Mash Preparation for Non-Sugar Based Feedstocks

The Alcohol School Concise

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WIRSPA

West Indies Rum & Spirits Producers' Association Inc.

Ethanol Technology Institute

Place: Caribbean Distilling Seminar, St Lucia

Date: 17th April 2024

Grain Processing:

MILLING

- Mill type, meal composition important

COOKING

- Gelatinization of grain starches
- Prepare starch for enzymatic conversion

CONVERSION

- Two step process:
  1. LIQUEFACTION
  2. SACCHARIFICATION

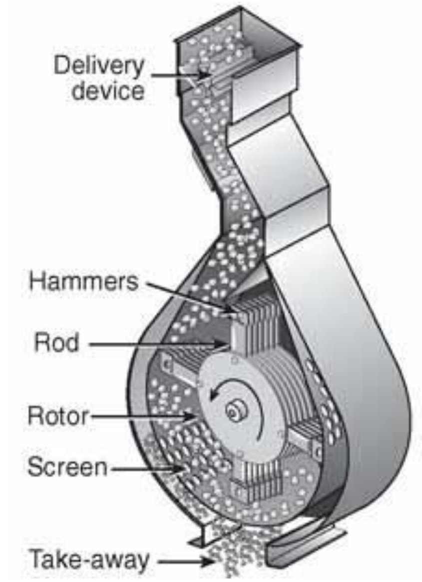
COOLING

- To fermentation temperature

Milling: To Expose Starch for Gelatinization & Conversion

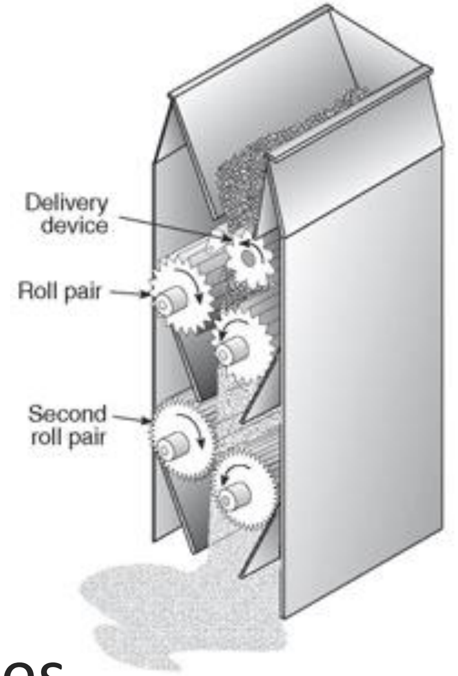
Hammer mill:

- Used in most ‘grains-in’ mash systems



Roller mill:

- Used mostly on malted grains ‘clear’ wort production (“grains-out mashing”)



- A finer grind will offer better yield but may cause processing issues
- Mill maintenance is critical & often overlooked

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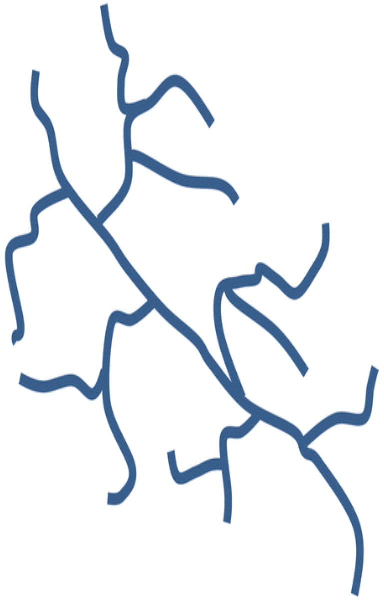
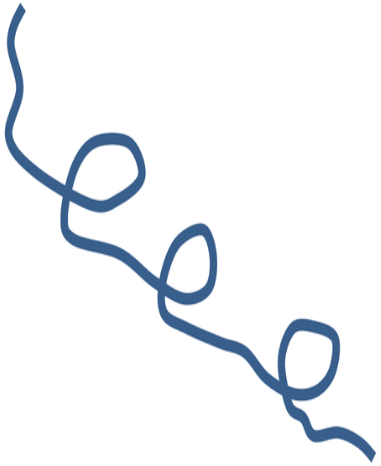
The Grain Substrate: Starch

What is Starch?

- A plant seed sugar storage mechanism, a carbohydrate found in the Endosperm of the grain
- Partially-crystalline granules of varying size
- Starch is comprised of various proportions of two glucose polymers
  - Amylopectin- branched chain (~80%)
  - Amylose – straight chain (~20%)
- Water insoluble at ambient temperatures



Amylopectin



Amylose

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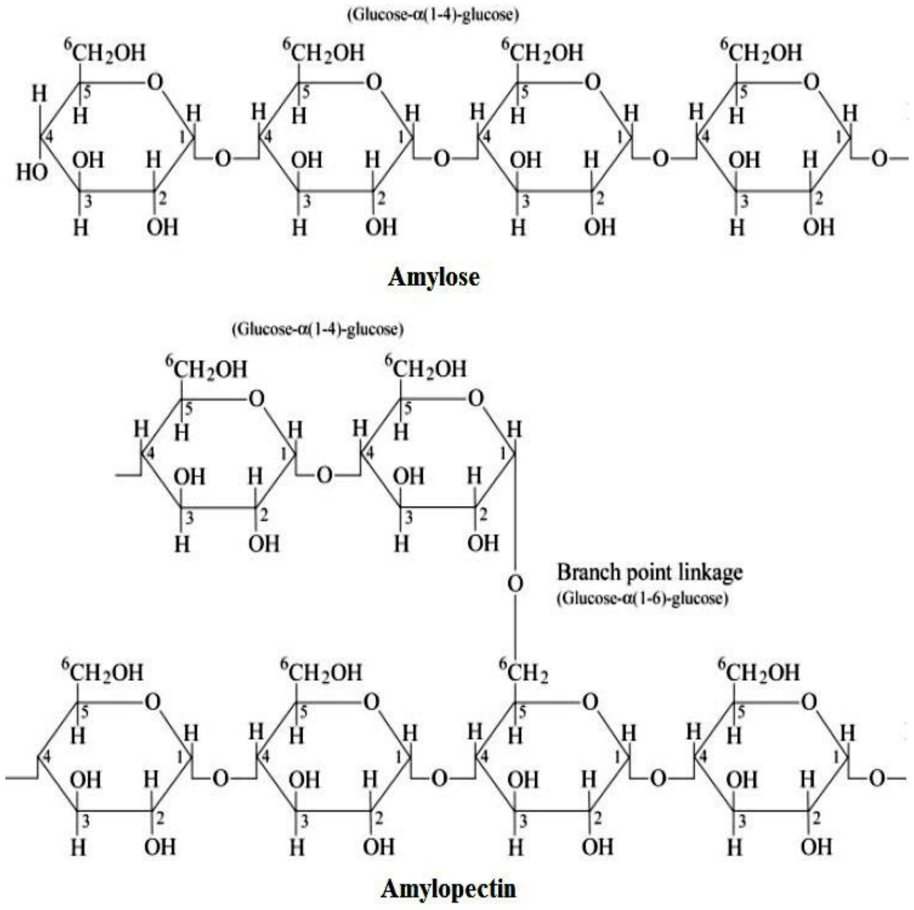
Starch Structure:



AMYLOSE

- α -1,4 linkage only straight chains
- 15-30% of total starch in most plants
- Soluble in water
- Molecular wt: 10,000 - 100,000

(mwt. of glucose = 180)

AMYLOPECTIN

- α -1,4 linkage plus α-1,6 linkage at branches
- 70-85% of total starch
- Insoluble in water
- Molecular wt: > 1,000,000

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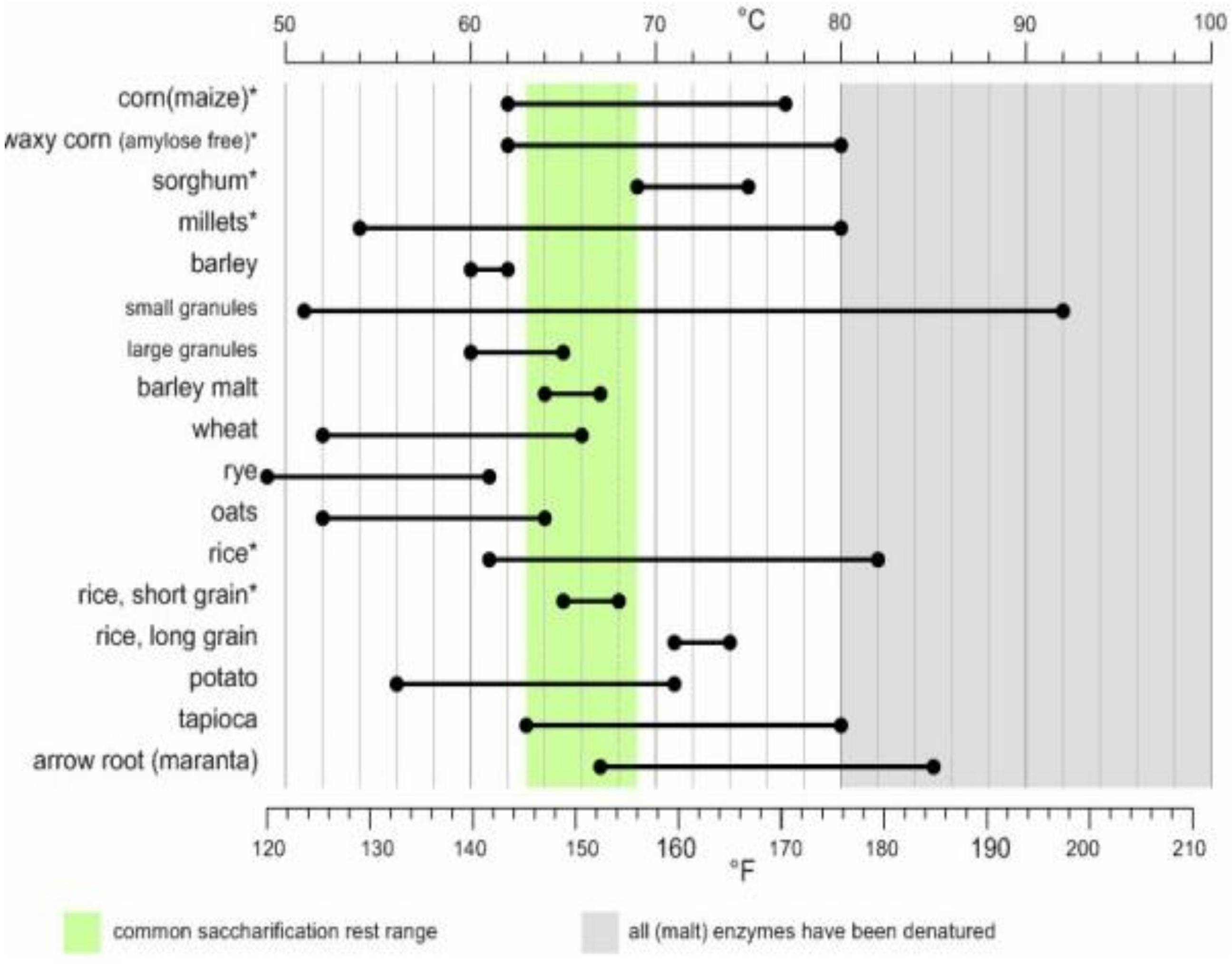
Cooking/Gelatinization

Why cook?

