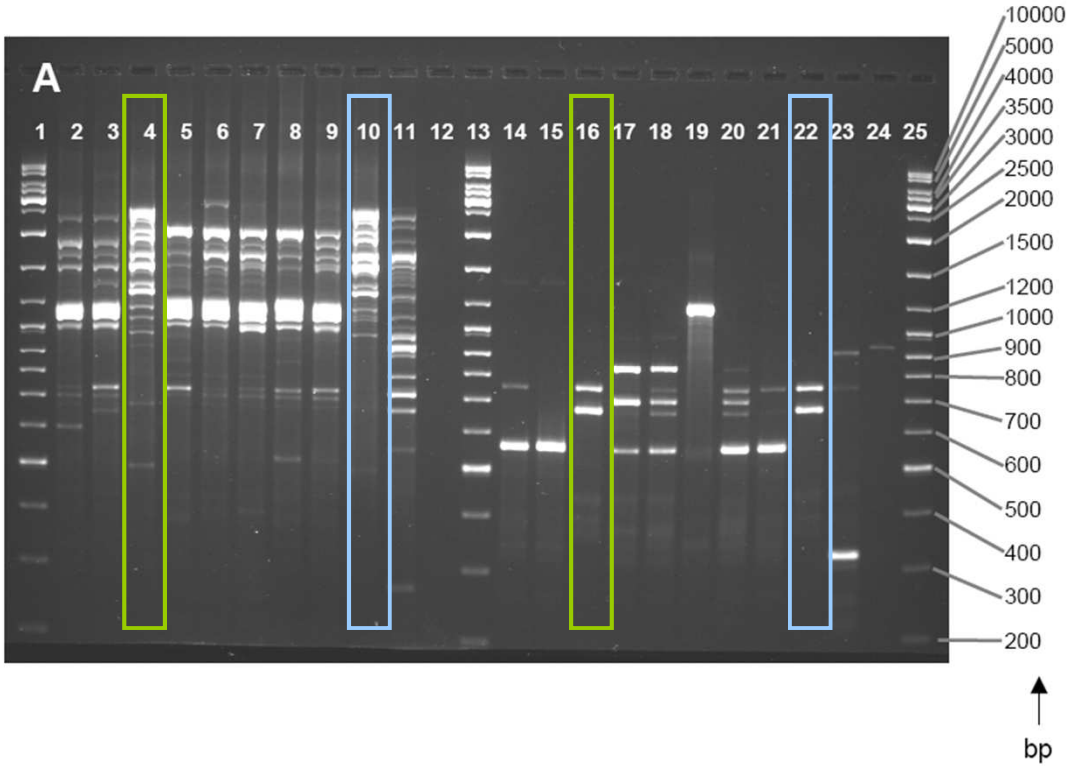
Table 8: Acidions-resistant yeasts in lambic fermentation (Verachtert and De Mot 1990)

