Selecting an Effective Yeast Strain for Your Feedstock

The Alcohol School Concise

Elena Fossati

New Product Development Manager

Lallemand Biofuels & Distilled Spirits

WIRSPA
West Indies Rum & Spirits Producers' Association Inc.

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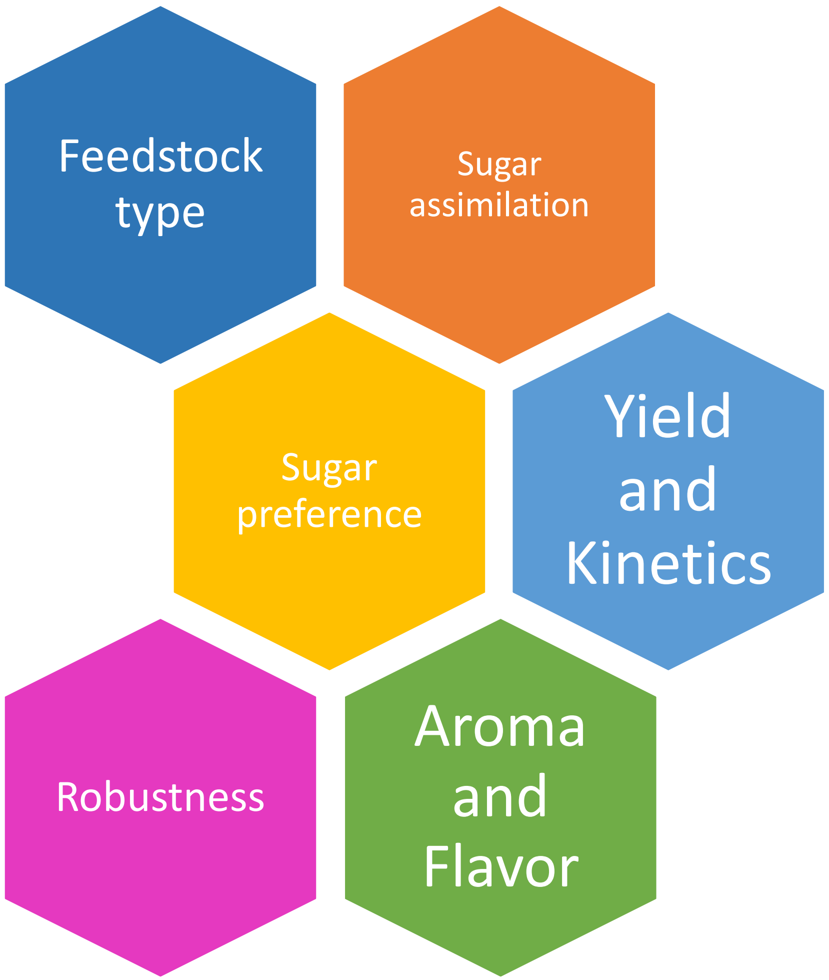
Place: Caribbean Distilling Seminar, St Lucia

Date: April 17th, 2024

Outline



- Distilling yeast characteristics
- Types of feedstocks
- Yeast sugar assimilation, fermentability and preference
- Performance and kinetics
- Yeast robustness to stresses
- Congener synthesis

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Distilling Yeast: Desired Characteristics

'Flavour' distilled spirits

- Complete fermentation
- Moderate or high fermentation rate
- Stress tolerance
- Congener production is key : source of flavors and aromas in final distillates

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- High yield and complete fermentation
- Fast Fermentation rate
- High stress tolerance
- Preferably low congeners production

