Brewing with *Brettanomyces*
the horse the goat and the barnyard

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Owner/Brewer

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Crooked Stave Artisan Beer Project

- Msc. Brewing and Distilling - ICBHD, Heriot-Watt University Edinburgh, Scotland
  - Dissertation titled: **Pure Culture Fermentation Characteristics of Brettanomyces Yeast Species and Their Use in the Brewing Industry**

- Brewed for Odell Brewing Company
  - Sabotuer
  - Friek
  - Deconstruction

- Opened Crooked Stave November 1st 2010
- Brewed our First Batch June 10th, 2011
  - 100% Brettanomyces primary fermentation in oak barrels... still aging!

- **Wild Wild Brett** - Series of 100% Brett primary fermented beers
  - Barrel-aged Sour Beers - L’Brett d’Or, Nightmare on Brett street, Reverentia
  - Petite Sour - Partial Brett primary in spontaneously soured wort blended in large oak foeder
  - Surette - Saison wild ale, mixed house culture fermented entirely in oak foeder
Brettanomyces / Dekkera

- Same organism, name can more or less be used interchangeably

- **Brettanomyces**
  - The asexual reproducing (budding) form, known as an anamorph

- **Dekkera**
  - The sexual reproducing form, known as a teleomorph

- Original nomenclature:
  - *Brettanomyces bruxellensis*
  - *Brettanomyces lambicus*
  - *Brettanomyces intermedius*
  - *Brettanomyces custersii*
  - *Brettanomyces claussenii*
  - *Brettanomyces anomalus*
  - the list goes on...
Current nomenclature:
- \textit{Brettanomyces bruxellensis*}
- \textit{Brettanomyces anomalus*}
- \textit{Brettanomyces custersianus}
- \textit{Brettanomyces naardenensis}
- \textit{Brettanomyces nanus}

and for the perfect state (teleomorph)
- \textit{Dekkera bruxellensis}
- \textit{Dekkera anomala}

Of these, \textit{Brettanomyces bruxellensis} and \textit{Brettanomyces anomalus} are the two species currently used by brewers, with a \textit{Brettanomyces custersianus} just starting to make its way into use.

*\textit{B. lambicus} has since been reclassified as a \textit{B. bruxellensis} and \textit{B. claussenii} as \textit{B. anomalus}.
Initial Discovery and Characterization

- The earliest published account came from a paper presented to the Institute of Brewing in 1904 by N. Hjelte Claussen.
  - He proposed the yeast be called *Brettanomyces* (British Brewing Fungus), as it was responsible for the secondary fermentation and development of characteristic flavors and aromas of the finest English stock ales.

- In 1940 the first systematic investigation was presented by M.T.J. Custers, and his findings on 17 strains of *Brettanomyces*.
  - At that time Custers believed *Brettanomyces* yeasts were only found in English and Belgian beers,
  - We now know it is a yeast that is found around the world and in every known wine making region. **NOT a Belgian yeast.** per se...

- Custers Thesis
  - Fermentation of glucose to ethanol occurred more rapidly under aerobic conditions than anaerobic conditions, Custers termed this “negative Pasteur effect”.
    - This was in no means a complete fermentation by a brewers standards, only measure of how quickly fermentation or metabolism started under the studies conditions.
  - Considerable amounts of acetic acid produced during aerobic conditions, while no appreciable amounts were formed during anaerobic conditions.
  - Custers to believed cells slowly became adapted to anaerobic conditions eventually resulting in a normal anaerobic fermentation.
Custers Effect

- The term “Custers effect” was later introduced in place of negative Pasteur effect and is best interpreted in terms of glycolytic activity;
  - as cells are switched from aerobic conditions to anaerobic, glycolysis (the break down of sugars to pyruvate) comes to a temporary stop. This transient lag phase was observed to last up to ten hours before the slow dissimilation of glucose and subsequent production of CO2 resumed.

- Lag phase during early anaerobic growth and/or fermentation is due to the continued drainage of NAD+ to NADH through an irreversible conversion of acetaldehyde to acetic acid.

