

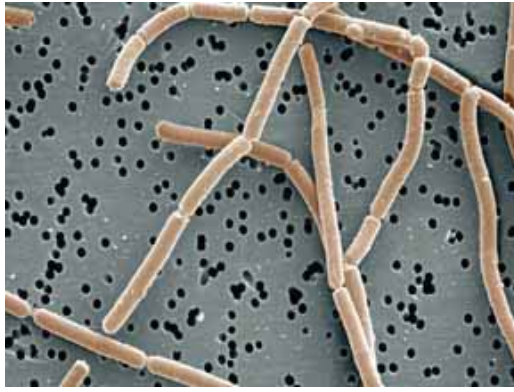
Pickled Beer: Concepts of Sour Ale

Raj B Apte

Matadero Creek Brewery

- What is sour ale: lambic, flemish red, oud bruin, berliner weiss, gose, jopenbier
- Cast of characters—lotsa Latin
- Fermentation: balancing food and environment
- Flemish sour ale
 - Historical basis
 - Flavor palette
 - Microbial machinery can make anything
 - Tandem fermentation: acetic acid, alcohol, lactic acid, phenols
- Procedures
 - Total acidity
 - Anti-oxidants and hop polyphenols
 - Sour mashing
 - Wood
 - Turbid mashing
- Summary of sour ale brewing

Cast of characters

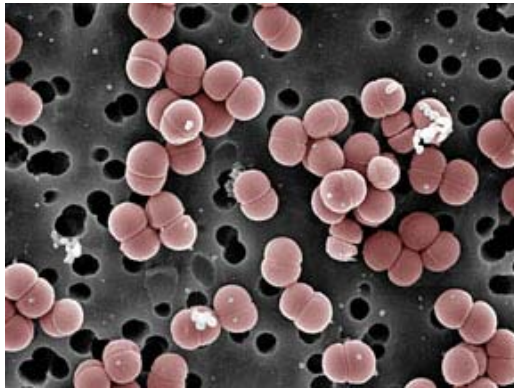


Lactobacillus

- basis of yoghurt, cheese
- lactic acid
- salt resistant; hop sensitive?

Pediococcus

- anaerobic
- lactic acid
- hop resistant
- secrete amylase?

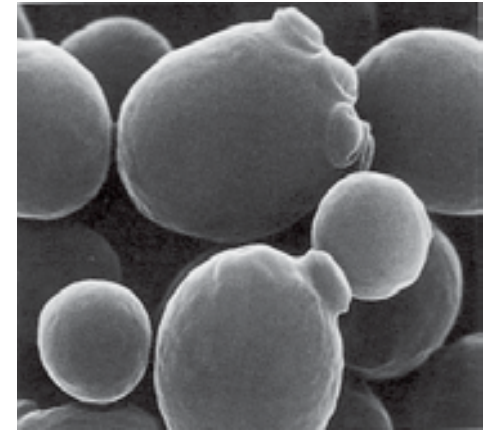


Acetobacter

- aerobic
- pellicle forming
- inhibited by salt

Enteric bacteria

- low alcohol tolerance
- low acidity tolerance
- produce mixed acids



Saccharomyces

- sugar fungus
- aerobic or anaerobic
- produces alcohol
- bakers, brewers, vintners; hundreds of strains

Brettanomyces

- british fungus; british flavor
- aerobic or anaerobic
- Bordeaux v. Davis
- produces alcohol or acetic acid
- several species; many strains

(all photos stolen from websites—please let me know if you want attribution)

Pickling

- Sauerkraut & Lactic Cucumbers
 - wild Lactobacillus
 - 1.5% brine, similar to animal fluids
 - anaerobic
 - produce 0.1-2% lactic acid
- Vinegar and Brine
 - inoculated Acetobacter in wine
 - salt toxic to Acetobacter; brined only after acetic fermentation is done.
 - produce 5-15% acetic acid
 - aerobic
 - re-use
 - Shao Xing cooking wine
- Pediococcus
 - sausage making
 - pickled cucumbers
 - silage
 - cheese



www.Sudkeramik.de



???

General Fermentation

| | salt | Alcohol tolerance | Lactic acid | Acetic acid | starch | time | oxygen | temp, F |
|--------------------|------|-------------------|-----------------|-------------|--------|------|--------|---------|
| Saccharomyces | - | 20%, P | pH 4.5 | 0.5% | - | 2 | + | 40-95 |
| Brettanomyces | - | 15%, P | | P | | 100 | + | 40-95 |
| Lactobacillus | 8% | 8% | 1% P | - | | 4 | | 60-150 |
| Pediococcus | | | 2% P, pH 3.4 | | + | 100 | -- | |
| Acetobacter | - | 6-18% | | 8% P | | 30 | ++ | 70-110 |
| Enterobacteriaceae | | 2%, P | pH 4.4, P | 0.5%, P | | 2 | + | |
| molds | | - | - | -- | | 5 | ++ | <100 |