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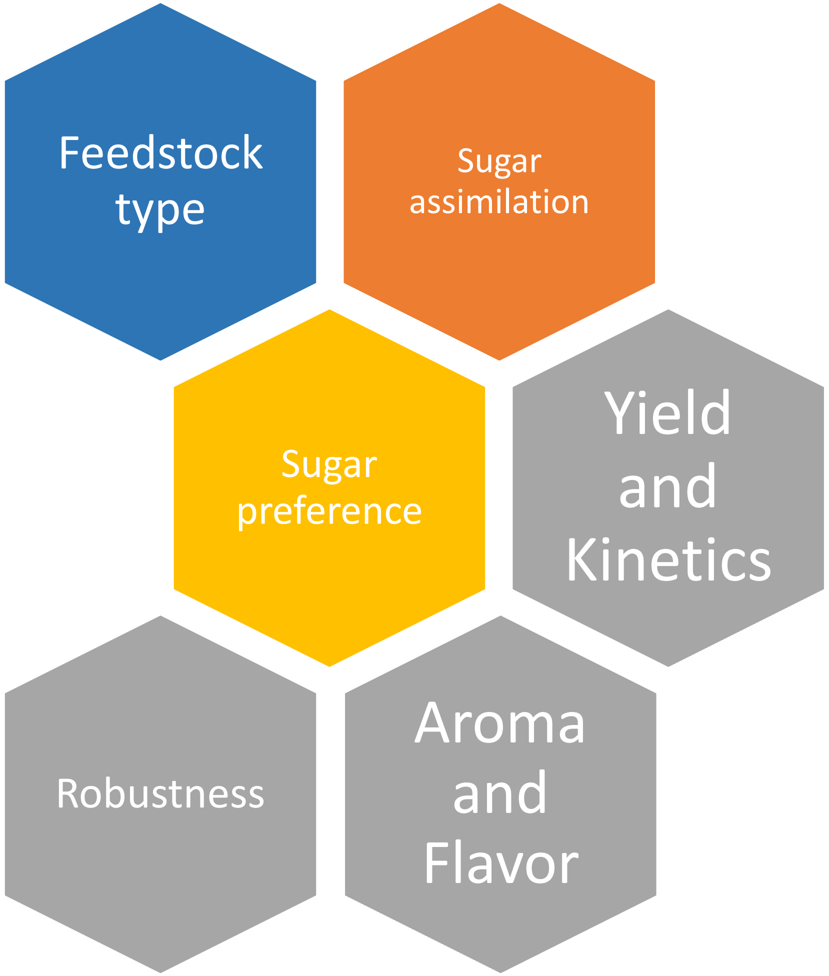
Feedstock Types and Yeast Sugar Assimilation



- Type of feedstocks
- Carbohydrate sources in the different feedstocks
- Sugar profile at the beginning of fermentation
- Are all yeast the same regarding sugar utilization?

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Feedstock Types

- Starch-based

- Sugar-based

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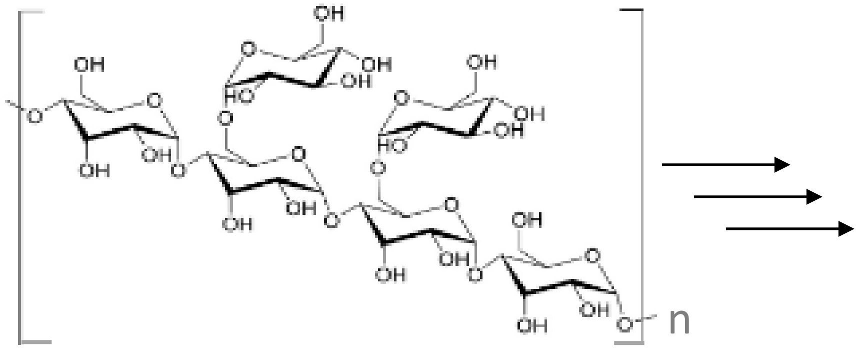
Starch Feedstocks

Starch
Non assimilable

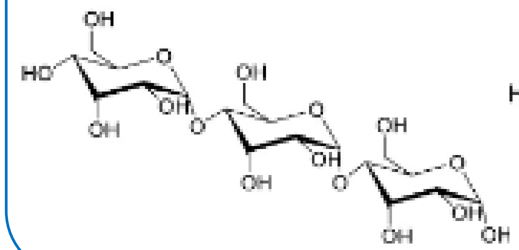


Non assimilable

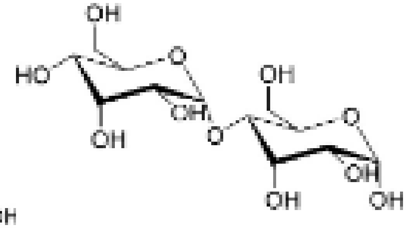
Dextrins
DP4-DPn

Completely assimilable by some yeast only

Maltotriose (DP3)

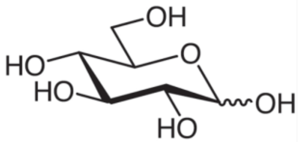


Maltose (DP2)