- There exists an inability for the cells to restore the redox balance via production of reduced metabolites, specifically glycerol.
  - This could be attributed to the absence of glycerol 3-phosphate phosphatase activity in some or all *Brettanomyces* species.

- The production of glycerol has been shown to be important in metabolizing NADH and restoring the redox balance during anaerobic fermentation in other yeasts including brewers yeasts.
  - Glycerol produced by yeast has a secondary role in enhancing the perception of body and mouthfeel in beer when 1-2 g/l are present. More on this later...

- The slow adaptation of *Brettanomyces* yeasts metabolism during anaerobic conditions following the lag phase was never understood, although the findings are in agreement with multiple studies that have suggested anaerobic fermentation is possible with sluggish to slow activity initially.
Fermentation Capabilities

• Two unique and important enzymes.

• α-glucosidase
  • Enzyme capable of hydrolyzing (breaking down) wort dextrins with up to 9 degrees of polymerization, a malto-oligosaccharide containing 9 glucose molecules.
  • Enzyme functions by cleaving off a glucose molecule forming the next lower malto-oligosaccharide.
  • Both extracellular and intracellular forms of the enzyme are produced by *Brettanomyces* yeasts.
  • Enzyme responsible for the over attenuation observed in Lambic and Sour beers.

• β-glucosidase
  • Enzyme capable of hydrolyzing multiple sugars and glycosidic compounds including;
    • Lactose - milk sugar
    • Cellobiose - sugar from cellulose in wood/plant material
    • Glycosides - present in various Hops, Fruit and Spices
  • Early studies conducted at Guinness in Dublin found strains of *Brettanomyces clausenii* were able to ferment lactose and cellobiose.
  • Research into ethanol production found a *Brettanomyces custersii* strain that was capable of fermenting cellobiose to ethanol, and exhibited a higher utilization of cellobiose then other *Brettanomyces clausenii* strains used in the study.
  • Present at varying levels in only certain *Brettanomyces* species.

• Interestingly *Brettanomyces* yeasts are most commonly cultured from beer conditioned in oak barrels and a symbiotic relationship has been assumed given the longevity of their existence in the barrels.
  • Strangely enough most of the *Brettanomyces* species which dominate during Lambic brewing do not generally have the necessary β-glucosidase enzyme present to hydrolyse cellobiose.
Most significant aspect of *Brettanomyces* yeasts is their ability to influence the flavor and aroma of beer.

Many organoleptic descriptors exist including; clove, spicy, horsey, barnyard, smokey, medicinal, band-aide, metallic, cracker biscuit, goat-like, cat piss, apple, floral, tropical fruit and citrus.

Research from the Wine and Brewing industries have explained a few of these compounds

- Precursor compounds come from raw materials with their production emphasized or limited through brewing techniques.
- Ability to produce various aromatic or flavor compounds varies greatly between strains. Strain Dependent!
Volatile Phenolics

- Responsible for some of the most recognized aromatic characteristics associated with *Brettanomyces*
  - 4-vinylguaiacol – Clove like
  - 4-ethylguaiacol – Spicy, Clove
  - 4-vinylphenol – Phenol, Plastic, Smokey
  - 4-ethylphenol – Spicy, Smoky, Horsy
  - 4-vinylcatechol – Plastic, Bitter, Smoky
  - 4-ethylcatechol – Band-aide, Medicinal, Barnyard

- Appear to have a synergistic or additive effect making their presence observable while the actual compound levels are below recognized threshold levels.

- Makes identifying one compound hard as they share similar organoleptic characteristics depending on the amount present in a beer.

