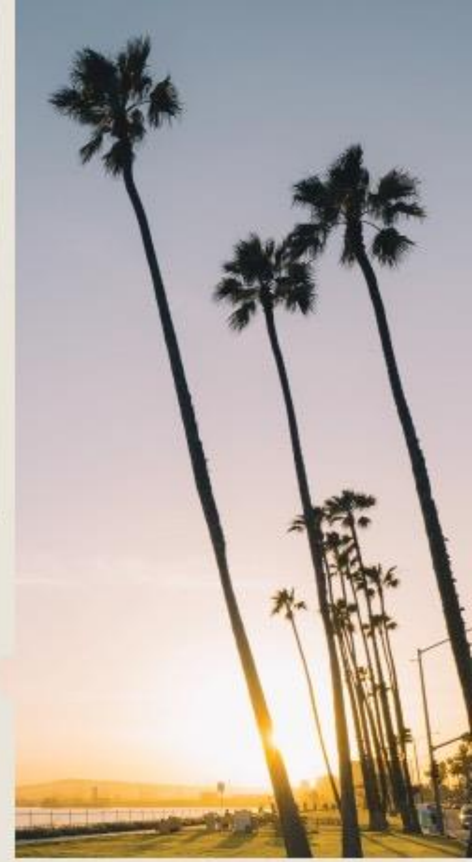

CA

CRAFT BEER SUMMIT 2019

CALIFORNIA CCBA CRAFT BREWERS ASSOCIATION

Total Fermentation of Beer

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2019

Welcome to the Golden Age

- The 20th Century saw more changes in brewing science that ever before in human history.
- pH!
- Stainless Steel
- Cellular microbiology
  - Yeast strains
  - Bacteria

200 years Ago…

- Breweries had their own malting house as part of the brewery.
- Barley had less diastatic power and took longer to malt – 7 days instead of 4.
- Protein rests and decoction mashing were invented to finish the malting process.
- Boiling released more starch = higher yield.
- Modification as Soluble to Total protein index = ~35%

Old Fermentation

- Prior to the 20th century, most fermentation was done in open vessels and transferred to wooden casks for maturation.
- Beer Spoilage was a problem.
  - Either make it strong and hoppy (English),
  - Or keep it cold (German),
  - Or sour it intentionally (Belgium).

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Today

- Malts are highly modified: S/T = 40-45%
- Malts have more diastatic power: 2X?
- A protein rest is not needed for further modification.
- Decoction will still produce tasty Maillard Reaction products without using a protein rest.
- Conversion will happen in 15-30 minutes.
- The yield is a function of Crush and Lautering.

New Fermentation

- Stainless steel tanks debut about 1950.
  - Enclosed and sanitary
  - Temperature controlled
  - No transfer necessary to prevent staling
- Closed, single vessel fermentation becomes standard. With a couple new problems…
  - Aeration
  - Pitching rate
  - Static pressure on yeast

Yeast Technology

- Ale vs. Lager vs. Brettanomyces vs. bacteria
- The yeast genome is being mapped.
- We can produce yeast with specific traits.
- But most importantly, we understand how the yeast cell lives.