- Disrupt the integrity of the grain to separate starches from proteins, exposing them for gelatinization
- Fully gelatinize (swell) the starch granules as they absorb water, making them available for enzymatic hydrolysis
- Sterilize/sanitize the mash, reducing bacteria levels to minimize spoilage in fermentation

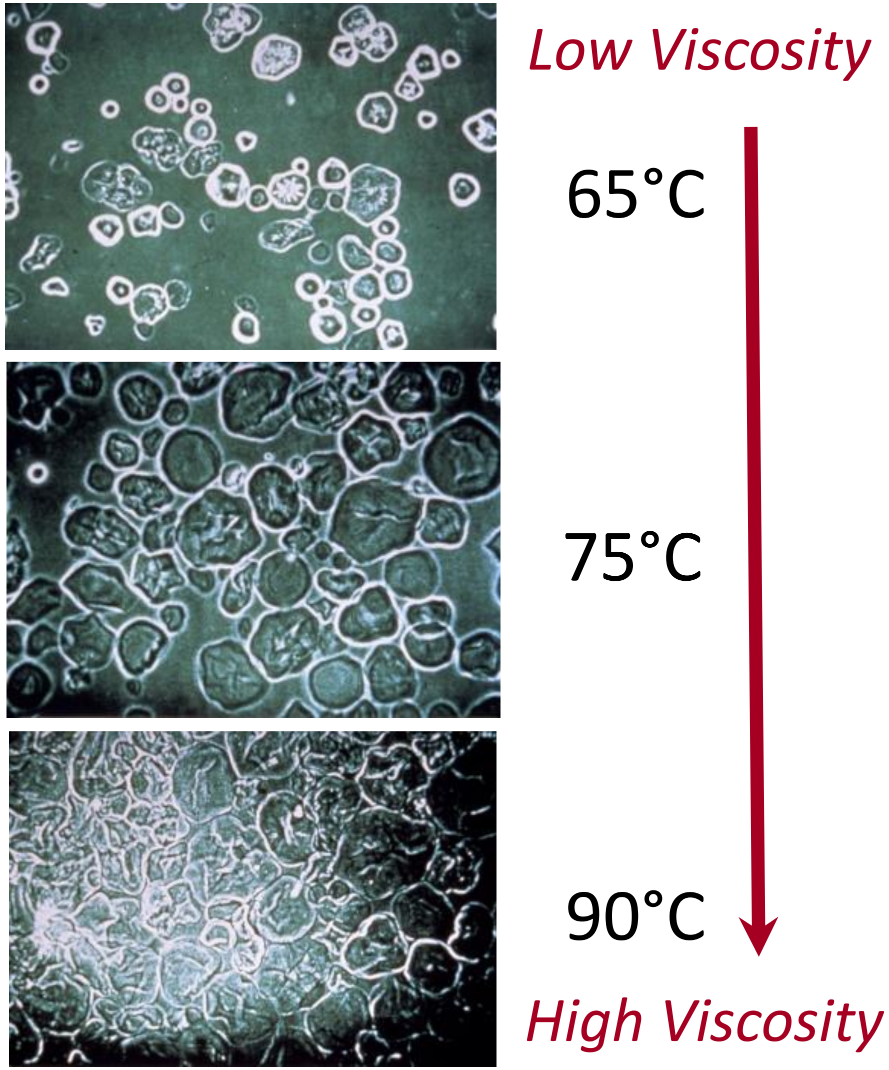
Gelatinization

- Process by which a granule of starch swells and solubilizes in the presence of water and heat.
- During gelatinization water gets inside the granule, making the starch structure more suitable for enzymatic hydrolysis.
- Gelatinization temperatures vary for the different grains, varieties, harvests & solids ratio



The peak of gelatinization is also the point of max viscosity of the mash

Starch Gelatinization



J. Murtagh, PhD thesis

- The granules take up water and swell, increasing the surface area and viscosity losing their crystallinity

- Gelatinization occurs over a range of temperature (50-80°C)

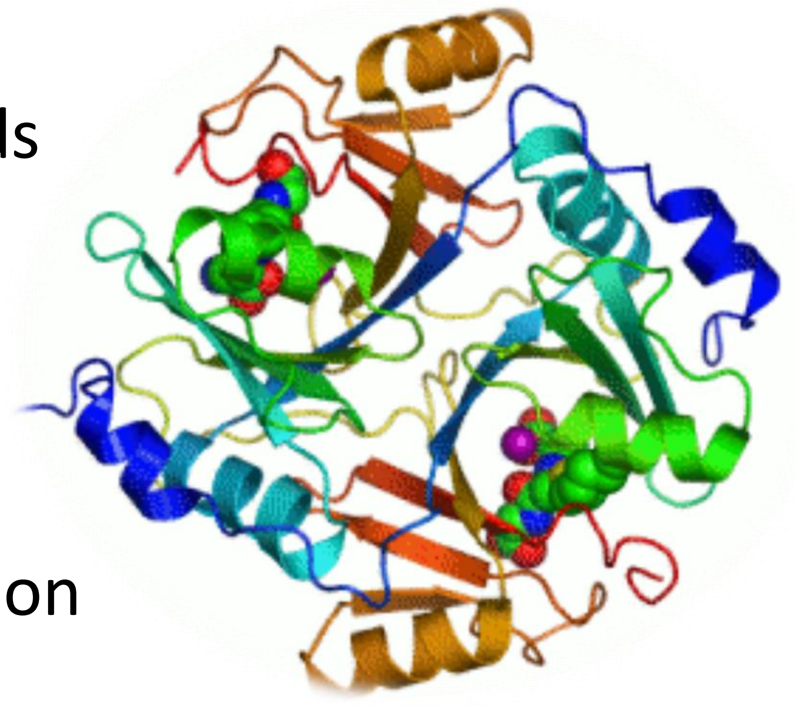
- As gelatinization progresses, solubilization occurs.

- BUT it is still starch – we need Enzymes!

What are Enzymes?

Enzymes are:

- Proteins made of ribbon wrapped/folded amino acids
- Biocatalysts that speed up reactions
- Used to reduce the energy required for a reaction
- Are not themselves consumed/changed in the reaction
- Very fragile at high temperatures
- Each have an optimum performance pH range, Temp range



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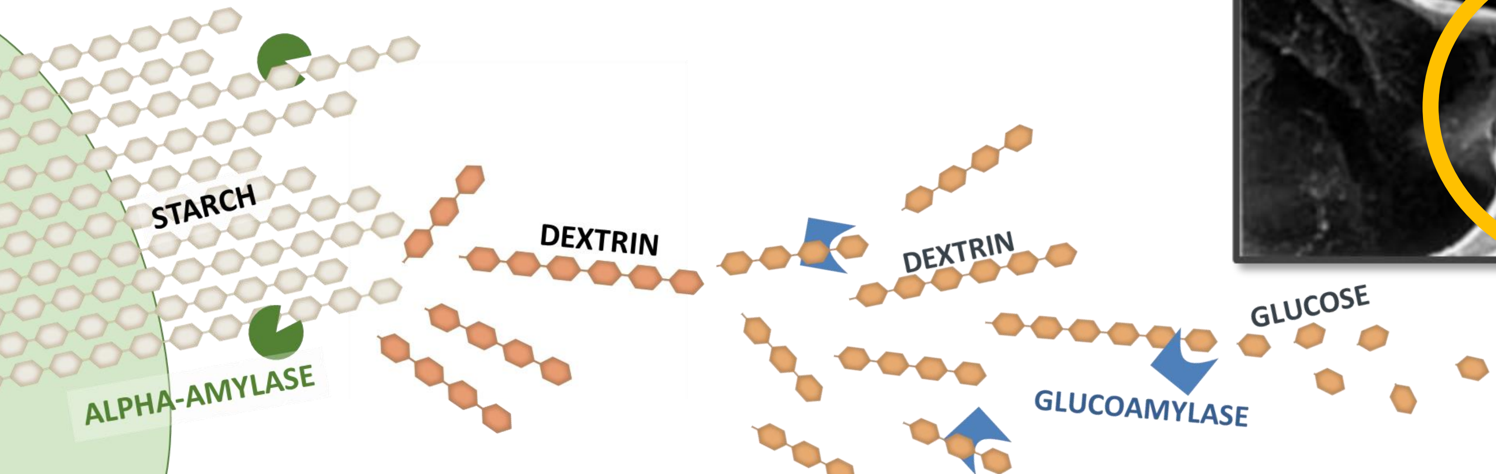
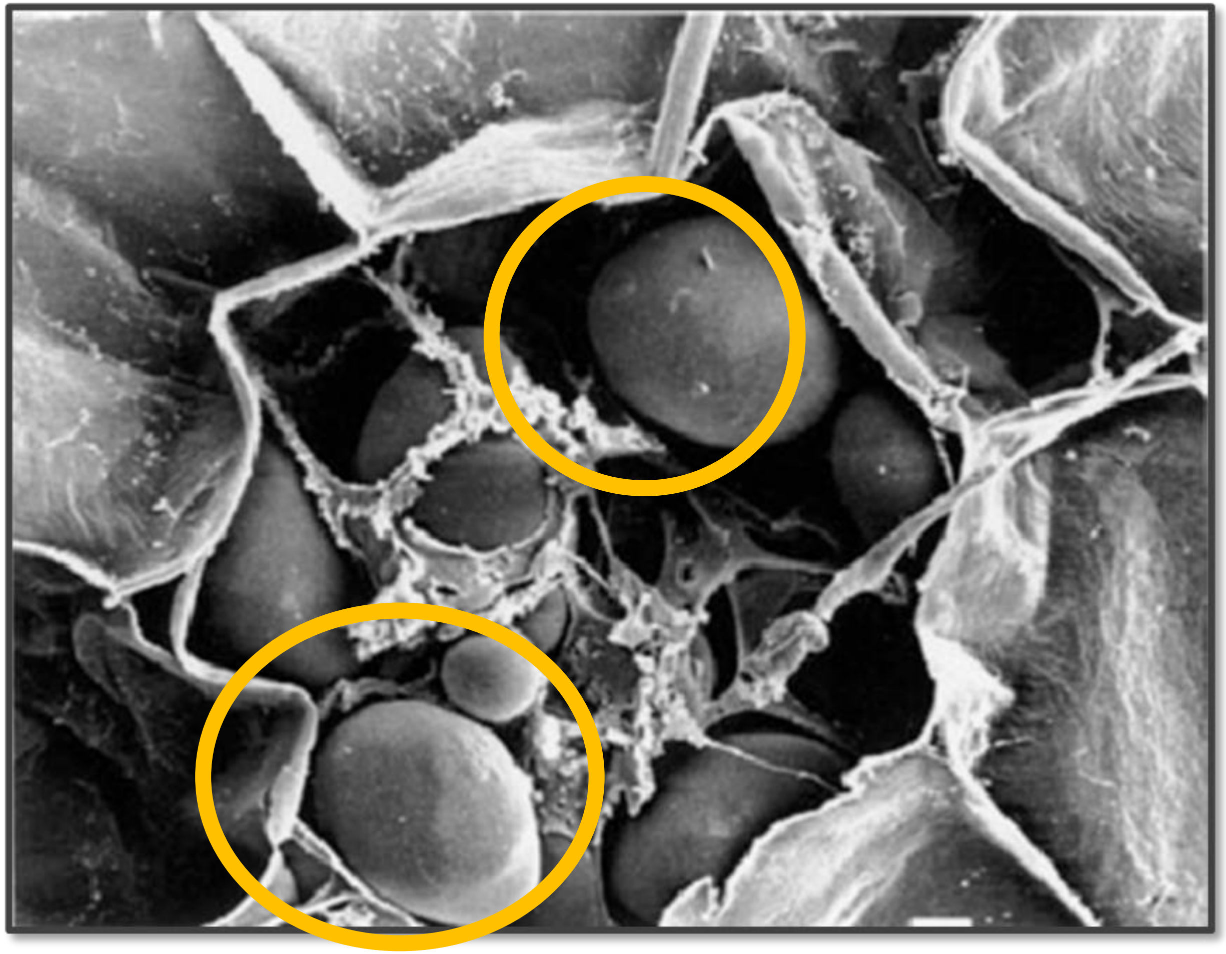
Enzymes for Starch Breakdown

- During liquefaction, α-AMYLASE breaks gelatinized starch into dextrins, short chains of glucose
- During saccharification / fermentation, GLUCOAMYLASE converts the dextrins into glucose