- The mechanism responsible for the metabolism of these volatile phenolics is a two enzyme system.
  - First is a phenolic (cinnamic) acid decarboxylase,
    - Responsible for the decarboxilation of hydroxycinnamic acids into the respective 4-vinyl derivative
    - Present in both *Brettanomyces* and various brewers yeast strains
  - Second, a vinyl phenol reductase responsible for the reduction of the 4-vinyl derivative into its respective 4-ethyl derivate.
    - The vinyl reductase enzyme is unique to *Brettanomyces* yeasts
Production of Esters

- Esterases present in *Brettanomyces* species have ester synthesizing activity with an increase of the following esters observed during the period *Brettanomyces* species dominate as active yeasts in Lambic and Sour beers.
  - Ethyl acetate - Fruity, solventy
  - Ethyl lactate - Fruity, buttery
  - Phenethyl acetate - Rose flower like

- The esterases decrease any isoamyl acetate present.

- High concentrations of C₈ to C₁₂ fatty acids accumulate in Lambic as well during the same period.
  - These Fatty acids then become esterfied into their respective ethyl esters.

- Recently brewers have talked about pineapple or tropical fruit like aromas produced by *Brettanomyces* yeasts.
  - Unknown which compounds are responsible for these flavors or aromas
  - Some people have suggested ethyl lactate, others ethyl butyrate.
  - No studies previously looked at a range of esters produced by pure cultures of *Brettanomyces* yeasts because up until recently it has almost exclusively been considered a spoilage organism.
### Important Aroma / Flavor Compounds

**Table 1.** Quantitative analysis of 16 standard fermentation compounds analysed from pure culture fermentations conducted with eight strains of *Brettanomyces* yeast. Fermentations were inoculated at a pitching rate of 12×10⁶.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Threshold (mg/l)</th>
<th>WLP645</th>
<th>WLP650</th>
<th>WLP653</th>
<th>CMY001</th>
<th>BSI-Drie</th>
<th>WY5112</th>
<th>WY5526</th>
<th>WY5151</th>
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<tbody>
<tr>
<td>Acetaldehyde</td>
<td>10</td>
<td>1.37</td>
<td>1.21</td>
<td>1.25</td>
<td>1.26</td>
<td>1.98</td>
<td>0.80</td>
<td>1.05</td>
<td>1.16</td>
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<tr>
<td>Ethyl Acetate</td>
<td>30</td>
<td>1.26</td>
<td>3.62</td>
<td>12.25</td>
<td>16.76</td>
<td>35.80*</td>
<td>1.88</td>
<td>8.38</td>
<td>2.68</td>
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<tr>
<td>Ethyl Lactate</td>
<td>250</td>
<td>0.28</td>
<td>0.18</td>
<td>1.81</td>
<td>1.44</td>
<td>1.29</td>
<td>1.72</td>
<td>0.75</td>
<td>0.59</td>
</tr>
<tr>
<td>Isoamyl Acetate</td>
<td>1.6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ethyl Butyrate</td>
<td>0.4</td>
<td>ND</td>
<td>ND</td>
<td>0.04</td>
<td>0.06</td>
<td>0.08</td>
<td>ND</td>
<td>0.05</td>
<td>ND</td>
</tr>
<tr>
<td>Isoamyl Acetate</td>
<td>1.6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ethyl Caproate</td>
<td>0.21</td>
<td>ND</td>
<td>0.16</td>
<td>0.25*</td>
<td>0.39*</td>
<td>0.36*</td>
<td>0.11</td>
<td>0.29*</td>
<td>0.04</td>
</tr>
<tr>
<td>Ethyl Caprylate</td>
<td>0.9</td>
<td>0.08</td>
<td>1.65*</td>
<td>4.13*</td>
<td>3.35*</td>
<td>3.00*</td>
<td>0.72</td>
<td>1.34*</td>
<td>0.22</td>
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<tr>
<td>n-Propanol</td>
<td>800</td>
<td>0.59</td>
<td>2.57</td>
<td>4.14</td>
<td>3.72</td>
<td>6.56</td>
<td>0.88</td>
<td>3.01</td>
<td>1.12</td>
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<tr>
<td>Isobutanol</td>
<td>200</td>
<td>0.70</td>
<td>3.83</td>
<td>2.32</td>
<td>7.04</td>
<td>8.00</td>
<td>2.46</td>
<td>3.16</td>
<td>1.63</td>
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<td>2-Methylbutanol</td>
<td>65</td>
<td>0.27</td>
<td>5.22</td>
<td>1.09</td>
<td>2.67</td>
<td>2.61</td>
<td>1.31</td>
<td>1.49</td>
<td>0.60</td>
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<tr>
<td>3-Methylbutanol</td>
<td>70</td>
<td>0.91</td>
<td>5.13</td>
<td>3.82</td>
<td>6.71</td>
<td>8.62</td>
<td>2.15</td>
<td>5.39</td>
<td>2.14</td>
</tr>
<tr>
<td>4-Vinylphenol</td>
<td>**</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4-Vinylguaiacol</td>
<td>0.3</td>
<td>0.0397</td>
<td>0.0529</td>
<td>0.0244</td>
<td>0.0390</td>
<td>0.0458</td>
<td>0.0400</td>
<td>0.0511</td>
<td>0.0849</td>
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<tr>
<td>Diacetyl</td>
<td>0.15</td>
<td>0.012</td>
<td>0.024</td>
<td>0.220*</td>
<td>0.029</td>
<td>0.029</td>
<td>0.056</td>
<td>0.051</td>
<td>0.038</td>
</tr>
<tr>
<td>2,3-Pentanediene</td>
<td>0.9</td>
<td>0.003</td>
<td>0.004</td>
<td>0.018</td>
<td>0.004</td>
<td>0.004</td>
<td>0.009</td>
<td>0.003</td>
<td>0.007</td>
</tr>
</tbody>
</table>