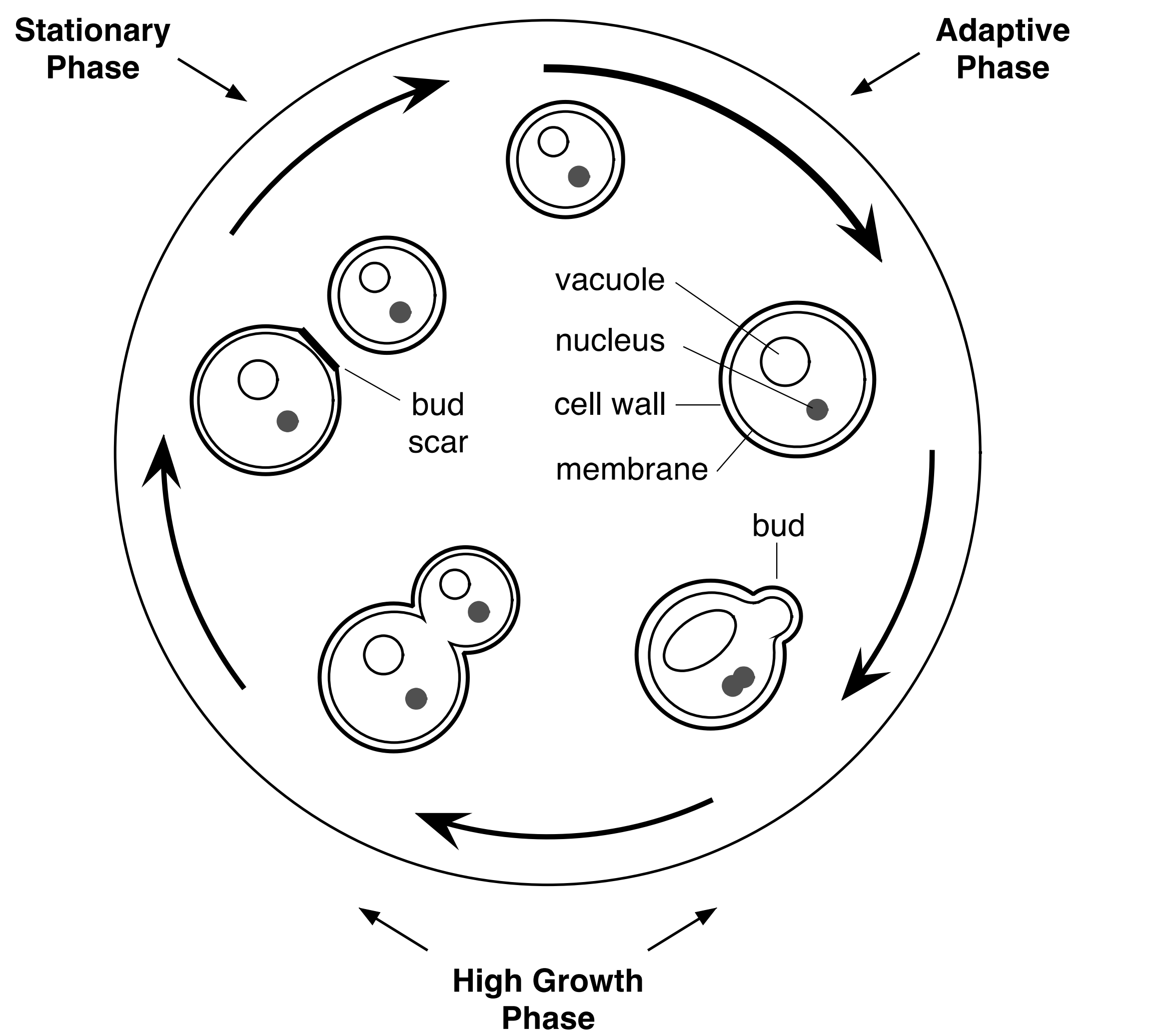
Yeast Life Cycle

3 Phases for Yeast:

a. <u>Adaptive</u>
   - physical growth

b. <u>High Growth</u>
   - reproduction
   - typically 2-4 daughters

c. <u>Stationary</u>
   - build glycogen reserves
   - flocculation
   - hibernation



Yeast Management

- The brewer makes the wort, the Yeast make the beer.
- You must have a sufficient amount of clean, healthy yeast.
  - Freshness (vitality)
  - Pitching rate (viability).
- Manage the yeast and you manage the beer.

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Vitality vs. Viability

- Vitality is the health of the yeast.
- Viability is the percentage of them that are healthy.
- To brew good beer, you must have healthy yeast, and enough of them to get the job done.

Two Options for Brewers

- You have two options when you are brewing
  - You can brew the same beer
  - You can brew a different beer
- To brew the same beer, you must have the same fermentation.
- To have the same fermentation, you must have the same rate and total amount of yeast growth.
- Change any condition, and you have brewed a different beer.