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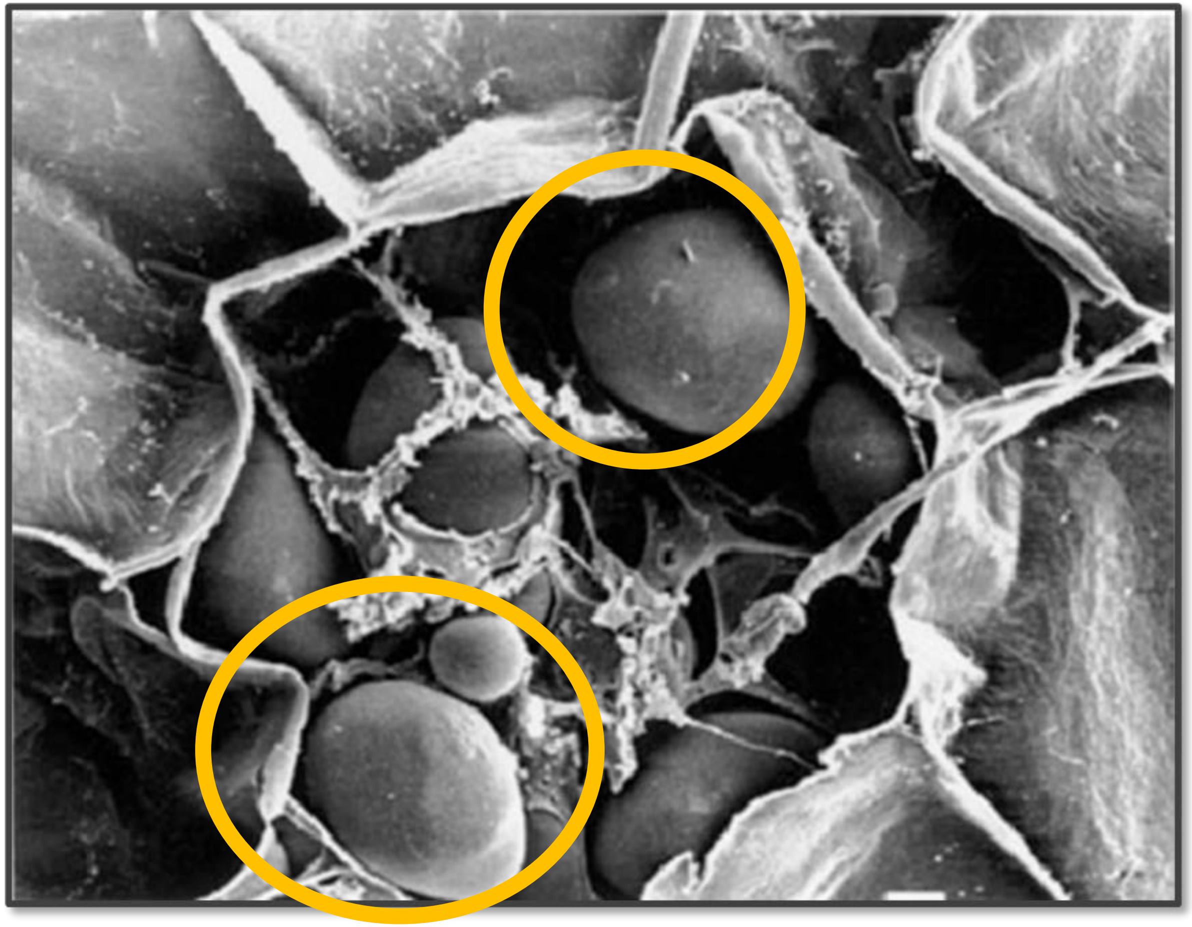
Enzymes that assist Starch Breakdown

- The Starch, if not in a well malted grain, may be intimately bound amongst Cellulose, Glucan, Xylan, Protein which can be accessed by Malt enzymes or exogenous enzymes such as B-Glucanases or Proteases etc

Component	% content of dry matter			
	Corn	Wheat	Barley	Rye
Protein	9-12	12–14	10-11	10-15
Fat	4.5	3	2.5-3	2-3
Starch	65-72	67-70	52-64	55-65
Ash	1.5	2	2.3	2
Total cell wall material (inc. lignin & cellulose)	9.6	11.4	14.0	14.6
Water extractable non-starchy polysaccharides:				
Arabinoxylans	0.03	0.6	0.3	1.4
Beta-glucans	0.05	0.14	2.4	0.8



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Enzyme Sources for Grain Conversion

- Naturally occurring (endogenous) enzymes in malted cereals

- Commercial (exogenous) enzyme concentrates of bacterial or fungal origin

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Malted Barley - Enzymes

Operation	Enzymes	Enzyme action	Function
Decoction vessel (cereal cooker)	α-amylase	Hydrolyse starch	Adjunct* liquefaction. Reduce viscosity
	β-glucanase	Hydrolyse glucans.	Aid the filtration.
Mashing	α-amylase	Hydrolyse starch.	Malt improvement.
	Amyloglucosidase	Increase glucose content.	Increase % fermentable sugar in "light" beer.
	Debranching enzyme	Hydrolyse α-1,6 branch points of starch.	Secures maximum fermentability of the wort.
	Proteases	Increase soluble protein, and free amino- nitrogen (FAN).	Malt improvement Improved yeast growth.
	β-glucanase	Hydrolyse glucans.	Improve wort separation.
	Pentosanase/xylanase	Hydrolyse pentosans of malt, barley, wheat.	Improve extraction and beer filtration.
	Fungal α-amylase	Increase maltose and glucose content.	Increase % fermentable sugar in "light" beer.
Fermentation	β-glucanase	Hydrolyze glucans.	Reduce viscosity and aid filtration.
	α-acetolactate- decarboxylase (ALDC)	Converts α-acetolactate to ace-toin directly.	Decrease fermentation time by avoiding formation of diacetyl.

Typically, lower optimal temperature ranges than commercial enzymes

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Benefits & Disadvantages of Malt Sourced Enzymes

- Single source of a variety of enzymes
  - Mandatory requirement for malt for some spirits types

    (E.G. Scotch Whisky)
- Contributor of a variety of flavor attributes, determined by barley variety, kilning profile
- Provide useable nitrogen for fermenting yeast with proteases releasing dipeptides from proteins in the grains

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- **Malt enzymes are heat sensitive and are inactivated at typically high cooking temperatures**
- **Malts are a source of microbial contaminants!**

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Commercial Enzymes

Bacterial or Fungal exogenous enzymes

Potential advantages over malt:

- Higher temperature tolerance, may have preferred pH optima
- Concentrated, relatively stable
- Easy to add to mash
- "Cleaner" (more sanitary) than malt
- Allows for step-wise conversion of starches to fermentable sugars: better process control

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Starch Conversion to Fermentable Sugars

Two-step process, involving two classes of enzymes:

Step 1: Liquefaction

- Alpha-amylase is used to thin (liquefy) the gelatinized starch by randomly hydrolyzing α-1,4 bonds into shorter chains called "dextrins"
- Dextrins show less viscosity and lower osmotic pressure than gelatinized starch

- Alpha-amylase is available as a commercial preparation (exogenous enzyme) and present in malted grains i.e. sacrificial malt addition to cook followed by malt inclusion on the cool down phase.

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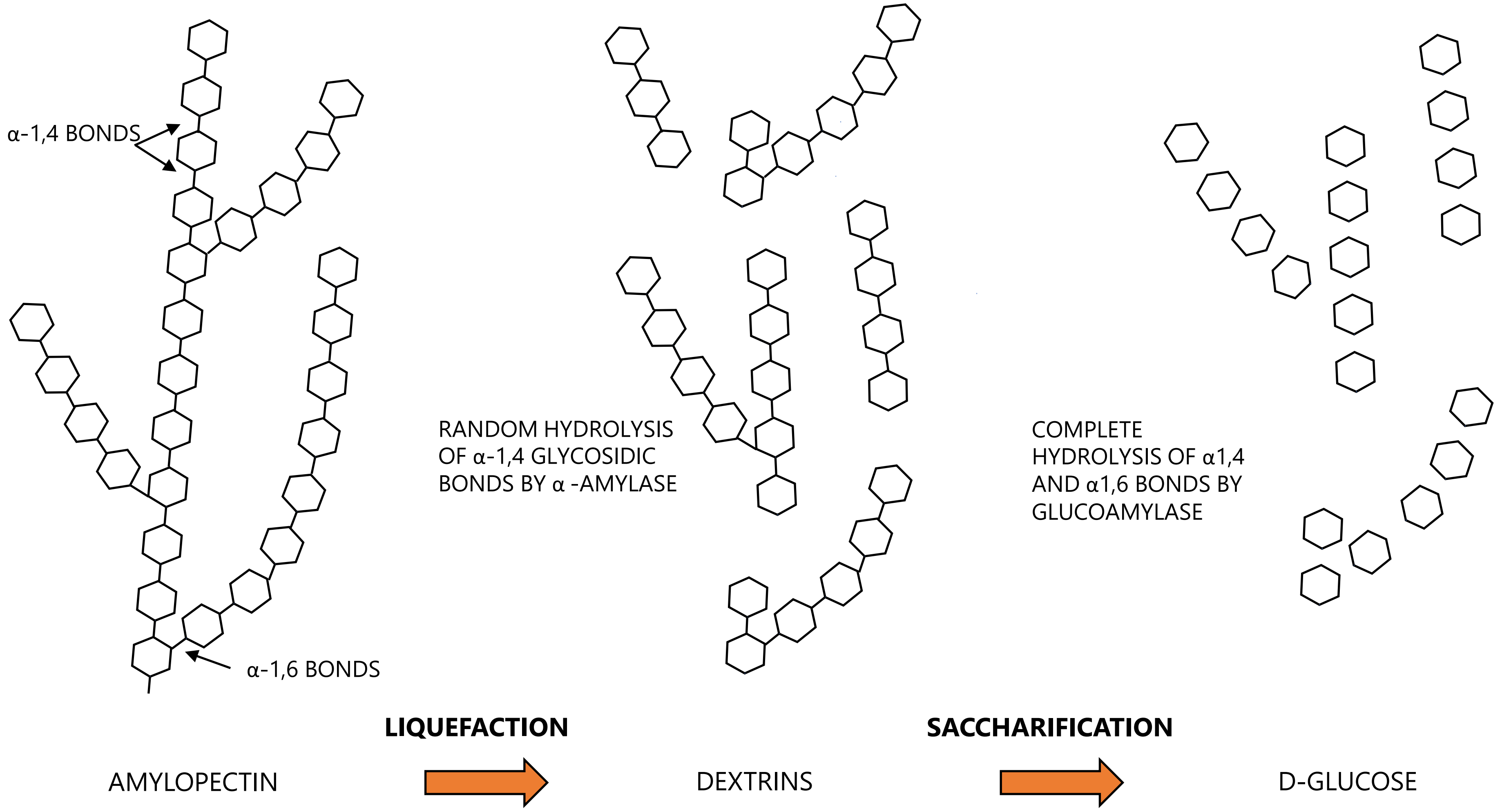
Starch Conversion

Step 2: Saccharification

- Glucoamylase (amyloglucosidase) hydrolyzes dextrins into sugars
  - Hydrolyzes α-1,4 bonds in a stepwise manner from the non-reducing end of each dextrin chain
  - Also breaks alpha α-1,6 branch points to allow complete conversion to glucose
  - Optimal temperature 55-56^{o}C

- Malt enzymes:
  β-amylase and limit dextrinase convert dextrins into maltose

Starch Conversion – Graphic



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Saccharification: Timing & Extent

Options:

1. Ensure natural malt enzyme survival in mash & fermentation
2. Add Glucoamylase to Saccharification Tank or allow "saccharification rest" at optimal temperature to provide partial or complete conversion to glucose prior to fermentation
3. Add Glucoamylase to fermentor - **Simultaneous Saccharification and Fermentation (SSF)**:
   Addition at a lower temperature to release glucose more slowly to match rate of uptake by yeast, limit glucose available for contaminants, provide lower osmotic pressure
4. **Delayed Simultaneous Saccharification and Fermentation (DSSF):**
   Add Glucoamylase later in fermentation

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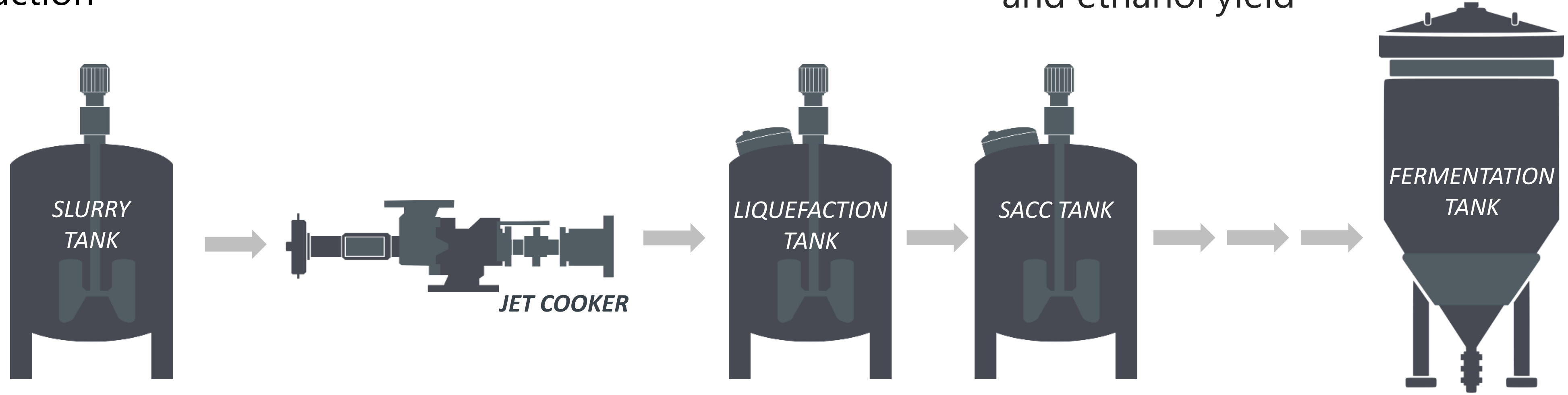
Enzymes for Starch Breakdown