ND, Not Detectable; *, Indicates levels at or above threshold; **, No conclusive data available. Fermentations were conducted in duplicate with samples of those fermentations analysed in duplicate. Data shown is the average of all figures attained.

**Threshold**
- Ethyl acetate (*only one strain*)
- Ethyl caproate - C₆ fatty acid ester
  - Sweet, Fruity, Pineapple
- Ethyl caprylate - C₈ fatty acid ester
  - Musty, Pineapple, Fruity, Waxy

**Low / Not Produced**
- Ethyl acetate
- Isoamyl acetate
- Higher Alcohols
- Diacetyl
- Acetaldehyde
How Crooked Stave Makes Brett Beers

- Things to take into consideration.
  - Fermentation type
    - Primary fermentation
    - Hybrid/Mixed fermentation
    - Secondary fermentation
    - Bottle conditioning
  - Raw Materials
    - Flabby / Weak mouthfeel
    - Malt / Adjuncts
    - Hops
    - Fruit
  - Brewing Technique
    - To rest or not to rest
    - Sour mashing/wort
    - Rest temp and time
    - Mash thickness
    - Sparge temp
  - Nothing new right?
American-Style Brett Ale

American Brett ales can be very light to black or take on the color of added fruits or other ingredients. Wood- and barrel-aged sour ales are classified elsewhere. Light to moderate and/or fruity and contributed by the Brettanomyces yeast. The evolution of natural acidity develops balanced complexity. Horsey, goaty, leathery, phenolic and light to moderate and/or fruity acidic character evolved from Brettanomyces organisms may be evident, yet in balance with other character. Acidity may also be contributed to by bacteria, but may or may not dominate. Residual flavors that come from liquids previously aged in a barrel such as bourbon or sherry should not be present. Wood vessels may be used during the fermentation and aging process, but wood-derived flavors such as vanillin must not be present. In darker versions, roasted malt, caramel-like and chocolate-like characters should be subtle in both flavor and aroma. American Brett ales may have evident full range of hop aroma and hop bitterness with a full range of body. Estery and fruity-ester characters are evident, sometimes moderate and sometimes intense, yet balanced. Diacetyl and sweet cornlike dimethylsulfide (DMS) should not be perceived. Chill haze, bacteria and yeast-induced haze are allowable at low to medium levels at any temperature. Fruited American-Style Brett Ales will exhibit fruit flavors in harmonious balance with other characters. Original Gravity (°Plato) Varies with style • Apparent Extract/Final Gravity (°Plato) Varies with style • Alcohol by Weight (Volume) Varies with style • Bitterness (IBU) Varies with style • Color SRM (EBC) Varies with style

• Brewers Association, 2011 Beer Style Guidelines, January 10, 2011
Brewing with Brettanomyces

- I deconstruct my beers from finish to start by coming up with the finished creation and working backwards.