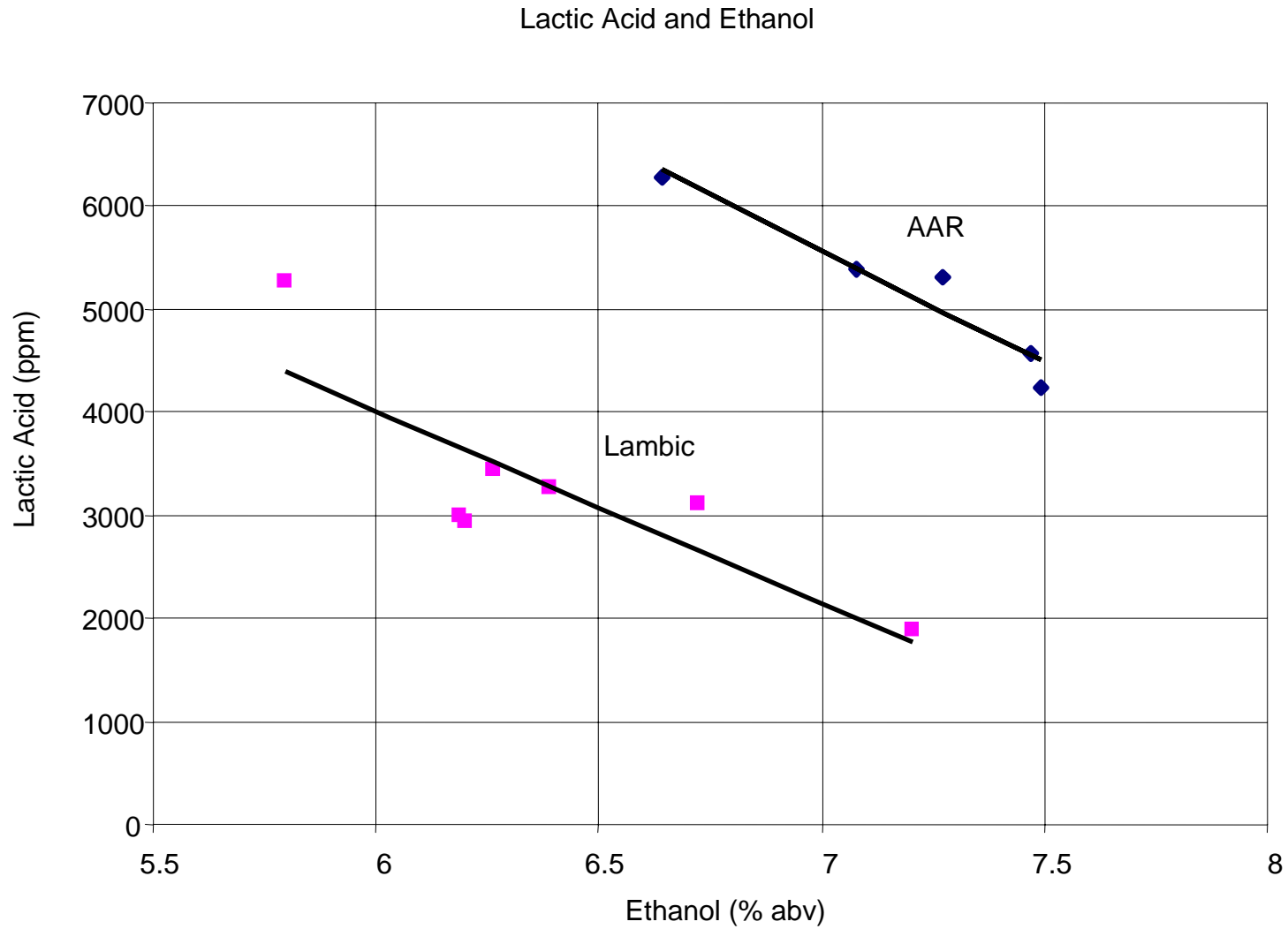
P produces

-- toxic

++ required

-, + minor effect

Ethanol toxicity in lactic bacteria



Historical context: everything was sour

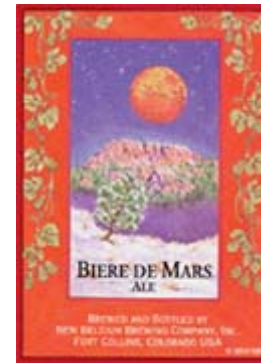
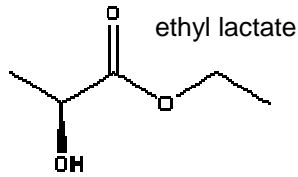
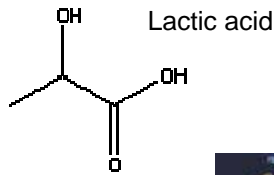
- Beer 1500-1900
 - Fresh ale: cheap, common
 - Aged beer: very expensive, 2 years
 - 5% abv sour ale. Today: Flemish sour ale
 - 10% abv stock ale. Today: Barleywine, Imperial Stout
 - Porter: a blending technique.
- Flemish sour ale
 - Dextrinous wort
 - Aged hops: anti-bacteria and anti-oxidant
 - “Spontaneous” or collateral inoculation
 - Wood: the role of oxygen
 - Time
- Tandem Fermentation
 - Saccharomyces: alcohol
 - Lactobacillus: lactic acid
 - Pediococcus & Brettanomyces: lactic & acetic acid, funk
 - Acetobacter: rising oxygen levels

| | Gueuze | Unblended AAR |
|-------------------------|-----------|---------------|
| Apparent Extract, Plato | 0-3.9 | 1.2-2.5 |
| Ethanol, v% | 4.6-5.7 | 6.6-7.5 |
| pH | 3.3-3.5 | 3.2-3.3 |
| Acetic Acid, ppm | 600-1200 | 1500-2500 |
| Lactic Acid, ppm | 1900-5300 | 4200-5400 |
| Ethyl Acetate, ppm | 60-170 | 90-130 |
| Ethyl Lactate, ppm | 360-480 | 220-290 |