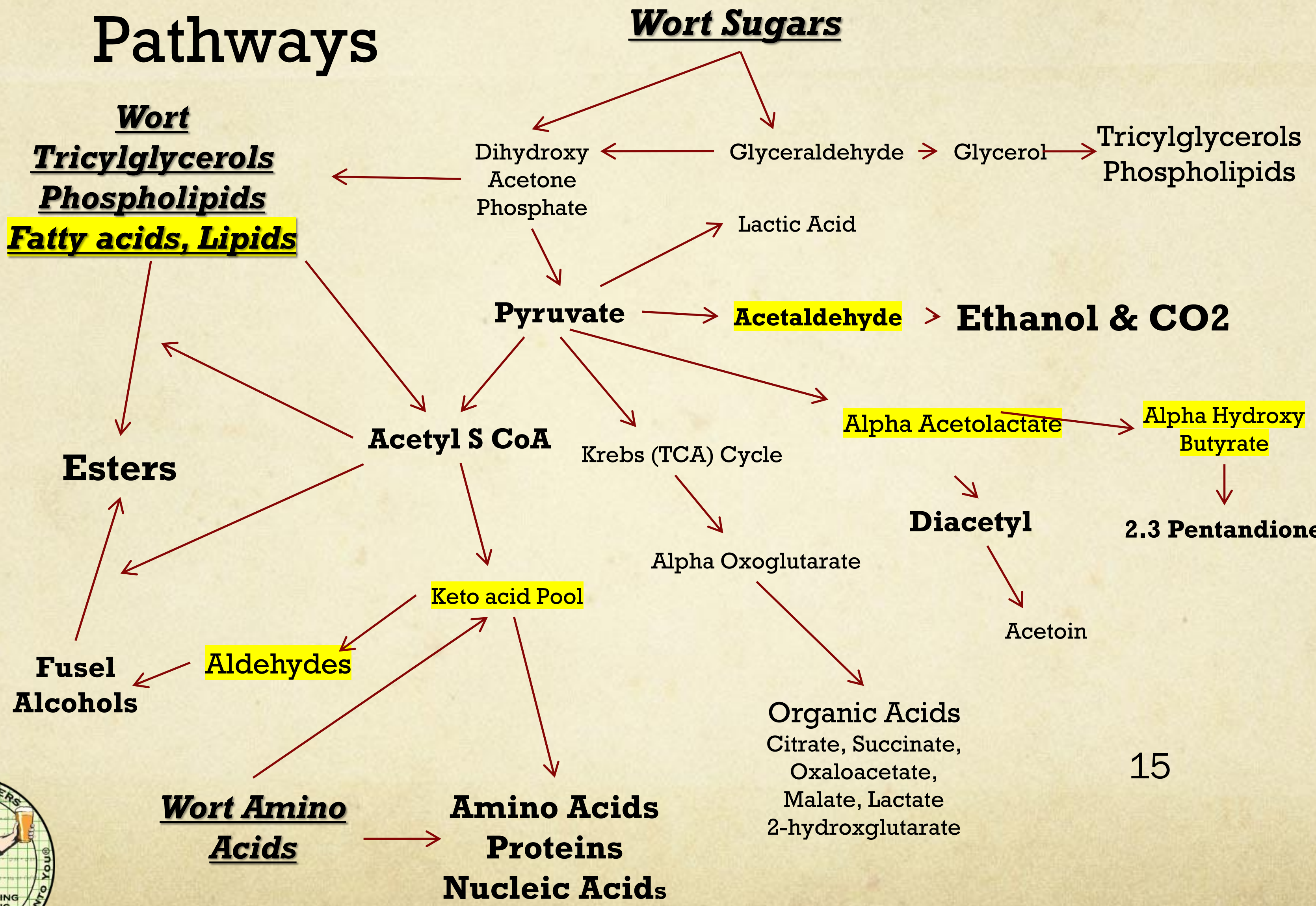
Brewery Fermentation Phases

- Adaptation (Lag) Phase
- Attenuation (High Growth) Phase
- Maturation Phase
- Clarification (Stationary) Phase

Yeast Don't Care about Beer

- Yeast do not have a maturation phase.
- It is your job as Brewer to create the conditions for it to occur.
- The Yeast produce waste products as they grow and reproduce.
  - Fatty acids, esters, acetohydroxy acids, alcohols, CO_2, aldehydes, lipids, etc.
- Rapid Growth produces lots of waste.

Highly Simplified Yeast Metabolic Pathways



Sythesizing Lipids and Sterols

- Yeast need a lot of wort nutrients to grow.
  - Tricylglycerols
  - Phospholipids
  - Fatty acids
  - Amino acids
- When they need them, they need them right now, and they have small mouths (figuratively).
- If they can't get it from the wort, they'll synthesize it from something else, which creates byproducts.

Lipid and Sterol Synthesis

- Lipids and sterols are essential building blocks for the yeast cell membrane. These building blocks are shared with daughter cells.
- Short chain fatty acids are produced during the synthesis of long chain fatty acids, which are incorporated into the synthesis of lipids and sterols that the cell needs.
- Short chain fatty acids are toxic waste to the yeast.

Ester Formation

- Waste products are excreted out of the cell.
- Esters are metabolic byproducts produced during growth as a detoxification step.
  - Esters are less toxic to the yeast cell than the short chain fatty acids and alcohols.
- Ester produced by action of 2 enzymes
  - Acetyl CoA enzyme
  - Alcohol Acetyl Transferase
- Different combinations of fatty acids and alcohols produce different esters, per yeast strain.

What Promotes Esters?