Assimilable and fermentable

Glucose



DP: Degree of Polymerization

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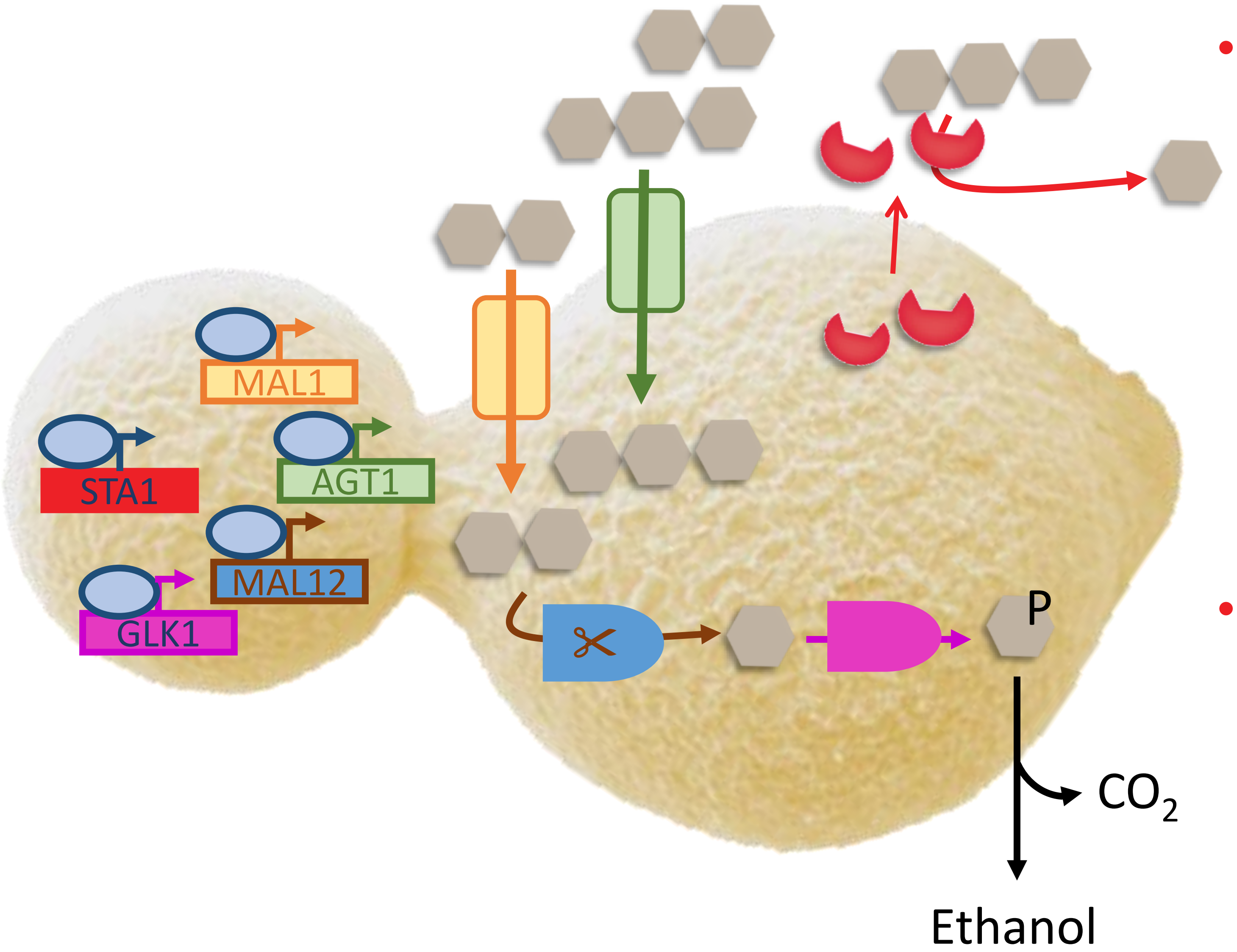
Maltose and Maltotriose Assimilation



- Several genes are required for maltose and maltotriose import and breakdown, encoding for:
  - ➢ Extracellular glucoamylase (Sta1)
  - ➢ Permeases (Mal1, Mtt1, Agt1)
  - ➢ α-glucosidases (Mal and Ima families)
  - ➢ Glucokinase Glk1: specific for Glc
  - ➢ Transcriptional activators (sugar sensing)