Martens

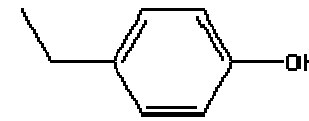
Landscape of sour ales

Lactobacillus
Pediococcus

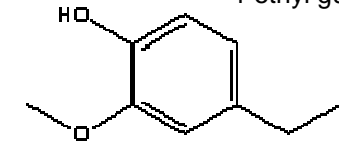


Brettanomyces

4-ethyl phenol



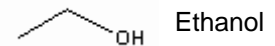
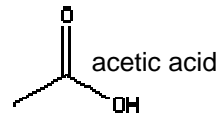
4-ethyl guaiacol



ethyl acetate

CCOC(=O)C

Acetobacter
Enterobacteriaceae



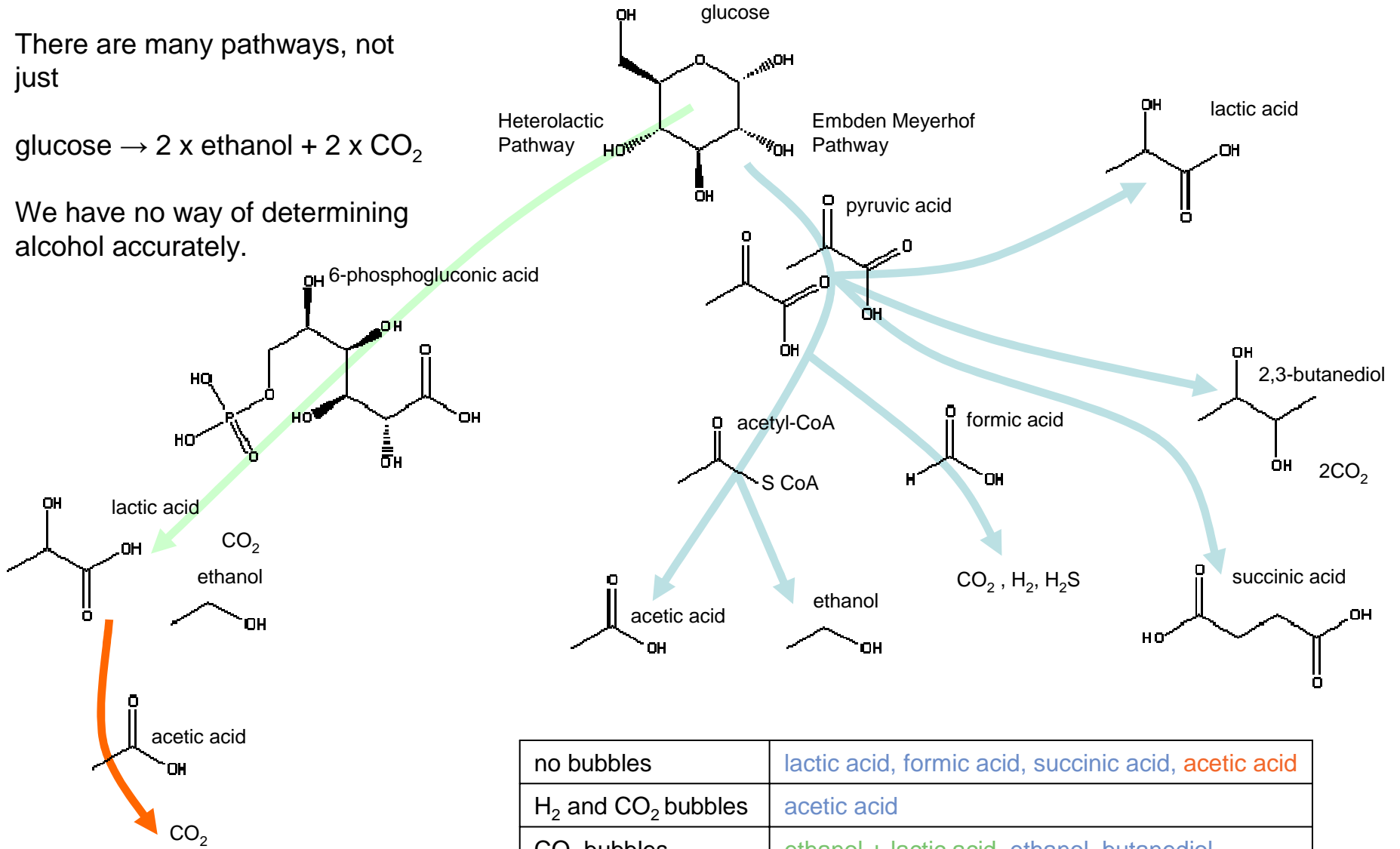
Saccharomyces

We are not in Kansas anymore

There are many pathways, not just



We have no way of determining alcohol accurately.