Alpha-Amylase (DistilaZyme AA) Performance in Liquefaction

- Key performance metrics are timely and sufficient **viscosity reduction** and **dextrinization** of starch
- AA performance contributes to the level of **residual starch**
- Optimal **temperature** range and AA **dosage** adjustments should be maintained to maximize performance in liquefaction

Glucoamylase (DistilaZyme GA) Performance in Fermentation

- Key performance metrics are timely and sufficient **saccharification** of dextrins to glucose, seen in DP4+ reduction
- GA activity and dosage should be **optimized to yeast kinetics -** if GA breaks down dextrins too fast, glucose may spike and affect yeast performance and ethanol yield



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Enzymes for Starch Breakdown

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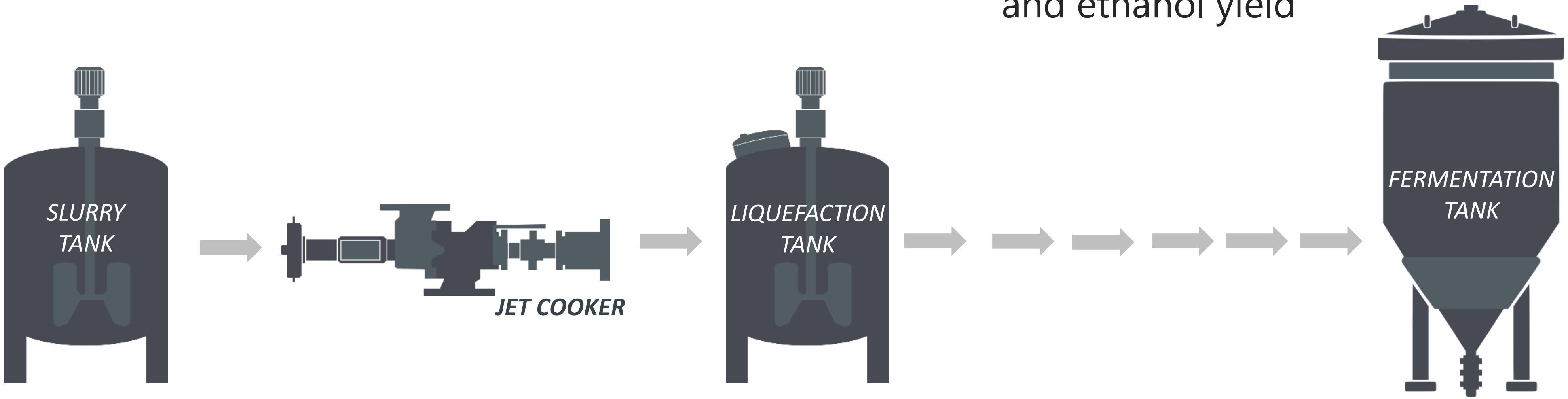

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Mash Preparation

Suitable mash requires both fermentable sugars and appropriate nutrients to support the nutritional requirements of the yeast!

➢ALL GRAIN MASHES ARE DEFICIENT IN NITROGEN (Except 100% Malt)

- Protease activity (added malt (added protease)) will provide some available nitrogen from the grains, but additional nitrogen may be added to ensure complete fermentation
  - Diammonium phosphate, Aqueous ammonia
  - *Target a Free Amino Nitrogen (FAN) content of ~250 mg/l*
  - *Add Commercial Protease Enzyme to release FAN through ferm*
- Mineral & vitamin additions can help ensure complete fermentation, improve efficiency

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Mashing Operations: Nomenclature

Infusion mashing:

- Low temperature cook maintains enzyme activity throughout mashing
- Grain residues may be removed, producing a "clear wort" (Grains-out mash)

Whole grain cooking (Grains-in mash):

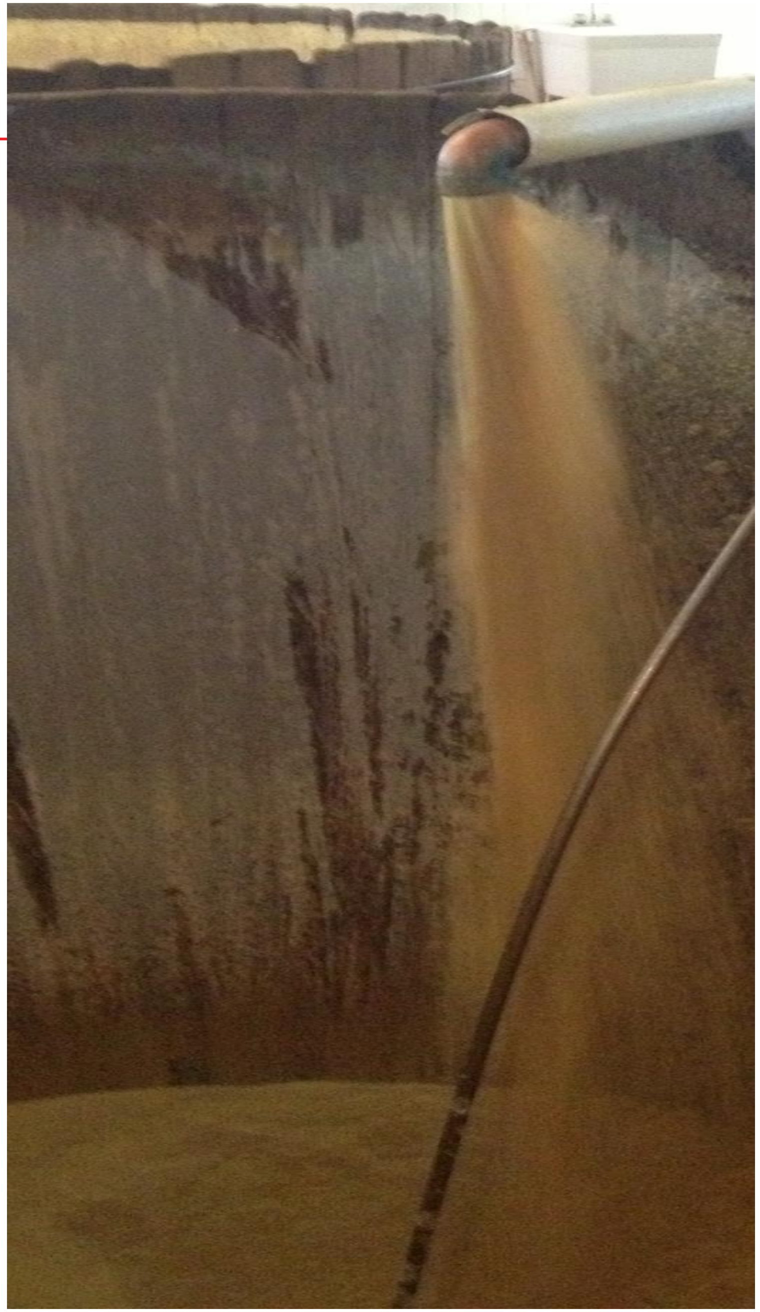
- High temperature cooking (high pressure or atmospheric pressure). Some Low temp. cooking.

Continuous cook

- Usually Grains-in, often high temperature

Batch cook

- May be high or low temp., grains-in or grains-out



Variations in Whisk(e)y Mashing

Whisky	Grains	Mashing Process
Scotch Malt whisky	Peated & un-peated malted barley	Infusion, clear wort (grain out)
Grain whisky	Wheat, maize, malted barley	Batch or continuous whole grain, infusion or high temperature cook
Irish Pot Still whiskey, Malt whiskey	Unpeated barley, malted barley, oats, rye	Infusion, clear wort (grain out), with / without prior mash conversion. ***Enzymes Option.***
Grain whiskey	Wheat, maize, malted barley	Batch or continuous whole grain, infusion or high temperature cook. ***Enzymes Option.***
Canadian Flavouring whisky	Rye, corn, barley, wheat, malted barley	Batch, whole grain, high temperature cook or whole grain infusion. ***Enzymes Option.***
Base whisky	Corn, rye, wheat, malted barley	Batch or continuous, whole grain cook, high temperature. ***Enzymes Option.***
American Straight whiskey ≤ 160° F	Corn, rye, wheat, malted barley	Batch whole grain, high temperature cook or infusion. ***Enzymes Option.***
Bourbon, rye, wheat Base whiskey > 160° F	Corn, rye, wheat, malted barley	Batch or continuous cook, whole grain, high temperature. ***Enzymes Option.***

Mashing Styles

Clear Wort (Grains Out):

- "Infusion cook"; retains activity of enzymes throughout cook
- Insoluble grain solids are separated, leaving "clear" fermentable wort
- Simultaneous low temp. cooking and conversion
- Malt only or malt/unmalted grain mixture
  - 100% malt used for malt whisky
  - Up to 60% un-malted barley used in many Irish whiskies

Whole Grain Mash (Grains In):

- Contains all grain solids: No removal of undigested particles
- Undergoes cook cycle to solubilize starches
- Cooking is higher temp. may be above or below 100°C
- Requires enzyme addition before and after cooking
- Malts and/or commercial enzymes can be used

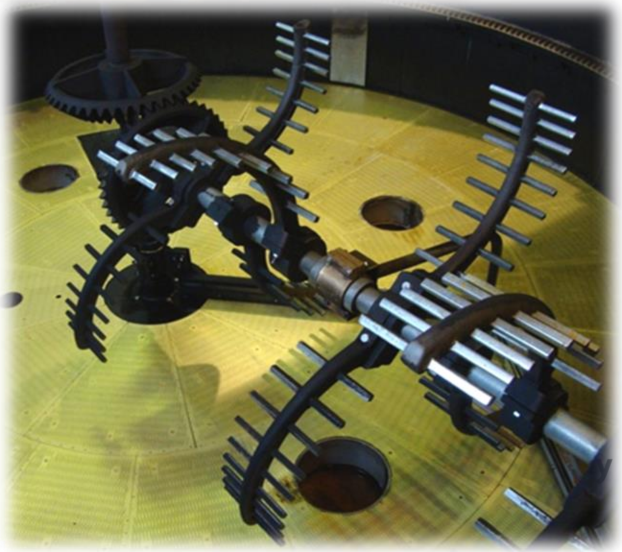
Batch Infusion Mashing: Older Traditional Mash Tun

Many have been replaced by modern Lauter tuns with higher throughput and improved efficiency

- Open, cast iron, uninsulated construction
- Rotating paddles mix the grain when water is added
- Smaller filter area, deeper bed than a Lauter
- No ability to sparge the grain bed

Irish Whiskey Mashing

Cereals are precooked in a mash conversion vessel and then 'lautered' to remove grain in a lauter tun



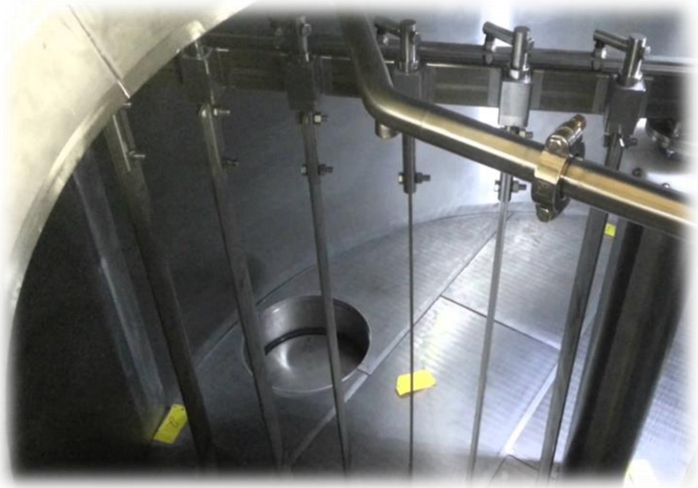
Batch Infusion Mashing: The Mash Lauter Tun

- Malt (or grain mixture) is mixed with the first water (4-4.5 T water/T malt) at 64-68^{o}C and held for up to 1 hour to solubilize starches and allow enzymatic starch conversion. Grain husk settles to form a bed.