- Once I know what I want the beer to look, smell and taste like, it’s easier to choose the ingredients/brewing techniques that will help to create the final product.

  - If it is “cleaner” Brettanomyces characteristics I’m after, subtle fruity aromas, or light clove with a slight tartness, then Primary fermentation with Brett would be the choice.

  - If I’m after a more funky beer with stronger complex characteristics gained through extended aging, secondary fermentation would be the choice, possibly in a barrel.

  - A mixed fermentation can give the best of both.. yeast with fruity esters from a chosen Saccharomyces strains as well as good attention quickly while the Brettanomyces strain delivers more primary type characteristics up front as well as delivering on the back end during extended aging.

  - It’s not black and white and there are many combinations to play with. In the end it will come down to the raw materials, Brewing techniques and Brettanomyces strain(s) chosen.
Quality Control

- Use separately marked designated equipment
  - As you retire equipment use it as *Brettanomyces* and sour brewing equipment

- *Brettanomyces* is not a super organism, it is a yeast and can be cleaned and removed like yeast. Heat killing or sterilizing will do the trick

- Recommended media agars
  - MYPG (Malt extract) agar
  - WLN agar
  - CuSO₄ agar
Propagation

• I would highly recommend propagating up an active starter if using *Brettanomyces* yeast for primary fermentation.
  • Proper cell counts needed for primary
  • Proper cell physiology will lead to a healthy active fermentation
  • Higher viability and vitality

• Same techniques used to grow *Saccharomyces* can be used with *Brettanomyces*, only more time is needed.
  • 24 hour lag phase
  • 3 days of exponential growth
  • 1-2 day lag or slowed growth
  • 2-3 days of near exponential growth
  • Generally took 7-8 days to reach stationary phase

• **Equipment needed**
  • 1 litre Erlenmeyer flask
  • Sterile breathable foam/film to cover flask (still need the exchange of O$_2$ and CO$_2$)
  • Warm environment (80° F is nice)
  • Stir plate will maximize cell counts (give a good swirl daily or often if you don’t have a stir plate)
  • Lightly hopped wort of 12°Plato or 1.048 Gravity
Figure 1. Growth curve for five strains of *Brettanomyces* during semi-aerobic batch culture. Cultures were grown in 500ml of wort substrate over a 288-hour period at 28°C with 80-rpm agitation. Viability was taken into account to reflect the actual cell number.
Primary Fermentation

- “Clean” primary fermentation
  - Brett primary fermented beer that does not use any souring organism or sour mashing.
  - Fermentation takes between 2 weeks and 1 month. **Strain dependent**
  - Little to no perceived tartness as anaerobic fermentation does not typically produce levels of acids above threshold.
  - Could develop a slight tartness from minimal acetic acid produced by the Brett after some extended aging or heavy oxygenating at knockout.

- “Sour” primary fermentation
  - Brett primary fermented beer that either had a sour mash, or souring organisms which develop during primary and with time and aging.
  - Acidulted malt is another option and I’ve heard of brewers using 10% or more in the mash
  - Can be a good idea as there seems to be a symbiotic relation ship with Brett and Lactic acid bacteria
  - Appears to improve attenuation and lower fermentation time.
  - Fermentation time still 10 days to 1 one month. **Strain dependent**
  - Most people associate Brett with tart/sour beers.
Primary Fermentation

- I knockout at 68°F (20°C) and let fermentation ramp up to 72°F (22°C)
  - I prefer to keep the fermentation around 70°F (20-22°C) for a stable sustained fermentation