- Extracellular glucoamylases and DP2/DP3 permeases are <u>only</u> functional in certain yeast strains (evolutionary adaptation)

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Ethanol from Starch

Starch

↓ ALPHA-AMYLASE

Dextrins

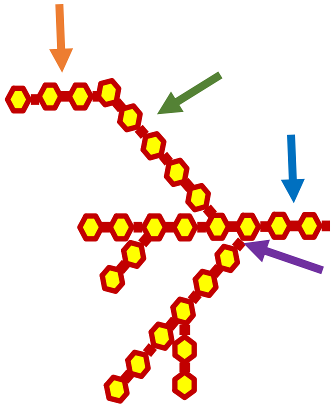
↓ **SSF** (Simultaneous Saccharification and Fermentation): **GLUCOAMYLASE**

Glucose

↓

Ethanol, CO_2

(and new cell growth)



Starch

Starch

↓ **MALT ENZYMES:**

- **ALPHA-AMYLASE**: endo α(1-4)
- **LIMIT DEXTRINASE**: (1-6) branch-links
- **BETA-AMYLASE** exo-α(1-4)
- **ALPHA-GLUCOSIDASE** exo-α(1-4)

Dextrins, DP3, DP2 and glucose

↓

- Secondary conversion: BETA-AMYLASE

Ethanol, CO_2

(and new cell growth)

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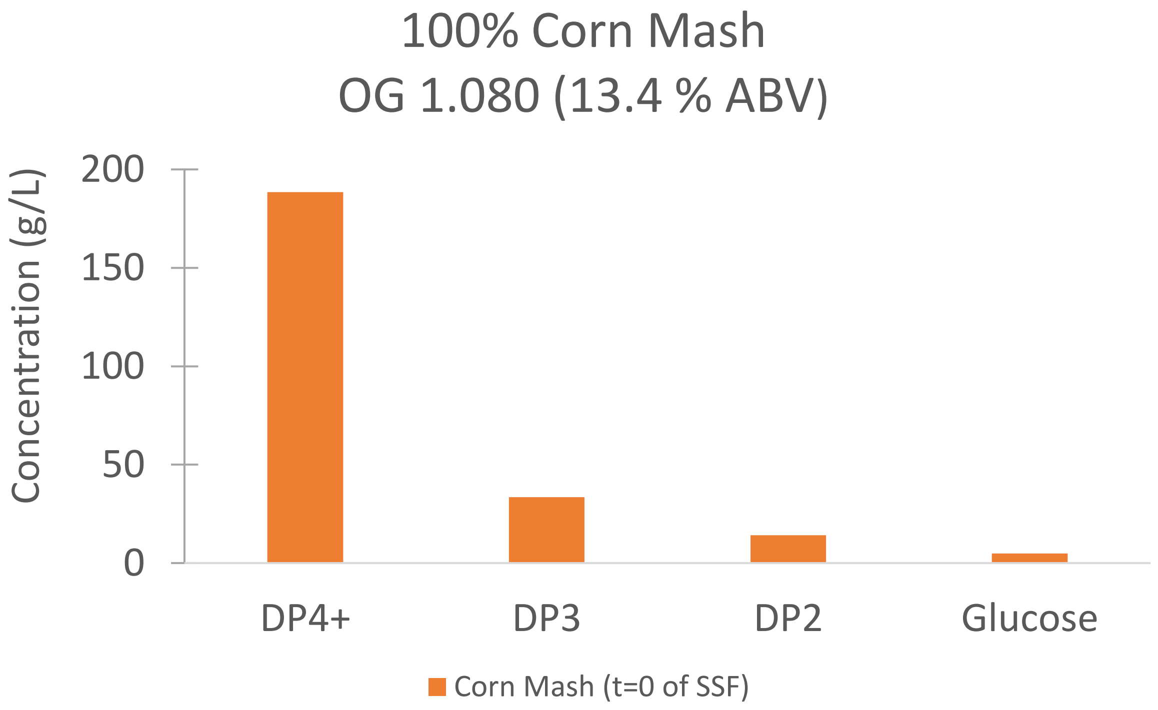
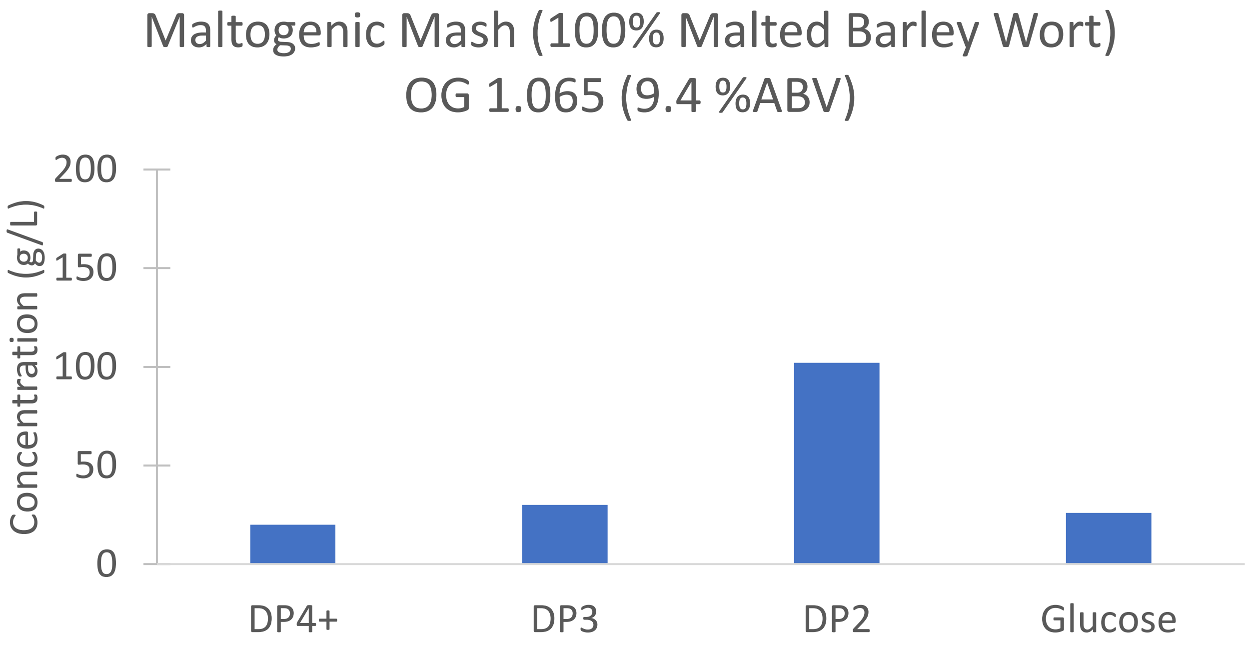
Starch Feedstocks: Sugar Breakdown at the Beginning of Fermentation (After Mashing)