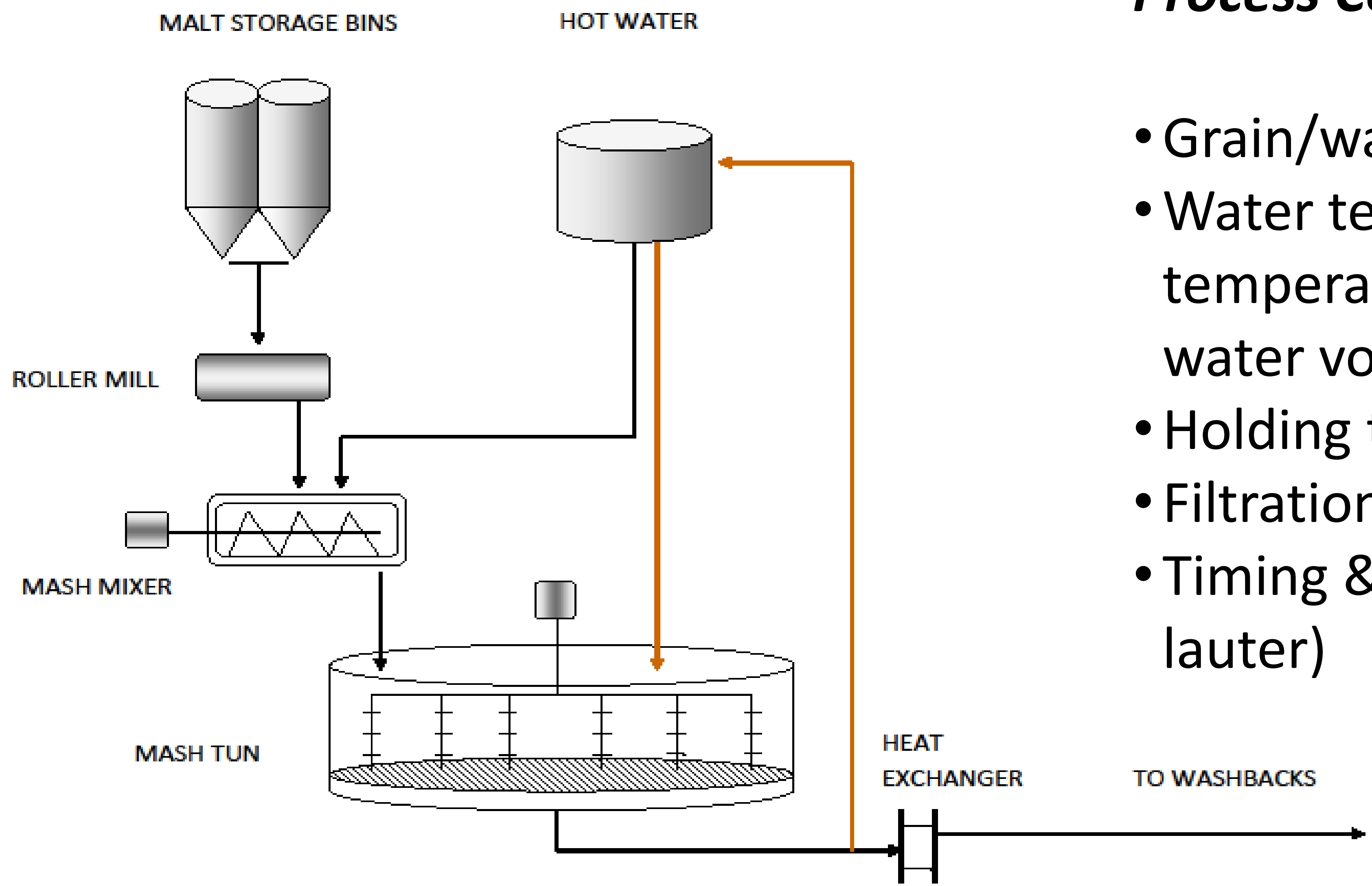
- Wort (extract) is drawn off through perforated bottom of mash tun, passing through grain bed (~ 1 meter depth) which provides filtering of wort. Clear wort is pumped through coolers to the fermentor (washback).

Batch Infusion Mashing: The Mash Lauter Tun

- Second water at 70-75^{o}C (1.2-2 T water/T malt) is sparged onto the grain bed and the extract is drawn off through grain bed to the fermentor
- Third and fourth (less common) waters (80-95^{o}C) are sprayed onto the grain bed to wash residual sugars from husks.
- This dilute extract is returned to the hot water tank for use as part of the first water of the next mash
- Residual grains removed from mash tun and sold as animal feed



Mash Tun Schematic



Process Control Variables:

- Grain/water ratio
- Water temperatures (mash temperature profile) and water volumes
- Holding times
- Filtration rate/wort clarity
- Timing & Rake height (full lauter)

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Mash Filter

- Alternative method for removing grain particles
- Gaining popularity by brewers and used by at least two malt distillers in Scotland
- Mashing, conversion done in stirred tank, then fed to filter
- Allows use of finer grind which may require hammer mill

Advantages:

- Faster cycle time
- Higher extract yield
- Higher capacity (smaller footprint)
- Drier spent grains

Disadvantages:

- Manual time to reset after each batch
- Higher extract of oils / cereal flavour
- Single wort profile produced

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A Common 100% Malt Process

Grist : Initial Water Ratio:

Typically in the range of 3:1 to 4:1 Litres/Kg

Example:

	KG MALT REQUIRED	LITRES OF LIQUOR REQUIRED	LITRES WASH PRODUCED
1ST WATER (4:1)	1000	4000	3500
2ND WATER (2:1)	NA	2000	1500
		TOTAL	5000

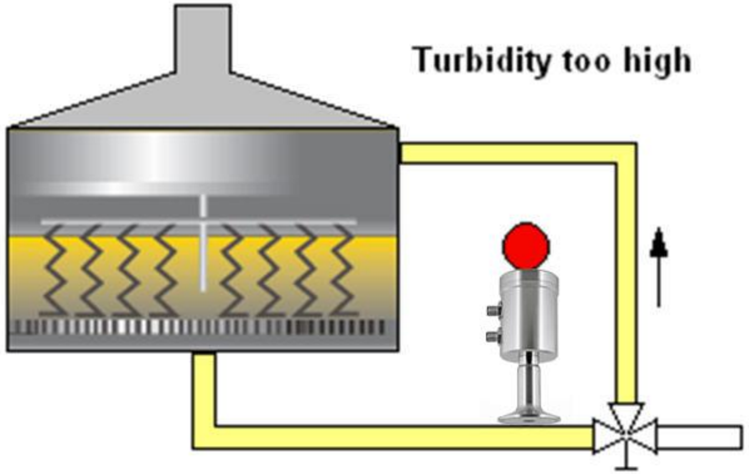
This example will produce 5000 L of wort at 1063 gravity, which when fermented will yield approximately 8.5% ABV wash using well modified distilling malt.

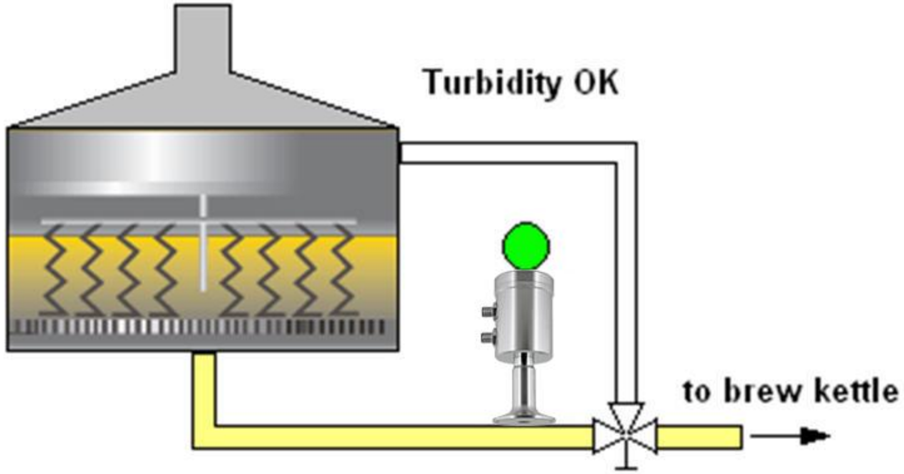
UK Peated malt would be the same but regional varieties may vary

Wort Clarity

Particles and haze in worts translate into cereal/biscuit character in spirit which persists in maturation and obscures Fruit/Estery character.

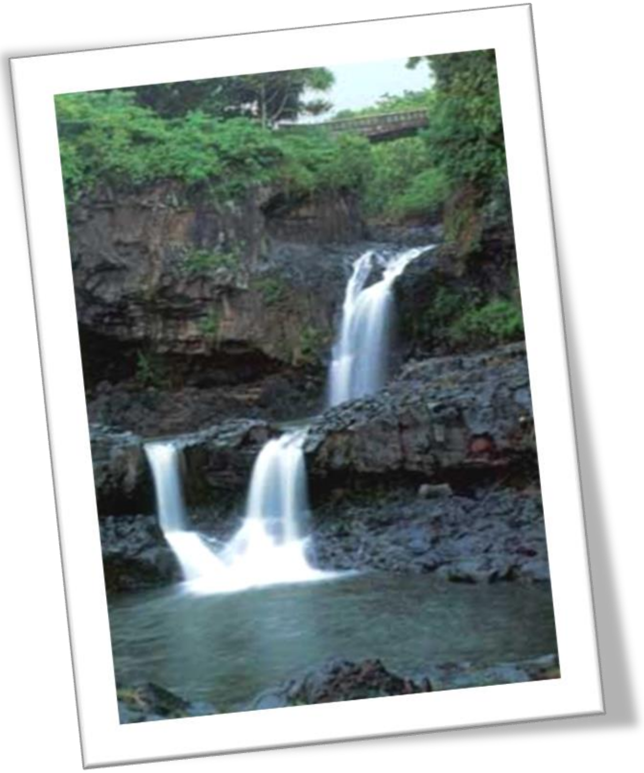
The solution is to recycle worts at start:





Whole Grain Mashing (1) Continuous Cooking

- Step operations are done in separate tanks
- Continuous liquid flow through system
- Retention time in each step determined by flow rate and tank volume
- High throughput
- Energy efficient: options for energy reuse
- Versatile for many grain types, and commonly used for corn, wheat
- Enables low temperature, atmospheric (100^{o}C) or pressure (110-130^{o}C) cooking



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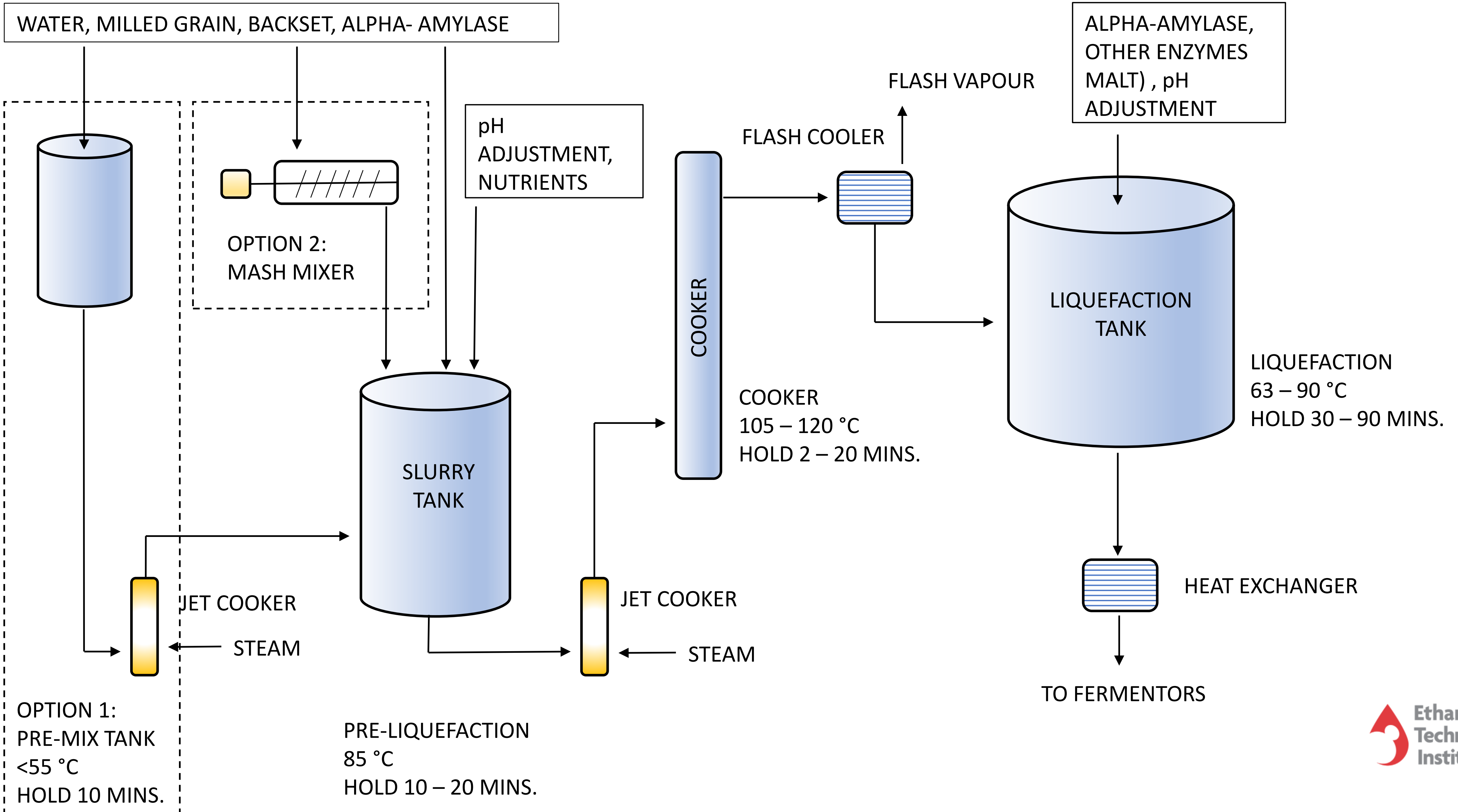
Corn Continuous Whole Grain Mashing System