- I like to heavily oxygenate at knockout - Always oxygenate!
  - Estimated dissolved oxygen of 12-15 pmm
  - Increases O₂ and increases acetic acid production
  - I like a slight acetic character as at 45-150 ppm acetic is a soft and sweet acid, very complimentary to the beer

- Pitching rate of 1×10⁶ cell/ml/°Plato
  - This is the standard rule of thumb pitching rate for ale yeast

- 14°Plato (1.056) beer attenuates down to 2.5°P (1.010) in 7-10 days

- Faster fermentation time when re-pitching with successive generations
- Greater ability to ferment high gravity beers
  - 22°P beer attenuated down to 4°P in 10 days
Oak Barrel Primary Fermentation

- Minute amounts of oxygen during fermentation in beneficial in stimulating fermentation.

- Can have a heavy H₂S aroma for some time if not able to breathe
Benefits of Pre-soured Wort

- Acidulated malt, Sour Mash, Sour Wort
- Pre-souring wort with lactic acid leads to better attenuation in a greater range of *Brettanomyces* strains.
- Increases flavor compounds and adds depth maybe even complexity
  - Both ethyl acetate and ethyl lactate increased as the lactic acid concentration increased, especially ethyl lactate (go figure).
  - Esters like ethyl caproate and ethyl caprylate saw a decrease in production while higher alcohols increased slightly.
  - An increase in higher alcohols is probably due to the increase in attenuation.

Conclusion

- Lactic acid appears to increase attenuation in *Brettanomyces* yeasts.
- A sour mash or some acidulated malt in the grist could be beneficial.
- Too much acid and the esters ethyl caproate and ethyl caprylate which are probably responsible for the fruity, pineapple, floral, tropical fruit, and sweet aromas/flavors decrease.
- In our first commercial batch we dropped the pH to 4.5 to start primary, it finished in under 3 weeks.
- Most recent sour wort batch dropped from 10°P to 1.5°P in 9 days. Lots of tropical fruit aroma and flavor
- Caution if making sour wort... drop pH to 4.5 before KO, KO @ 120°F and sustain around 100° for a few days, Do Not oxygenate... Purge with CO₂ through wort. Pitch a culture.. You can re-boil after to kill the lacto.
Impact of Pre-souring Wort

- Further analysis concentrated on the changes in fermentation behavior due to pre-souring wort with lactic acid.

  Wort was acidified with an initial lactic acid concentration of:
  - 100 mg/l, starting pH of 4.55
  - 500 mg/l, starting pH of 4.05
  - 1,000 mg/l, starting pH of 3.75
  - 3,000 mg/l, starting pH of 3.08

![Graph showing the impact of lactic acid concentration on apparent attenuation of Brettanomyces strains.](image)

**Figure 5.** Concentration of Lactic acid in wort prior to on-set of fermentation and its impact on apparent attenuation of eight *Brettanomyces* strains with a pitching rate of 12×10^6 cells/ml.
Hybrid/Mixed Fermentation

- This is the same idea as with Saison mixed fermentations.
- One yeast is used to get the desired flavors and the other for attenuation.

- *Saccharomyces* primary for a few days, then pitch *Brettanomyces* while the active fermentation is still going. More similar to secondary fermentation.
  - Could also underpitch in primary with *Saccharomyces* strain, then when fermentation slows, rack off the yeast and transfer to another tank with a large pitch of *Brettanomyces* to finish out fermentation.

- *Brettanomyces* yeast pitched first for the primary and after pitch *Saccharomyces* to finish out and attenuate the fermentation.
  - Improves mouthfeel depending on the strain chosen

- Mixed fermentation
  - Pitch both *Brettanomyces* and *Saccharomyces* at the same time let each one do its thing.
  - Relatively clean Brett character develops after a few weeks.

- Once fermentation is complete it’s good to rack the beer off the yeast(s) and mature the beer with only the yeasts in solution. Mostly Brett as it’s not too flocculent at room temps.
Most popular use of Brettanomyces is in secondary fermentations. Can add more complexity or a new direction to a beer.