| | |
|--|--|
| no bubbles | lactic acid, formic acid, succinic acid, acetic acid |
| H ₂ and CO ₂ bubbles | acetic acid |
| CO ₂ bubbles | ethanol + lactic acid, ethanol, butanediol |

Tandem Fermentation

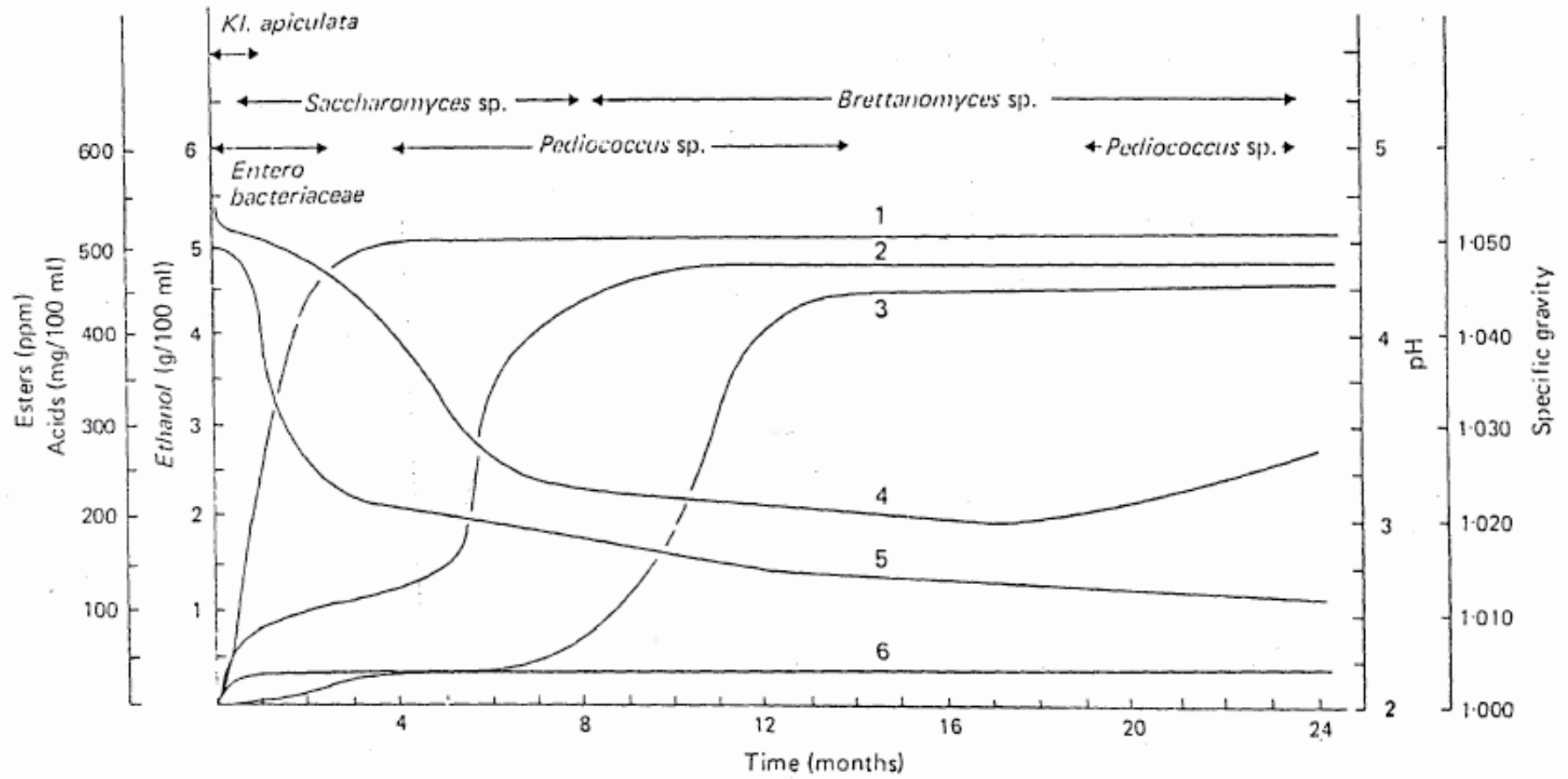
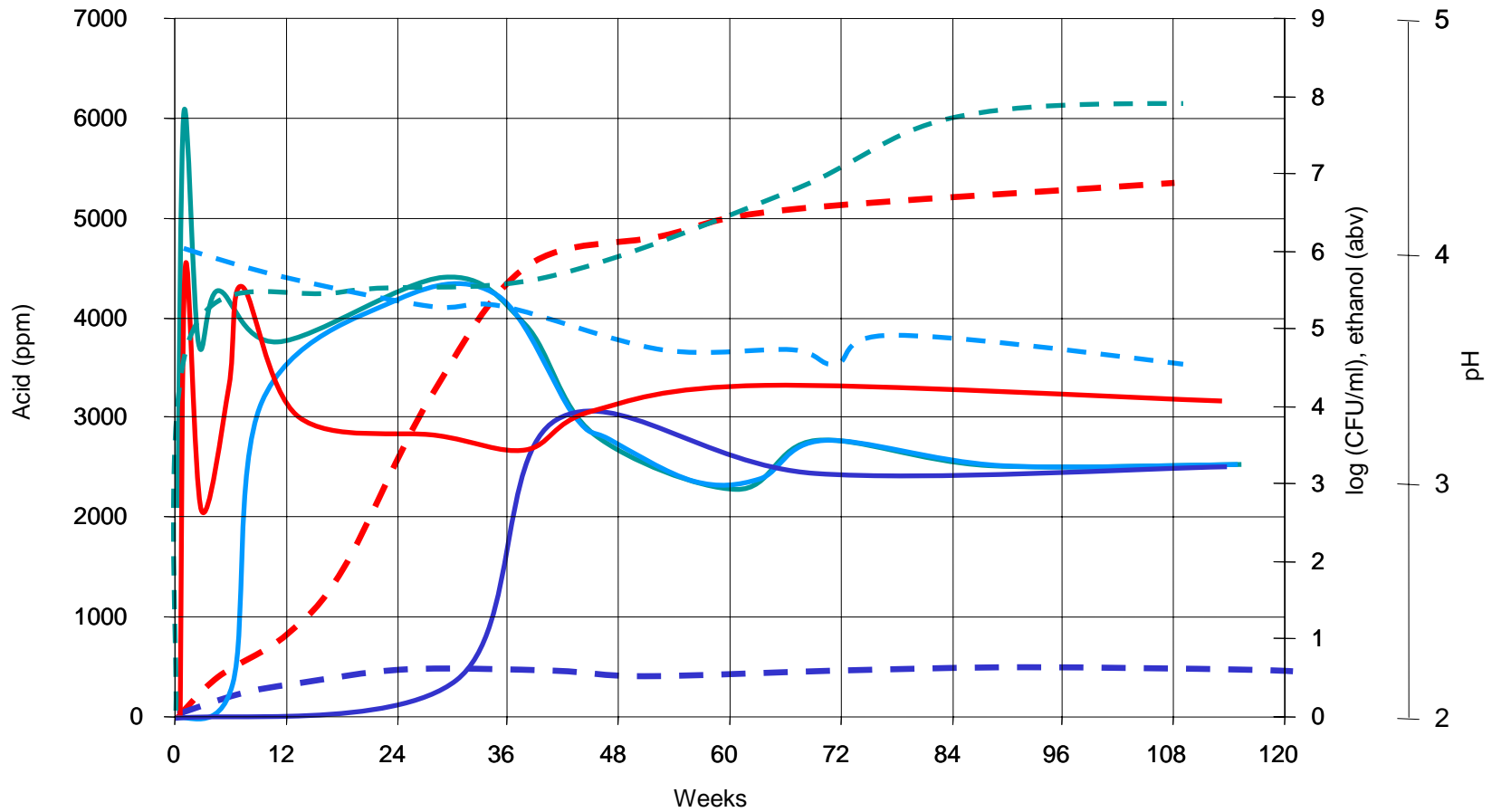