Months of fermentation of lambic

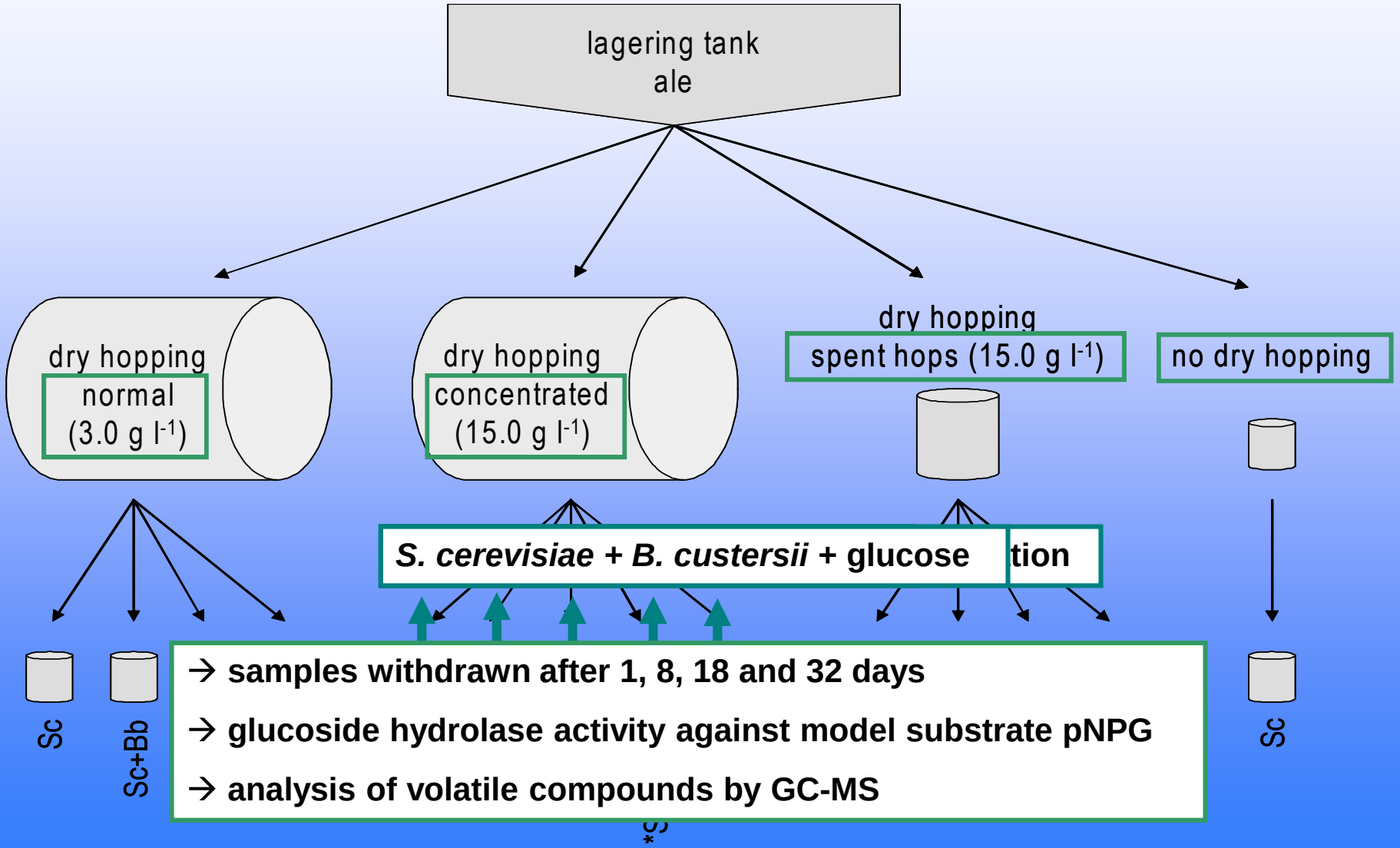
species	1-4	5-8	9-12	13-18	19-24	*Brettanomyces*[a]
abstinens	0	0				
anomalus	3	6				
bruxellensis	0	0				
custersianus	1	0				
custersii	0	0				
intermedius	0	0				
lambicus	0	0				
naardenensis	0	0				
nanonanus						
Number of species	2	1				
Kloeckera	26	4				

[a]: indicated by italic type

% of total



Experimental set-up of dry-hopping trial



HCN potential in sour cherry stones

Regulation of HCN in foodstuffs → EU-directive 88/388/EEC (1988)

↓

1 mg HCN per (v/v)% alcohol

Example: cherry beer → 4 (v/v)% alcohol
→ maximum 4 mg HCN / litre

200 g cherries / litre beer

↓ stones = 5-7% of total weight
(Chaovanalikit & Wrolstad 2004)

10 - 14 g stones / litre beer

↓ amygdalin in stones: 0.22 (w/w)%
(Chandra *et al.* 1993)

22.0 – 30.8 mg amygdalin / litre beer

↓ MW_{HCN} = 27 g/mol MW_{amyg} = 457 g/mol

1.30 – 1.82 mg HCN / litre beer **< 4 mg HCN / litre !**

Lethale dosis → 42 mg HCN / 70 kg body weight

Released benzaldehyde:
5.1 – 7.1 mg / litre
threshold: 2 mg / litre

Considering:
- maximum release
- no evaporation