A few factors to consider
- Assess what changes are likely to take place and how it will affect the beer.
- Residual sugars
  - Research conducted on secondary fermentations with the same eight strains of Brettanomyces, showed a decrease in both maltose and maltotriose.
  - The beers started with a gravity of 2.5°P (1.0093) and saw further attenuation between 1.8°P (1.0062) and 0.9°P (1.003)
  - Maltose decreased from 5 g/l residual sugar to <1 g/l sometimes as low as 0.1 g/l.
  - Maltotriose decreased from 7 g/l residual sugar to between 4 and 2 g/l.

In general it's important to have some extra dextrins in the beer as well as a specialty malts or adjuncts that contribute to body otherwise the beer can turn out watery and flabby, lacking depth.

At the same time I'm not a fan of taking a bad beer that didn't ferment out and had fermentation issues and tossing Brettanomyces at it to see what happens. Won't make the best beer, only for something interesting at best...
Secondary Fermentation

- Extraction of polyphenols and hydroxycinnimic acids from the mash.

- Higher levels in Secondary fermentations than Primary fermentation.
  - Hydroxycinnimic acids are present in the cell walls and cells which make up the aleurone layer and the husk.
  - Protein rest will create the pre-cursors.
  - Excessive handling of the malt/mash could impart some of these compounds.
  - Careful with over sparging and sparging too hot.
  - Some will boil off in the mash, but what is left behind can leave aggressive flavors after *Saccharomyces* along and *Brettanomyces* do their thing.
  - In secondary after using a POF+ *Saccharomyces* strain the 4-vinyl compounds will already be present.

- It always been my philosophy to limit the phenolic characteristics of these beers.

- Some brewers like them, and purposely mash to get the pre-cursors and create spicy, smoky flavors.
  - Ferulic acid rest at about 109 - 111°F or 43 - 44°C.
Secondary Fermentation

- Pitching rate in Secondary fermentations
  - Brewers have their theories why a high pitch count produces more flavor and aroma and other Brewers will tell you that lower pitch counts will produce more flavor and aromas.
  - Research with eight strains showed variability from strain to strain. Some times higher pitch counts lowered higher alcohols, some times they raised them. Same was true for esters.
  - I have not found much of a correlated effect on over attenuation either.
  - Don't add too much *Brettanomyces* yeast slurry otherwise you're just blending in *Brettanomyces* flavor, created during the propagation.
    - High in Acetic Acid.
  - General cell counts to add in secondary can be anywhere from 500,000 cells/ml to 2,000,000 cells/ml.
Bottle Conditioning

- Popular technique to get the Brett character by adding the yeast in the bottling bucket. Need extra sets of all gaskets and porous material on the bottle filler.
  - Best practice is to have a second bottling unit, unless you don’t mind all your beers possibly having Brett.

- Can be used to put the final touch on a beer and allow for a light integrated *Brettanomyces* character instead of a Brett bomb.
  - See what 100,000 cells/ml can do to a beer in bottle conditioning.
  - Time to reach the desired character will be strain dependent. New Belgium is typically two weeks, others take 3 months.
  - Careful over time as autolysis occurs in *Saccharomyces*, trehalose is released which is a fermentable sugar for *Brettanomyces* and further carbonation will occur.
  - It has also been suggested that unfiltered beers where there is a large portion of *Saccharomyces* in contact with *Brettanomyces* will produce goaty aromas due to caproic, caprylic and capric acids released from the cell wall of *Saccharomyces*.
    - *Brettanomyces* could turns those acids into esters then over time.
    - Would need to sit on the beer after bottling to develop these esters.

- Good practice is to let the beer drop bright or filter before secondary unless you want these aromas and flavors.
Raw Materials

• Developing a beer that will be fermented with *Brettanomyces* involves developing a recipe that will stand up to the yeast.

  • No one sets out to make a flabby beer or one lacking aroma and flavor, but can be the case with *Brettanomyces* fermented beers.
    • Usually associated with dryness due to over attenuation.