Figure 3. Evolution of some important parameters of spontaneous lambic fermentation: 1 = ethanol; 2 = lactic acid; 3 = ethyl lactate; 4 = pH; 5 = real extract; 6 = acetic acid, and sequence of microorganisms involved (Reprinted from Van Oevelen et al. 1977. *J. Inst. Brew.* 83:356-360). Guinard

AAR Fermentation Dynamics

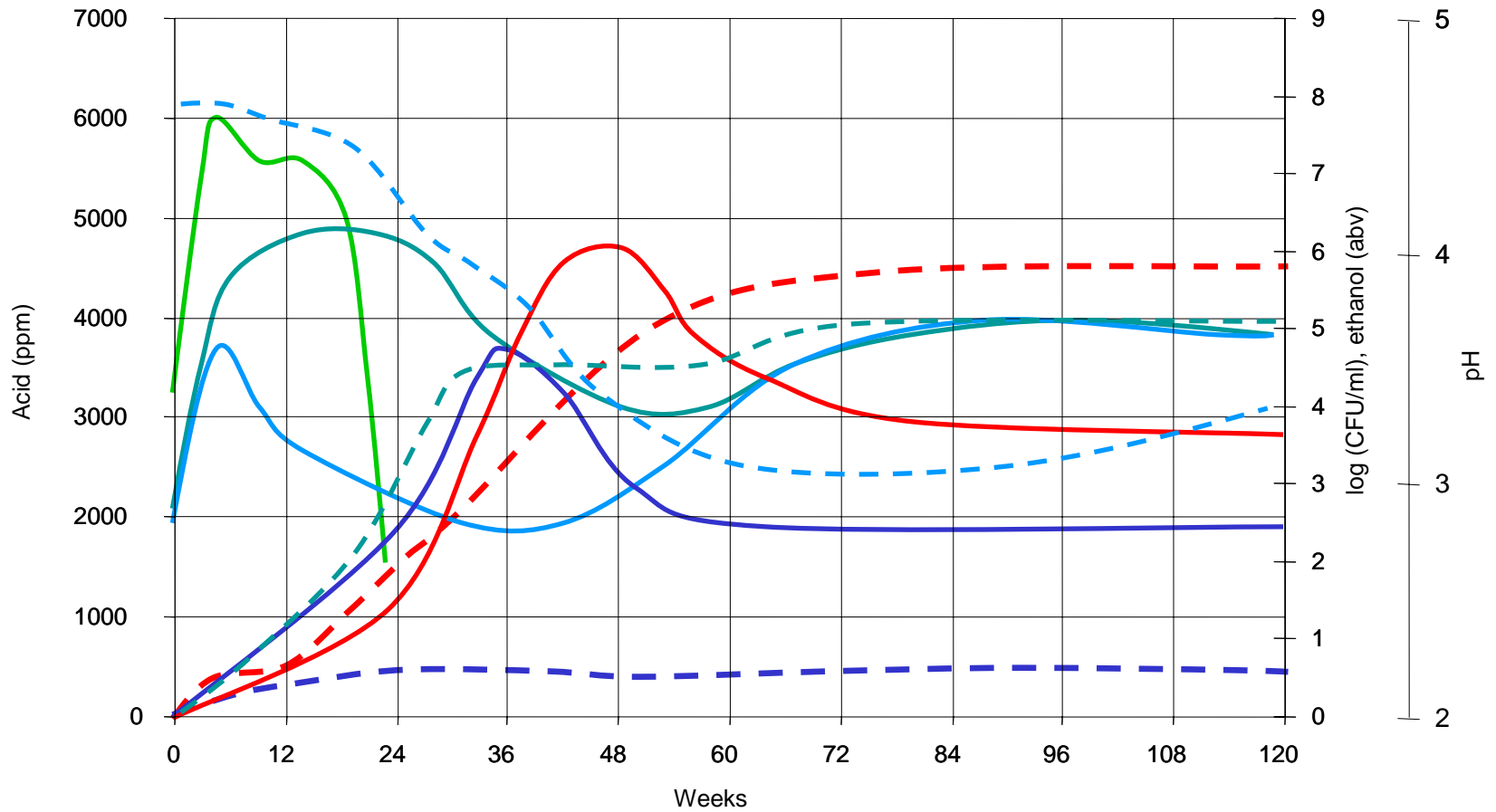


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- enterobacteria
- total yeast
- actidione-resistant yeast
- acetic acid bacteria
- lactic acid bacteria
- - - ethanol
- - - pH
- - - acetic acid
- - - lactic acid

data taken from Martens

Lambic Fermentation Dynamics



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- enterobacteria
- total yeast
- actidione-resistant yeast
- acetic acid bacteria
- lactic acid bacteria
- - ethanol
- - pH
- - acetic acid
- - lactic acid

data taken from van Oevelen

Titrateable acidity (TA) and pH, defined

| | |
|--------------------------------|---|
| pH | TA |
| like temperature | like heat |
| $-\log_{10}([H^+])$ | how many molecules of acid, by weight |
| measures strength of acid | measures quantity of acid |
| determines biological activity | determines taste |
| use pH paper or meter | titrate with base to neutrality "how much drano do you add to your beer to get pH 7.0" |

10,000ppm = 1%

1ml of 0.1N NaOH is equivalent to 9mg lactic acid

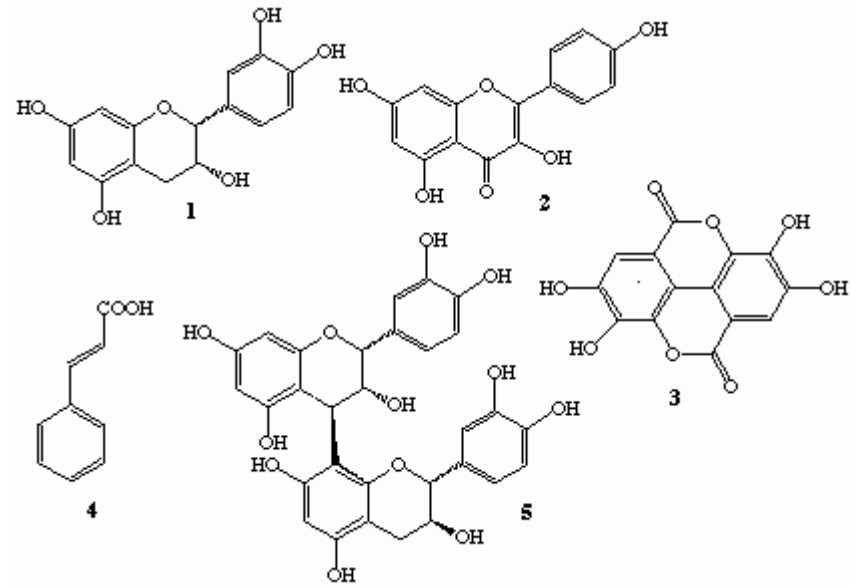
| Acid | pK _{a1} | pK _{a2} | Mass/Mole |
|----------|------------------|------------------|-----------|
| Tartaric | 3.02 | 4.54 | 150 |
| Citric | 3.03 | 4.74 | 192 |
| Malic | 3.40 | 5.05 | 134 |
| Lactic | 3.86 | -- | 90 |
| Ascorbic | 4.04 | 11.8 | 176 |
| Acetic | 4.76 | -- | 60 |