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Slurry Step Details

- Thorough mixing and wetting of all grain particles
- Operate above or below gelatinization temperature of grain
- Option 1. Below gelatinization temp:
  Mix then heat to slurry temperature
- Option 2. Above gelatinization temp:
  Mix in mash pre-mixer to avoid grain clumping
- Alpha-amylase added for partial liquefaction
- Backset addition will reduce water use
- pH adjustment for optimal enzyme activity
- May choose to add mineral/vitamin mix here

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Cooking

- Jet cooker (Hydroheater) provides rapid temperature rise
- Helps to shear and disrupt starch granules, releasing protein-bound starch
- Cook up to 120^{o}C and hold for 4 - 20 minutes in stirred tank or pipe system
- Flash and cool to conversion temperature

Liquefaction

- Liquefy the exploded/sheared starch granules by hydrolysis with α-amylase
- Conditions
  - Time; 60 – 120 minutes
  - pH; 5.2 – 6.0
  - Temperature: 82 – 85^{o}C
- α- amylase added at 0.04 – 0.06% by wt of grain
- Glucose content at end of liquefaction: 1 – 2% *(enough to start a ferm!)*

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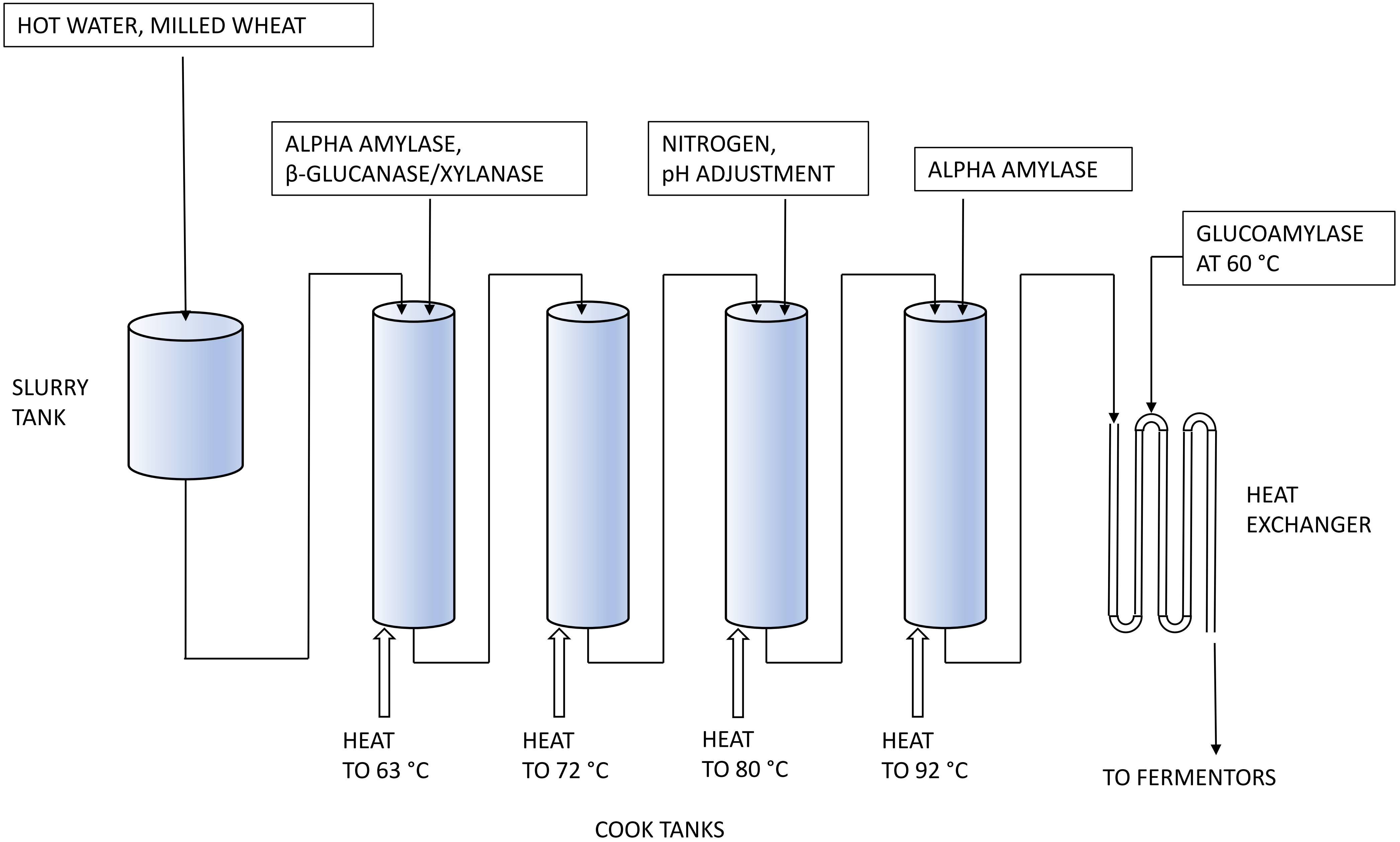
Continuous Wheat Mashing



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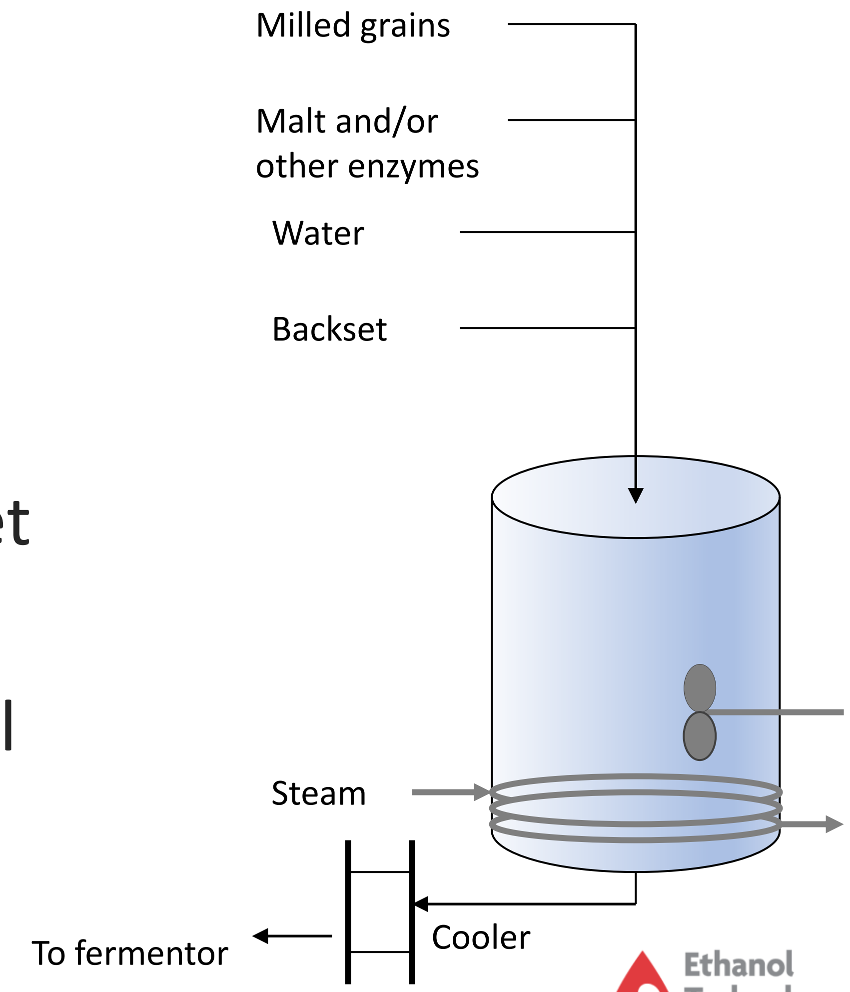
Whole Grain Mashing (2) Batch Cooking

- Traditional method for Bourbon, Tennessee and Canadian whiskies
- Some vodka producers use batch cooking
- Often used by smaller artisanal distillers
- Cooking may be atmospheric or high pressure
- Direct steam injection, steam coil or heating jacket
- Requires good mash agitation
- Lower cook temperatures allow survival of natural flora; Desirable for flavour development in some whiskies



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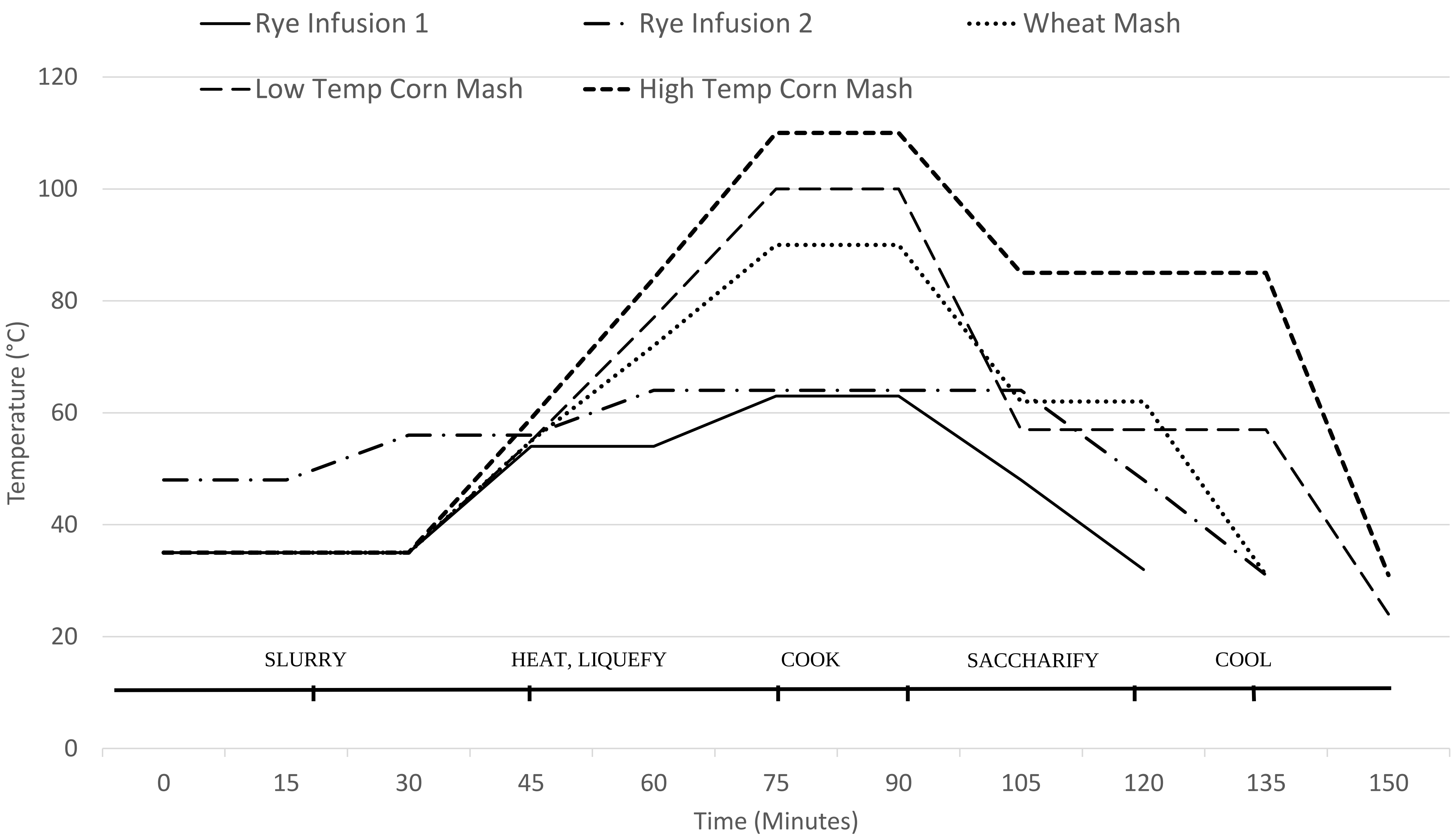
Batch Cooking Profiles