  • Not exactly the case...

    • One reason why *Brettanomyces* is such a fickle organism is due to the lag phase seen when switching from aerobic to anaerobic conditions. This is due to the inability to restore the redox balance between NAD+ and NADH. This is appears to be caused by the lack of glycerol production during fermentation, a metabolic step which acts to restore the balance.

    • Glycerol produced by yeast has a secondary role in enhancing the perception of body and mouthfeel in beer when 1-2 g/l are present.

      • Some studies have observed slight glycerol production occurring under anaerobic conditions in a variable amount of strains.

      • Glycerol production between strains could indicate the ability of the redox to be restored more rapidly, in which case strains would likely be capable of full fermentation with a decrease in the time required.
Raw Materials

- Body and the overall beer profile have to be built to suit the *Brettanomyces* yeasts and their ability to over attenuate as well as the lack of glycerol production.

- Body can also be taken care of in the brewhouse.
  - Mashing in at a higher temp
  - Lower mash rest time
  - Both could favor a longer end to fermentation, If you’re looking to make a beer that is ready for bottling sooner these techniques might not be the best

- Adjuncts
  - Oats - I prefer Steel-cut oats, gives a silky, creamy mouthfeel, light flavor contribution.
  - Flaked Wheat - Adds more complex starches, high protein level, could aid head retention.
  - Cara(pils)(hell)(foam) - supposed to aid head retention as well as build body in beer, little flavor or color pick-up.
  - Rye - can add a nice character at 5-10% of the grist.
  - Spelt - Suggested for pure mouthfeel addition.

- Acidulated malt
  - I’ve heard of usages up to 10% which give a very nice lactic acidity.
  - Acidity creates mouthfeel, possibilities for new flavors/aromas.
  - Could do a sour mash, or sour the wort.
Primary fermentation with *Brettanomyces* can create relatively clean beers. Each strain will have its own characteristic flavors and aromas but much can be done to enhance the appeal of those characteristics and enhance the *Brettanomyces* type aromas.

- Vienna and Munich make a nice contribution on top of the base malt.
- Cara Vienna, Cara Munich, Cara Aroma, Carmel, Honey, Special B – all these malts can add a nice character in a Brett beer.
  - Careful about adding malts which increase the bready flavor/aroma as *Brettanomyces* will create these on its own.
- Roasted malts – I find a dark Brett beer to be quite complex, lots of aromas and flavors develop.
  - Consider debittered of dehusked malts like Carafa special I,II, or III.
  - Too much roasted malt character brings out astringency and doesn't work well with some *Brettanomyces* strains which can have a metallic astringent character.

- Hops
  - *Brettanomyces* can do some very interesting things with aromatic hop glycoside compounds.
  - Brett aromas can be complimented by aromatic hops with fruity, spicy, piney, and citrus type aromas.
Brewing Techniques

- Single infusion mash - not sure there is anything to be gained by a step mash.
  - Ferulic acid - pre-cursor for 4-vinylguaiacol and 4-ethylguaic acid
  - Want spicy, smokey, phenolic characteristics?
    - Ferulic acid rest at 43-44°C (109-111 °F) enzymes release ferulic acid and other hydroxycinnamic acids into the mash

- Mash Temp - 65-67°C (150-153°F) adequate conversion with highly modified malts, large starch granule gelatinization.

- Mash rest - 30 minutes, enough time for conversion.
  - Re-circ (vorlauf) till solids are cleared

- Mash thickness - I like 2.5:1 liquor to grist ratio (Liters:Kilograms)
  - Too high promotes dryness.

- Sparging - Too hot of a sparge temp extracts husk and tannin flavors as well as over sparging.
  - Creates astringency and the possibility for phenolic pre-cursors to be leached into the wort
  - 168°F (75°C) will do the job
  - Final running depends on the beer but don't go below 2-3°P
Questions?

www.crookedstave.com
www.brettanomycesproject.com