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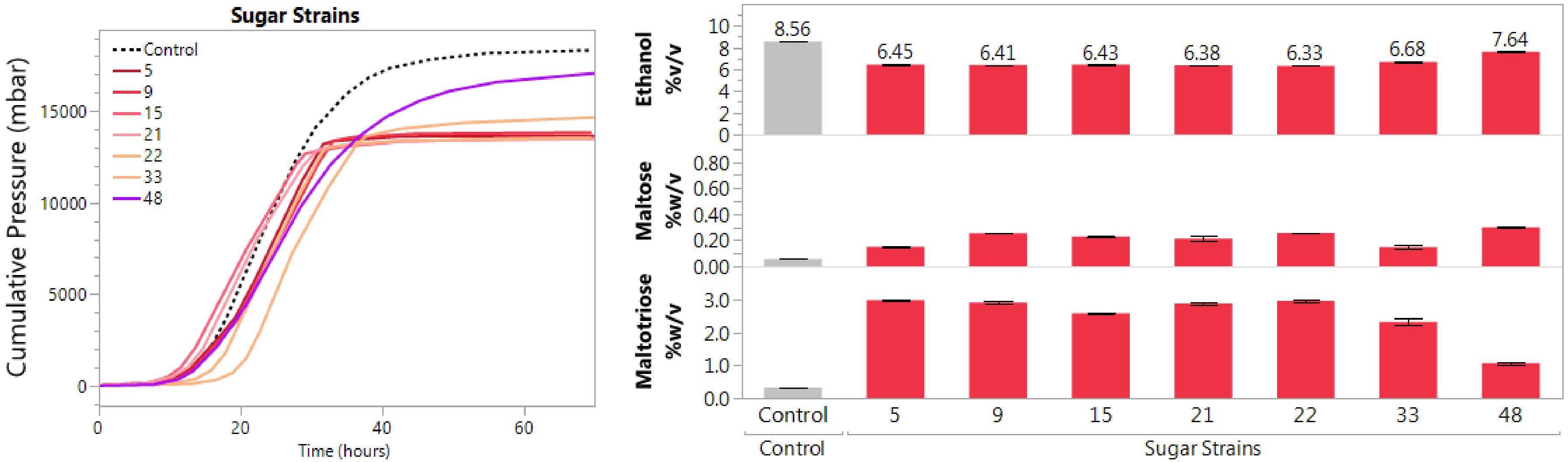
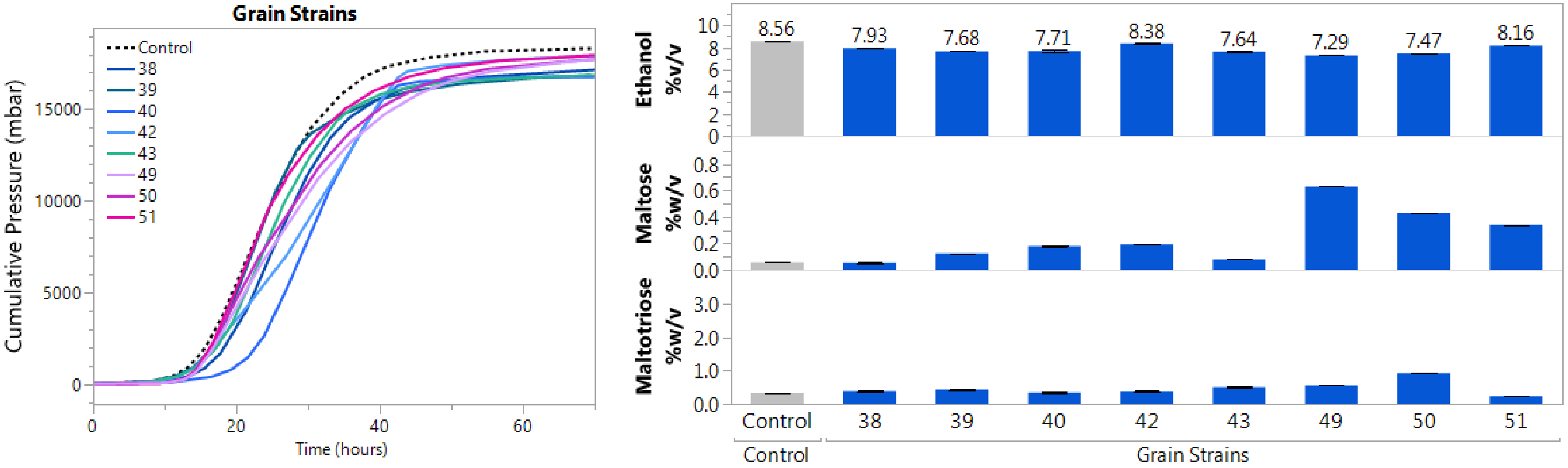