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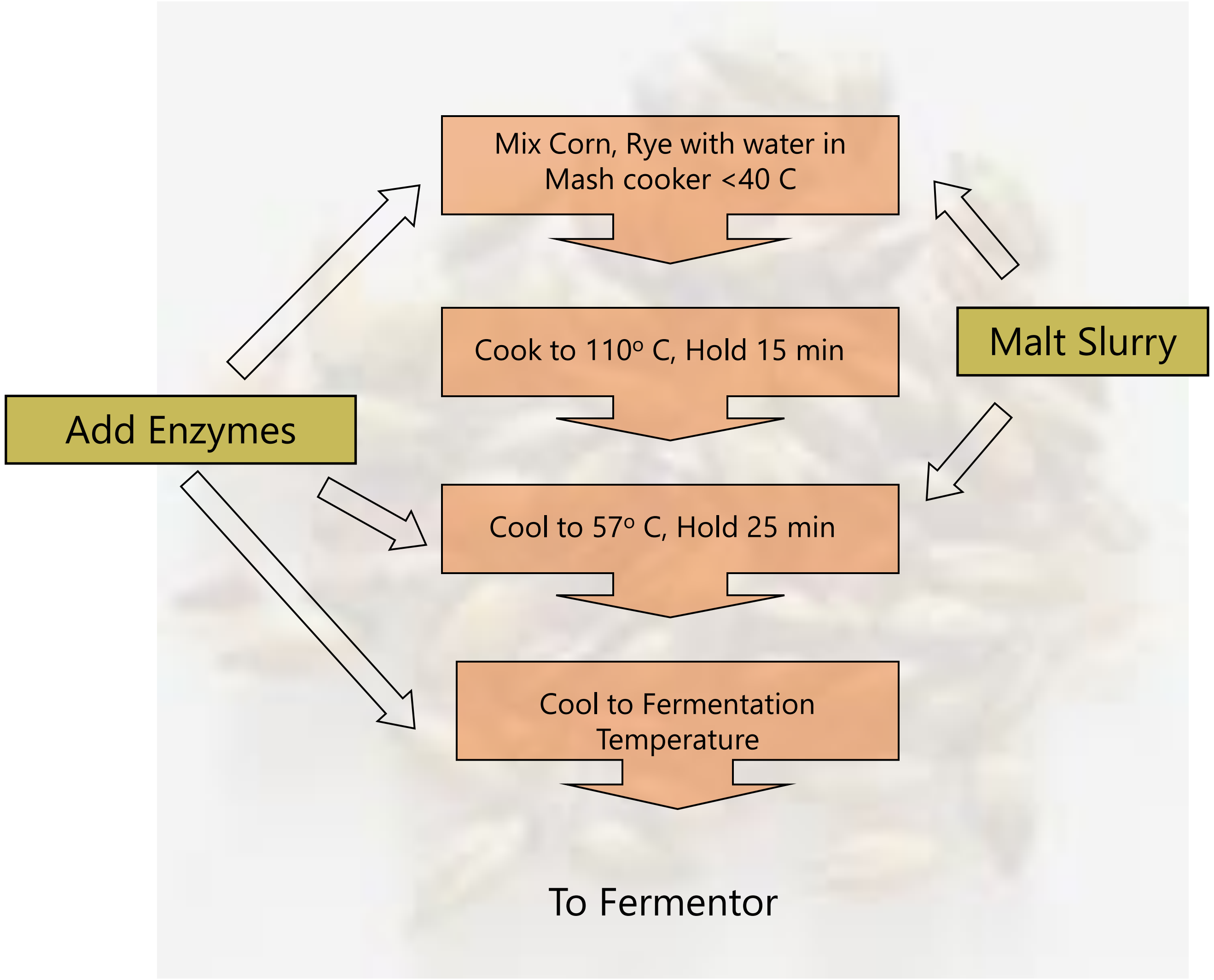
Bourbon Mashing Example

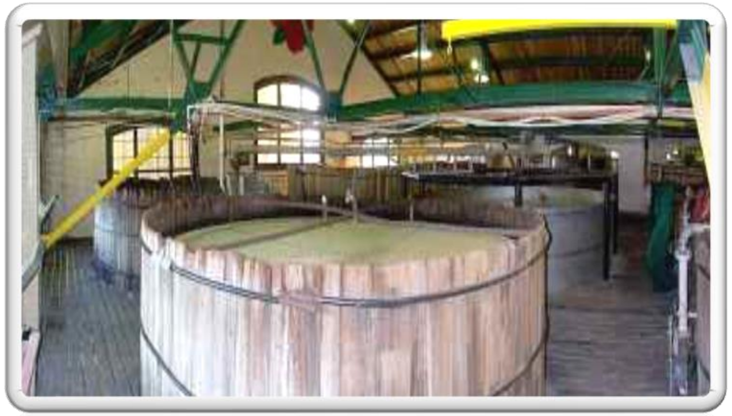
Bourbon Mashing Process



Typical American Straight Whiskey Mash Bills (%)

	Bourbon	Tennessee	Rye
Corn	70	80	39
Rye	15	10	51
Malt	15	10	10

R. Ralph, The Alcohol Textbook, 2003



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Process Variables: Whole Grain Batch Cooking

- Grain types, meal composition
- Cooking temperatures, hold times
- Heat source: direct steam, indirect
- Enzyme types; malt / commercial, viscosity-reducing enzymes
- pH adjustments
- Nitrogen, other nutrient addition
- Backset use
- Delayed Saccharification: *SSF*

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Summary

- Wide variety of mashing methods used by distillers
- Complete conversion of starches to sugars essential for good fermentation yields
- Important to ensure nutritional needs of yeast are being met
- Consistency, energy efficiency, cost effectiveness all important.
- All understood for many years …

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Mash Preparation for Non-Sugar Based Feedstocks

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Robert Fotheringham
Global Technical Manager
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WIRSPA
West Indies Rum & Spirits Producers' Association Inc.

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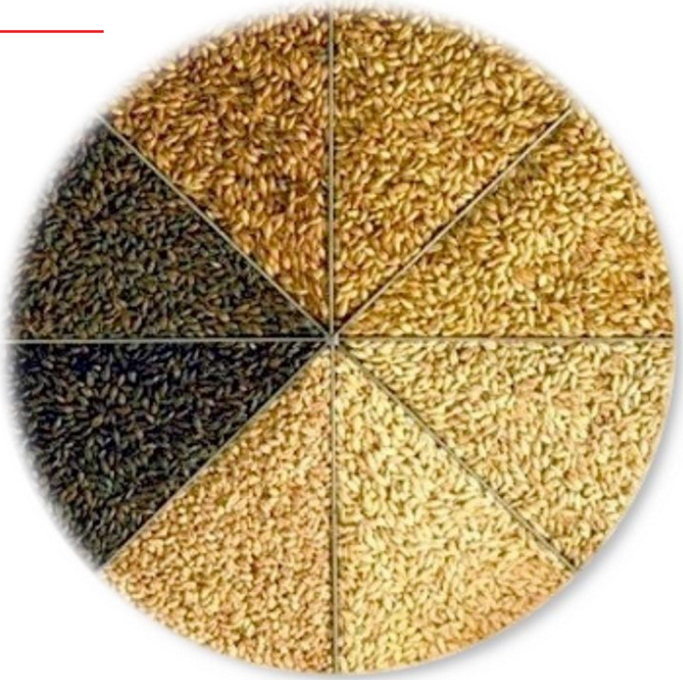
Place: Caribbean Distilling Seminar, St Lucia

Date: 17th April 2024

Time Bonus: Malt

A malted grain is any grain that has been allowed to germinate in order to stimulate the formation of endogenous enzymes

Most malts are kiln dried at a specific stage in the germination process to halt germination, to conserve most of the enzyme activity



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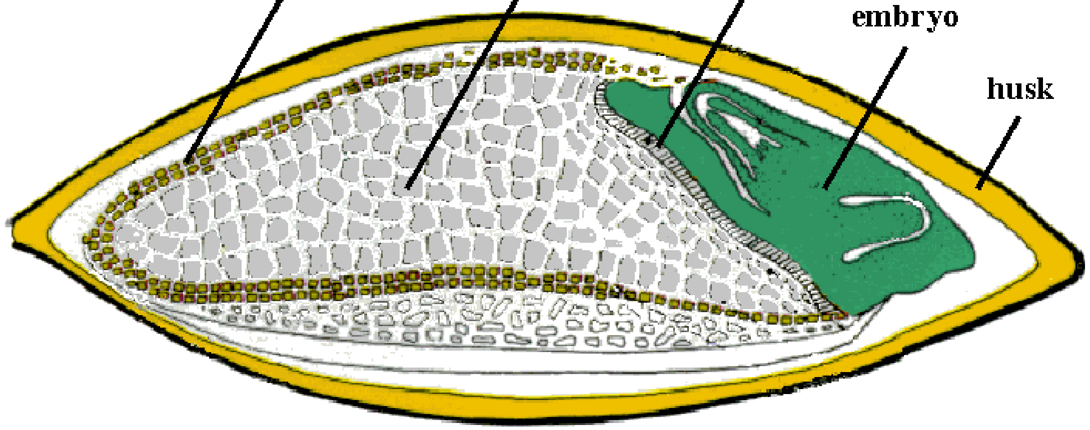
Malting: Barley Composition

- Endosperm contains the store of carbohydrates and proteins to support the initial growth of the germinating embryo



- Aleurone layer; a living cell layer that surrounds the endosperm and provides source of enzymes during germination

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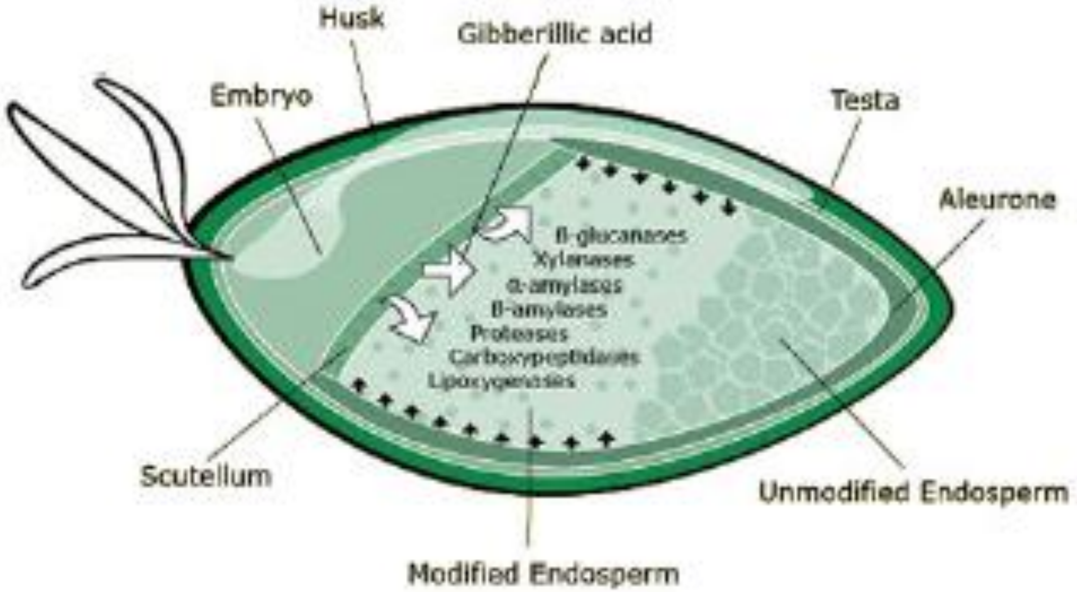
Process of Malting

- From the embryo and down the aleurone layer enzymes are expressed
- These enzymes start to modify the endosperm and breakdown some starch
- Some energy is used to drive these processes & growth
- During drying rootlets are removed (part of malting loss)



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Barley Malting Process

Steeping

Grain takes up water in steeping vessel for 24 hrs with controlled aeration to allow proper respiration

Germination

Grain transferred to malting box or a rotating drum where germination rate is controlled for 4-5 days. Temperature, aeration and moisture are controlled (moisture 43 – 49%), and bed is turned to prevent matting and entanglement of developed rootlets