| | % abv | pH | TA tartaric w% |
|---------------------------------|-------|-----|----------------|
| Leinenkugel lager | | 4.1 | |
| Preston Merlot, 2000 | 13.5 | 3.5 | 0.6 |
| Landskroon Chardonnay, 2003 | 13.0 | 3.0 | 0.7 |
| Verhaeghe Duchesse | 6.2 | 3.1 | 1.5 |
| Petrus Aged Pale | 7.3 | 3.4 | 1.0 |
| Cantillon Iris | 5.0 | 3.4 | 1.4 |
| Hanssens Oudbeitje | 6.0 | 2.8 | 1.5 |
| Morte Subite Peche | 4.3 | 3.6 | 0.7 |
| Kriek de Ranke | 7.0 | 3.5 | 0.7 |
| Matadero Creek Cask Porter (me) | 6.0 | 3.3 | 0.6 |
| Matadero Creek Kriek (me) | 6.0 | 3.5 | 0.8 |

Why use hops for low bitterness beer?

Anti-Oxidant polyphenols

- Polyphenols come from hops and malts (tannins are polyphenols).
 - high usage of aged hops
 - high sparge temp.
- Anti-oxidants: no conclusive human health effects, but very important for flavor stability
- Saaz: 6% polyphenols, 3% alpha acids
- Bottle conditioning with yeast has anti-oxidant effect
- Cardboardy, oxidized flavour is prevented
- Contribute to haze (never drink a clear lambic)



catechin (1), camphorol (2), ellagic acid (3), cinnamic acid (4), procyanidine B1 (5)

| Polyphenol, Czech Saaz | mg/100g |
|------------------------|------------|
| Hydroxy benzoic acids | 1 - 10 |
| Hydroxy cinnamic acids | 100 - 450 |
| Proanthocyanidins | 100 - 600 |
| Flavanoles | 30 - 200 |
| Flavonoles | 1 - 10 |
| Quercetine flavonoids | 50 - 250 |
| Camphorol flavonoids | 50 - 300 |
| Prenyl flavonoides | 100 - 1000 |

Sour Mashing, Papazian—NOT!

Lactic Souring

In gallon jug, mix 3L brewing water at 46C with 800g unmilled malt, seal with fermentation lock, hold at 46C for 2-3 days. If possible, inoculate with *Lactobacillus delbrueckii* or *amylolyticus*; or inoculate with grain.

- *Lactobacillus* is oxygen-tolerant; oxygen is toxic to *Pediococcus*
- Some L., eg *L. amylolyticus*, can digest starch and do not require mashing.
- At 46C very few other things will grow
- standard method of mash acidification for Reinheitsgebot brewing (addition of synthetic acids forbidden). Widely practiced for pale beers in Germany
- low ethanol, low IBU, low oxygen give more sourness

Acetic Souring

Orleans process: put beer in a jar, cover with cloth, and keep warm and dark. I have Brett also in the pot. Very useful for blending

Wood, Plastic and the Role of O₂

| Material | Oxygen Permeability cc-mil/m ² -day-Bar |
|--|---|
| Wood, Oak | 7200 |
| High-density polyethylene HDPE | 2325 |
| Low-density polyethylene LDPE | 8586 |
| Polyethylene terephthalate copolymer PETG | 400 |
| Polypropylene PP | 2526 |
| Polycarbonate PC | 4650 |
| Nylon (not oriented) | 79 |
| Saran | 2 |

Flextank.com

200L barrels admit 20cc/L of oxygen per year = 100cc/L air. For 20L carboy, that's 2L of air.