- Esters are produced under stressful growth conditions, i.e., lots of cell waste.
- Optimal growth = low esters
- Stress can be either famine or feast:
  - Famine: lack of essential nutrients, or low temperature, low oxygen = more synthesis = more esters
  - Feast: Overabundance of specific nutrients, or high oxygen, or high temperature. High growth leads to shortages which leads to synthesis and esters.

Building Maturation

- The other two primary byproducts that can be cleaned up by the yeast are:
  - Diacetyl
    - From acetolactate, an acetohydroxy acid.
    - Diacetyl and 2,3 pentanedione are Vicinal Diketones (VDKs)
  - Acetaldehyde
    - Intermediate step in the production of ethanol.
    - Excess is excreted from the cell but can be taken up later and processed to ethanol for energy.
    - Zinc is important cofactor to acetaldehyde reduction.
- Fermentation is done when Maturation is done.

What is Diacetyl?

- Diacetyl is a buttery, or stale milk flavor and aroma. (like microwave popcorn)
  - Pentanedione is sweeter, like honey-butter.
- Diacetyl and 2,3 Pentanedione are Vicinal Diketones (VDK) that should be reduced by the yeast during maturation.
- These VDKs are NOT produced by the yeast.

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How Much Can You Taste?

- Taste threshold depends on style.
  - Generally accepted to be 10-40 ppb.
  - Beers reported to range from 8-600 ppb.
- Acceptance Requirements vary:
  - 50 ppb at 10 IBU for American Light Lager
  - 80 ppb at 30 IBU for German Pils
  - 150 ppb at 30 IBU for Boston Lager
    - Disclaimer: All numbers are anecdotal and intended for illustration.

Diacetyl Formation

- Diacetyl and 2,3 Pentanedione are formed by chemical oxidation (decarboxylation) of acetohydroxy acids.
  - Acetolactate – (CO_2 + 2H) = Diacetyl
  - Acetohydroxybutyrate – (CO_2 + 2H) = 2,3 Pentanedione
- The acetohydroxy acids are created by the yeast as an intermediate compound in the synthesis of the amino acids, Valine and Isoleucine.

Yeast and Valine/Isoleucine

- Valine and Isoleucine are Group B Amino Acids
  - They are absorbed slowly during the first 12 hours of fermentation, and moderately thereafter, after the majority of the Group A amino acids have been consumed.
  - Think of boarding an airline…
- If they can't get through the door, they will be synthesized by the yeast cell.

Uptake of Amino Acids

- Amino Acids are organized into Group A, B, C, and D.
- All amino acids use the same transport channels into the cell.
- Group A are taken up first, then B, then C.
- Group B use the same transport channels as Group A, so some make it in during first 12 hours, but most don't.

Diacetyl and Yeast Growth

- The total amount of excess acetohydroxy acids in the wort depends on the amount of yeast growth that occurred, and how it grew.
- Therefore, the production of the acetohydroxy acids depends on:
  - Yeast growth rate (the need for FAN)
  - The availability of FAN

How much FAN?

- 150 ppm total FAN is generally considered to be the sweet spot. (Your mileage may vary.)
  - 100 ppm is generally considered to be a minimum for good fermentation.
  - FAN levels below 50 ppm are problematic.
  - FAN levels in excess of 200 ppm tends to produce off-flavors from excessive yeast growth.

Adjusting Total FAN

- Moderate adjunct worts have moderate levels of total FAN, meaning that Group A are eaten quickly allowing Group B (valine and isoleucine) to be presented to the cell and reduce the need for valine synthesis.
- High Adjunct worts have low levels, meaning that overall valine levels are low and more synthesis is needed.
- Low Adjunct worts (ie., all-malt) mean that there is a lot of Group A, which suppresses Group B take up, which means more synthesis.

Conversion of Aceto to VDK

- Yeast cannot maturate acetohydroxy acids.
- The conversion of acetohydroxy acids to diacetyl and 2,3 pentanedione is a strictly chemical reaction.
  - Higher temperature = faster conversion.
  - Lower pH (3 vs. 4 vs. 5) = faster conversion.
- For example: the conversion of acetolactate to diacetyl takes:
  - pH 4.2: 42 hours longer at 8°C than 10°C.
  - pH 4.6: 66 hours longer at 8°C than 10°C.
  - Therefore, Raise the temperature for maturation!

Step 1: Control Growth Rate

- The first step to good maturation is to control the growth rate of the yeast to control the amount of waste generated.
  - Sufficient Nutrients (aeration, lipids, FAN, zinc)
  - Moderate Temperature (start cool)
- Think of Goldilocks and the Three Bears…

Step 2: Control Amount of Growth

- The second step is to have enough active yeast to clean up the waste.
  - Pitching rate vs. original gravity (OG)
- Typically, yeast mass will increase 4X during fermentation, then their lipid reserves are exhausted, and they enter Stationary Phase.
  - Pitch enough yeast, and control growth, so that there are active, hungry yeast when the sugars run out.