Kilning

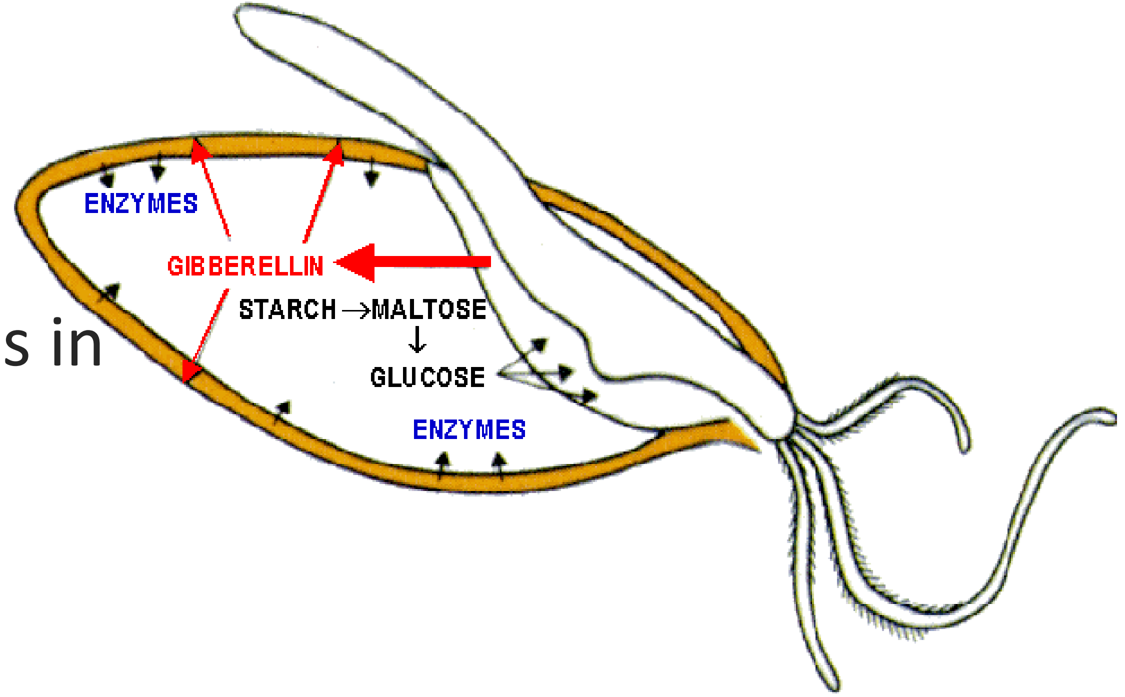
Germination is halted by drying with forced hot air. Enzymes are partially inactivated by heat (49 – 60^{o}C), and malt colour and flavour are developed.

"Peated" malts are infused with peat smoke or peat extracts during kilning

Physiological Changes During Germination

Germination:

- Rootlet (chit) forms, which stimulates production of hormones
- Hormones stimulate production of enzymes in the scutellum and aleurone, which are released into the endosperm
- Enzymes break down cell walls and protein matrix, exposing starch granules
- Germination is stopped at point when grain is "fully modified" = complete degradation of endosperm cell walls



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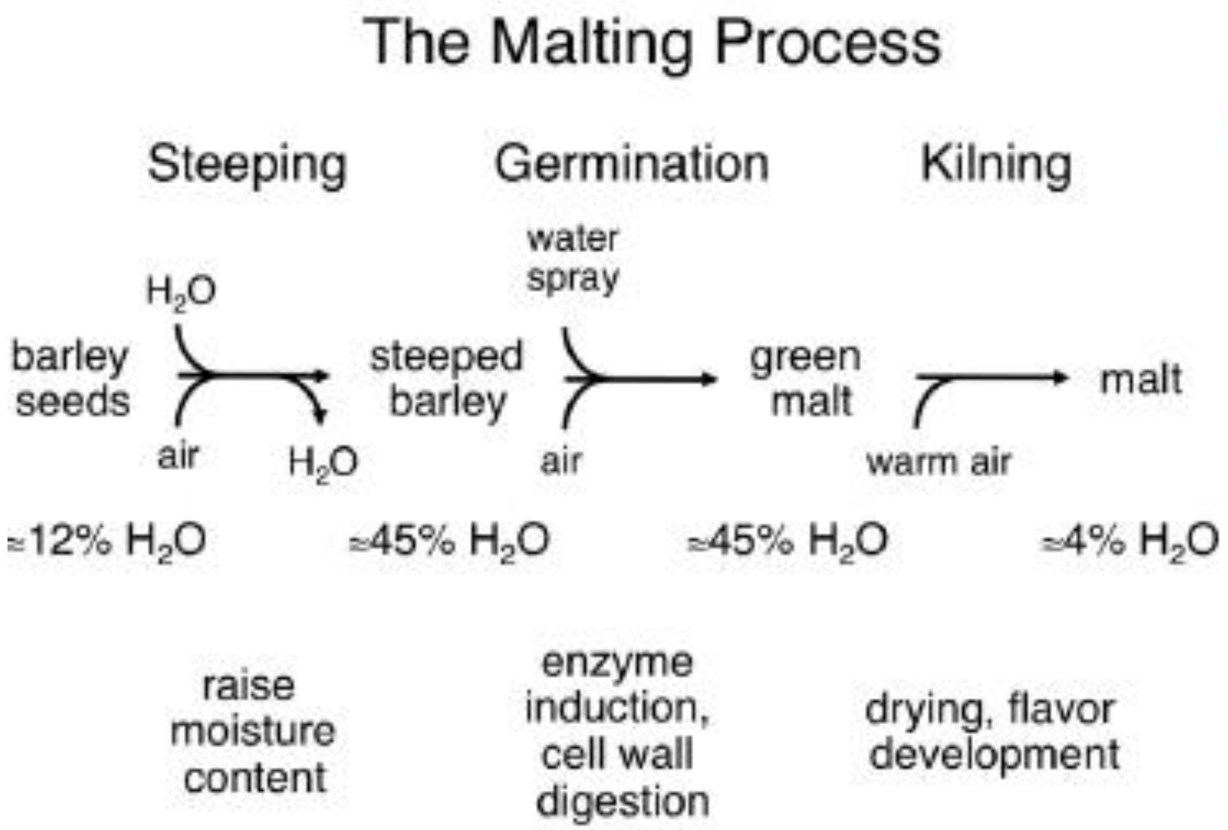
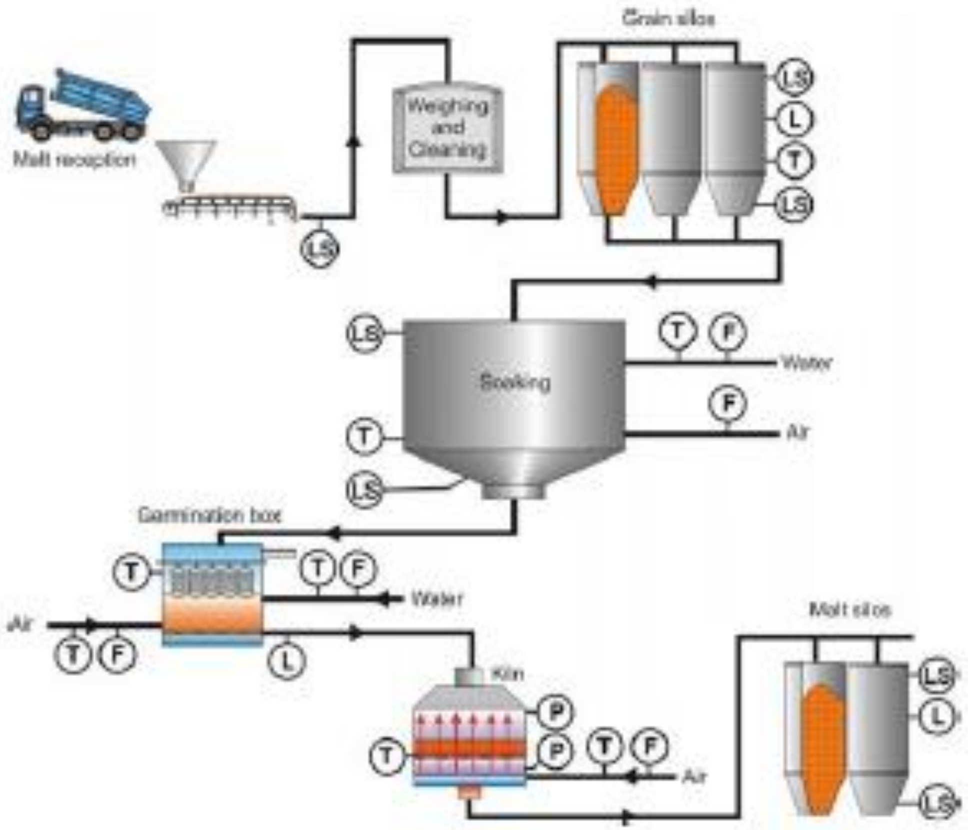
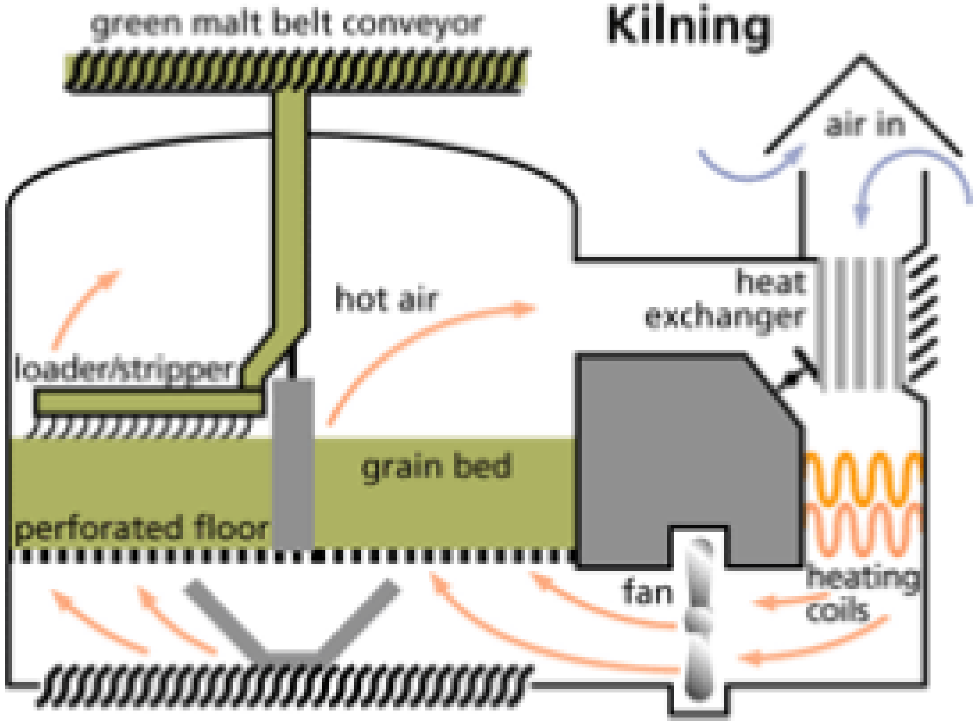
The Malting Process





Kilning Process

- Typically heated air is forced through a bed of "wet" malt
- As moisture is removed temperatures can be increased
- Typical parameters measured are
  - Air on (ºC)
  - Air off (ºC)
  - Air off Humidity



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Kiln Drying

Objectives:

- Stop seed growth and modification
- Create shelf stability by reducing moisture to 4-5%
- Conserve enzyme activity to be used later in mashing
- Create malting flavors and aromas and amber color (of interest to brewers) through Maillard reactions

Peating:

- Original kilns were peat-fired and today conventional forms of hot-air heating are used
- Some distillers retain the peaty flavours by burning peat and blowing smoke across the grain bed during kilning

Malt Enzymes

Malt Enzyme	Optimum Temperature Range (°C)	Optimum pH Range
β-glucanases	40 - 60	4.5 – 6.3
Proteases	45 - 50	3.9 – 5.5
β-amylase	58 - 62	5.5
Limit dextrinase	40 - 50	5.5
α-amylase	65 – 70	5.2

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Mash Preparation for Non-Sugar Based Feedstocks

The Alcohol School Concise

Robert Fotheringham

Global Technical Manager

Lallemand Biofuels & Distilled Spirits

WIRSPA

West Indies Rum & Spirits Producers' Association Inc.

Ethanol Technology Institute

Place: Caribbean Distilling Seminar, St Lucia

Date: 17th April 2024