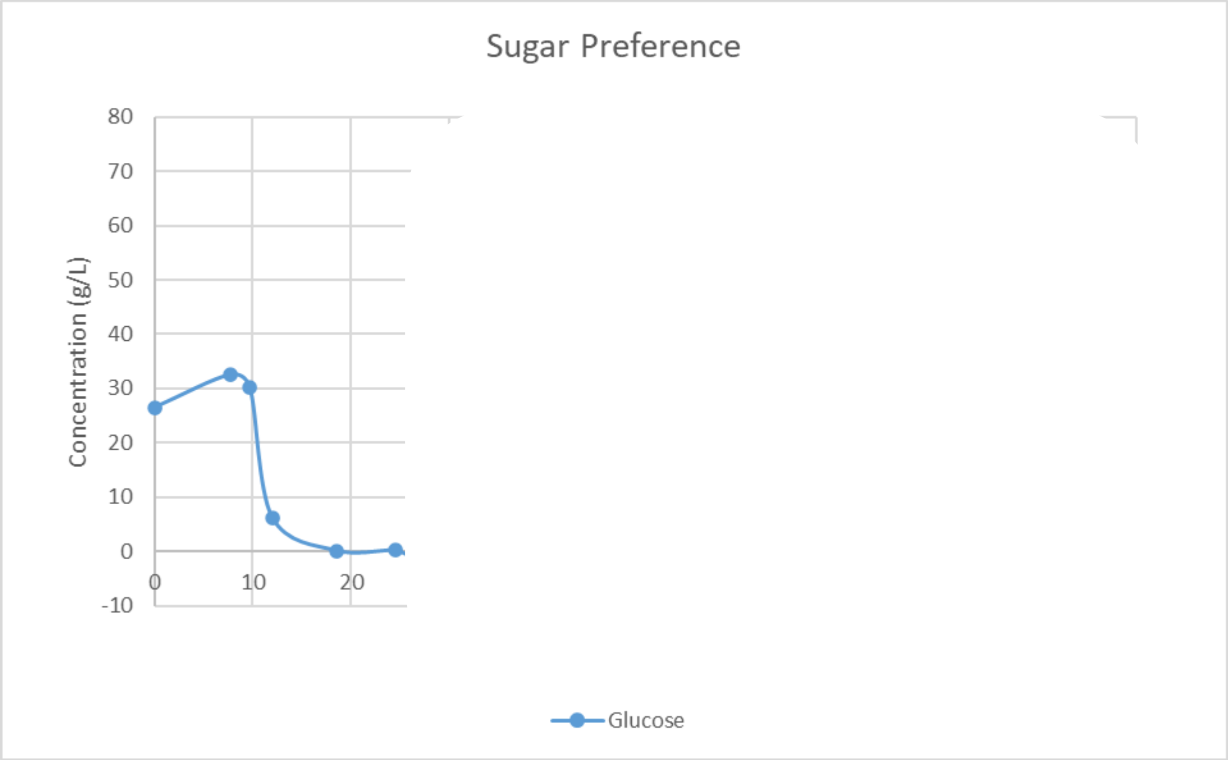
Yeast Screening for Maltose and Maltotriose Utilization