Pitching Rate

- Typical guideline: 1 Billion cells per liter per °Plato.
  - Ales: 0.75 billion/liter/°P
  - Lagers: 1.5 billion/liter/°P
- A little more doesn't hurt, i.e., 1 billion for ales, or 1.75 billion for lagers, but don't double it.
  - It is hard to overpitch, but it can happen.
- High Gravity beers (>1.075 OG), benefit from higher pitching rates.

Third Step: Control Activity

- Raise the fermentation temperature by at least 3°C/5°F towards the end of fermentation to keep the yeast active.
- Allow the fermentation to rest at that temperature for as many days as the high growth phase lasted. Typically 2-4 days.
- The sugars should be gone, but the yeast are still active and will consume the VDK's and acetaldehyde.

Diacetyl Cleanup

- Yeast can reduce diacetyl 10X faster than chemical conversion can make it.
- After conversion to diacetyl or pentanedione, the yeast can take in these compounds and reduce them for energy.
  - The VDKs are reduced to high threshold (tasteless) compounds, such as acetoin.
- Most diacetyl reduction occurs during primary growth phase, with final 30% occurring during maturation.

Bottling Acetohydroxy Acids

- Do not chill and package too soon!
- If the beer is cold, the acetohydroxy will not convert to diacetyl and the yeast won't touch it.
- Do a VDK Test:
  - Take a sample of the beer, heat it to 65-70°C in the microwave, and smell it.
  - If you smell butter, there is still acetohydroxy in the beer.
  - Keep doing the Diacetyl Rest.

Managing Diacetyl

- Manage Diacetyl by managing fermentation.
- Control the yeast growth.
  - Pitching Rate, FAN Levels, trub, oxygen,
  - TEMPERATURE (start low, finish higher)
- Control the maturation.
  - RAISE the temperature 5°C to help convert the precursor to diacetyl.
  - Give the yeast TIME (days) to take up the diacetyl.

Acetaldehyde Reduction

- Zinc is a cofactor for the enzyme alcohol dehydrogenase that reduces acetaldehyde to ethanol.
- Zinc also plays a role in yeast membrane interactions, aiding in flocculation.
- Yeast requires between 0.050 and 0.300 ppm available zinc. Turbid wort supplies more zinc than clear wort.
- Zinc should be added to the wort on the cold side, immediately before pitching.
- High levels of zinc may be detrimental to yeast health and fermentation performance.

Sequential Wort Additions

- Ensure good mixing of added wort!
  - Filling big fermenters can take 2 or 3 brews over a 24 hour time period.
  - New, aerated wort generally means new growth, which means more acetohydroxy acids.
  - New wort contains 100-200 ppb of acetohydroxy acids before seeing yeast!

After Maturation

- Dry Hopping
  - Dry hopping can result in more diacetyl.
  - Allow time for maturation prior to cooling.
- Cold Conditioning/Lagering: Clarification
  - **Maturation needs to be finished before clarification!**
  - Maturation is a yeast function, warmer is better.
  - Clarification is a physical (temperature) function, colder is better.

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Dry Hopping Can Cause Diacetyl

- Why? How?
- Hops are plants, containing amylase enzymes.
- Amylase generates more fermentable sugar.
- Oxygen from dry hopping and circulation of the hops causes more fermentation.
- Low FAN means more acetohydroxy acids.
- Typically, most breweries would chill after dry hopping without further maturation.

Dry Hopping & Hop Creep

- Dry hopping can cause further fermentation and attenuation, up to 1°Plato.
- If the beer is has been pasteurized or filtered, before dry-hopping, then the beer will get sweeter, up to 1°Plato.

Diacetyl Summary

- Diacetyl is produced by the CHEMICAL reaction of acetohydroxy acids.
- Acetohydroxy acids are produced during yeast growth from the synthesis of valine and isoleucine (Group B FAN).
- The conversion of acetohydroxy acids to diacetyl is often the rate-limiting factor for reduction.
- Therefore, raise the temperature and give the beer time to clean up.

Maturation Summary

- Maturation is a yeast function.
- The brewer creates the conditions for the yeast to maturate the beer.
  - Pitching Rate, Growth Rate, Total Growth
  - Raise the Temperature to maturate
  - Give the beer Time to maturate
- After maturation, the beer can be chilled for clarification in 1 week.
- No more long lagering.