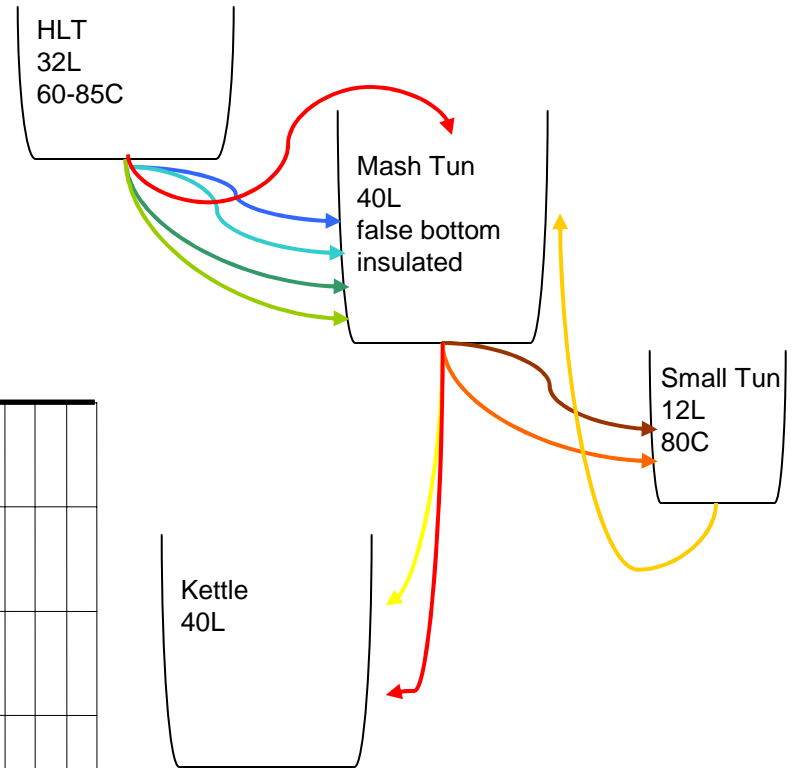
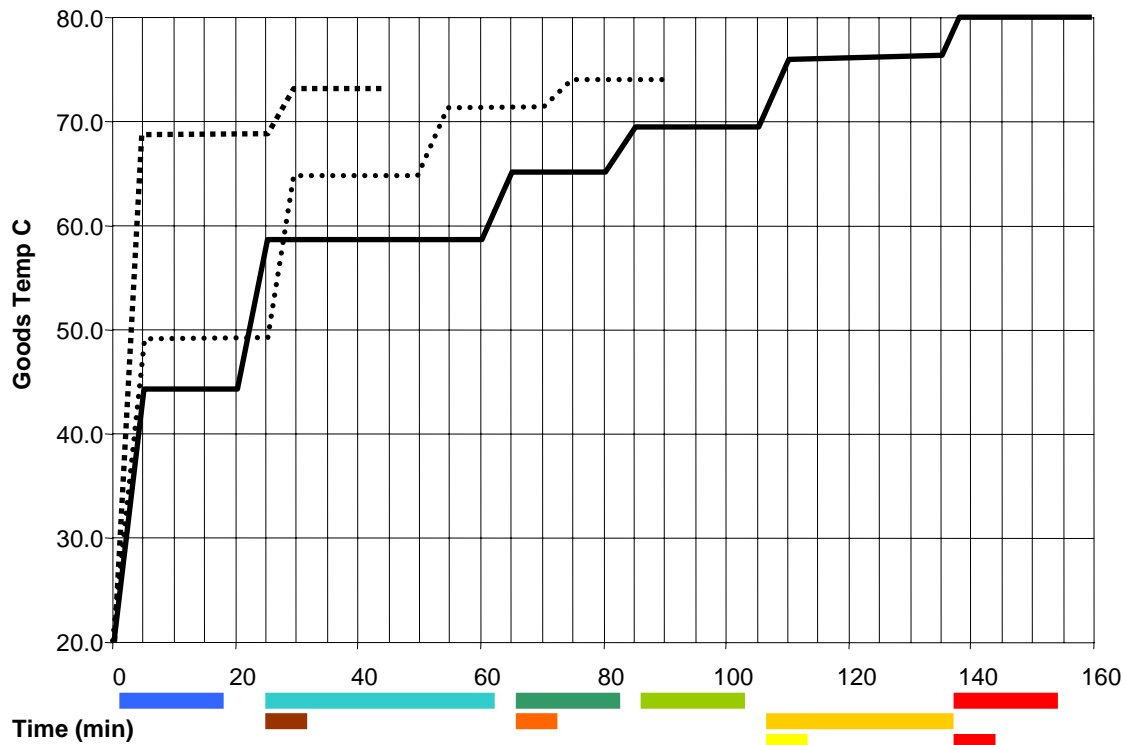
Brett will not thrive without oxygen. If you use cultured Brett, the large cell population can overcome this.

Custers effect = negative Pasteur effect = Brett needs O₂

Large casks at Rodenbach admit much less oxygen than 20L HDPE pail, much more than glass.

Turbid Mashing

Lambic/plambic only
 30-40% raw wheat
 Free Amino Nitrogen (FAN)
 starch
 tannins
 high extraction eff.
 works for very poorly modified malt



Brewing sour ales

- Mashing
 - Turbid or infusion mashing (high sacch. temp)
 - raw wheat, 30-40%, dark crystal
 - protein rests, high sacch. rest
 - hops 30-120g/20L. <15 IBU.
 - gypsum/carbonate to buffer (100ppm)
- Starting Fermentation
 - slow cooling: chill to 50C, pitch sour, hold until pH < 4.5
 - open window inoculation. enterobacteria—the sponge experiment
 - large doses of pure culture v. small inoculations
- Aging
 - primary or secondary lees
 - wood/plastic. bungs and topping off
 - late carbohydrate additions (corn or wheat starch, malt)
 - good ol' summertime
- Blending & Fruit
 - hard lambic, soft lambic, acetic lambic
 - running vinegar culture
 - traditional or novel fruits
 - late, do not age
 - carbonation levels, dryhopping

Bibliography

1. Kunz, Technology of Brewing and Malting, 2nd Ed. VLB (1999)
2. Dawoud, Characteristics of Enterobacteriaceae involved in Lambic brewing, Dissertationes de Agricultura, KUL. (1987)
3. Martens, Microbiology and Biochemistry of the Acid Ales of Roeselare, KUL. (1996)
4. van Oevelen, Microbiology and Biochemistry of the Natural Wort Fermentation in the Production of Lambic and Gueuze, Dissertations de Agricultura, KUL. (1978)
5. Kumara, Microbiology and Biochemistry of Lambic Beer Overattenuation, Dissertationes de Agricultura, KUL. (1990)
6. Battcock and Azam-Ali, Fermented Fruits and Vegetables, A Global Perspective, FAO Agricultural Bulletin 134. (1998)