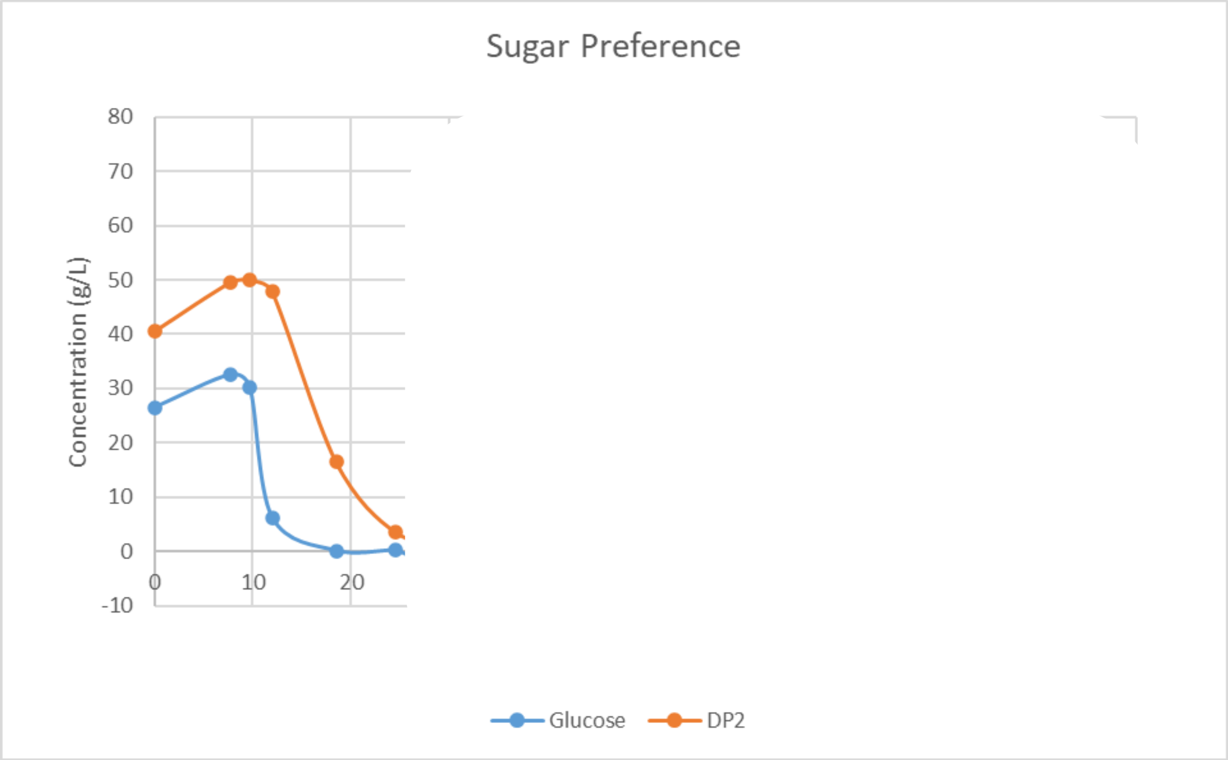


- A strain able to utilize both maltose and maltotriose efficiently is required for maltogenic mashes with no exogenous enzyme addition
- Ethanol loss of up to 25% when using strains unable to assimilate maltose and maltotriose

Yeast Sugar Preference: Bourbon Fermentation Example



Yeast Sugar Preference: Bourbon Fermentation Example



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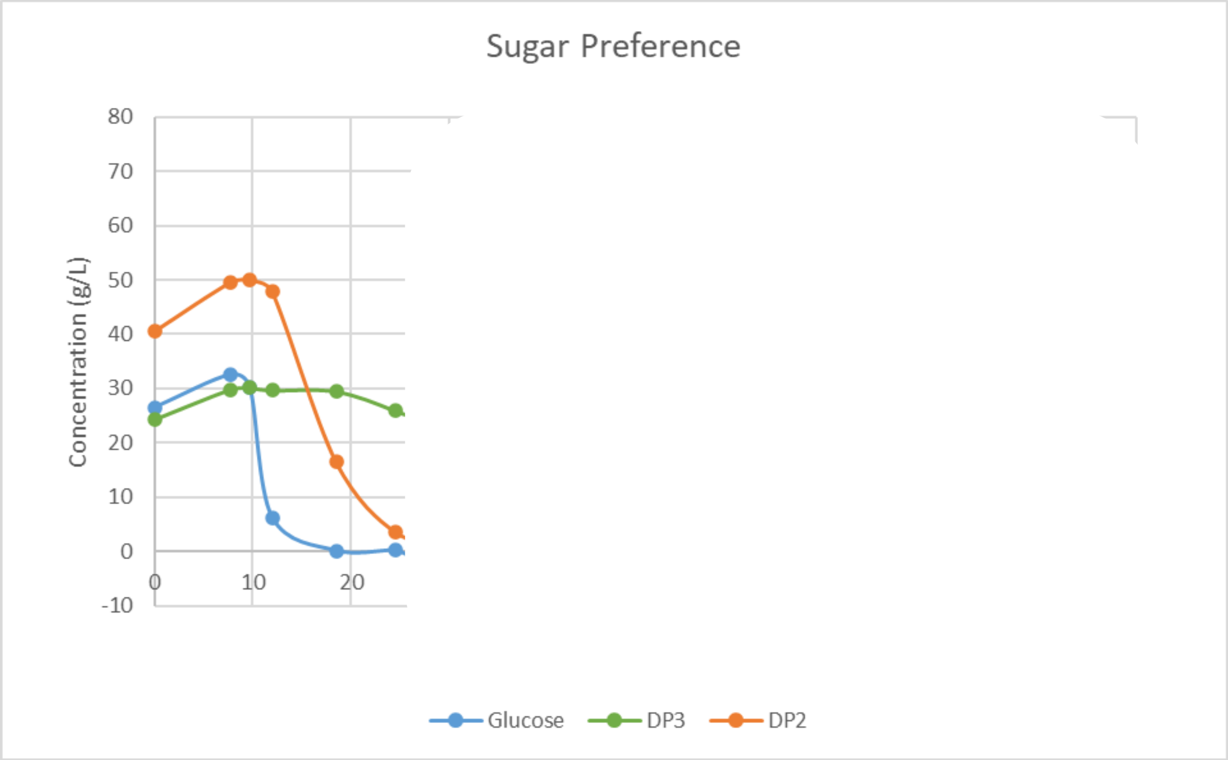
Yeast Sugar Preference: Bourbon Fermentation Example



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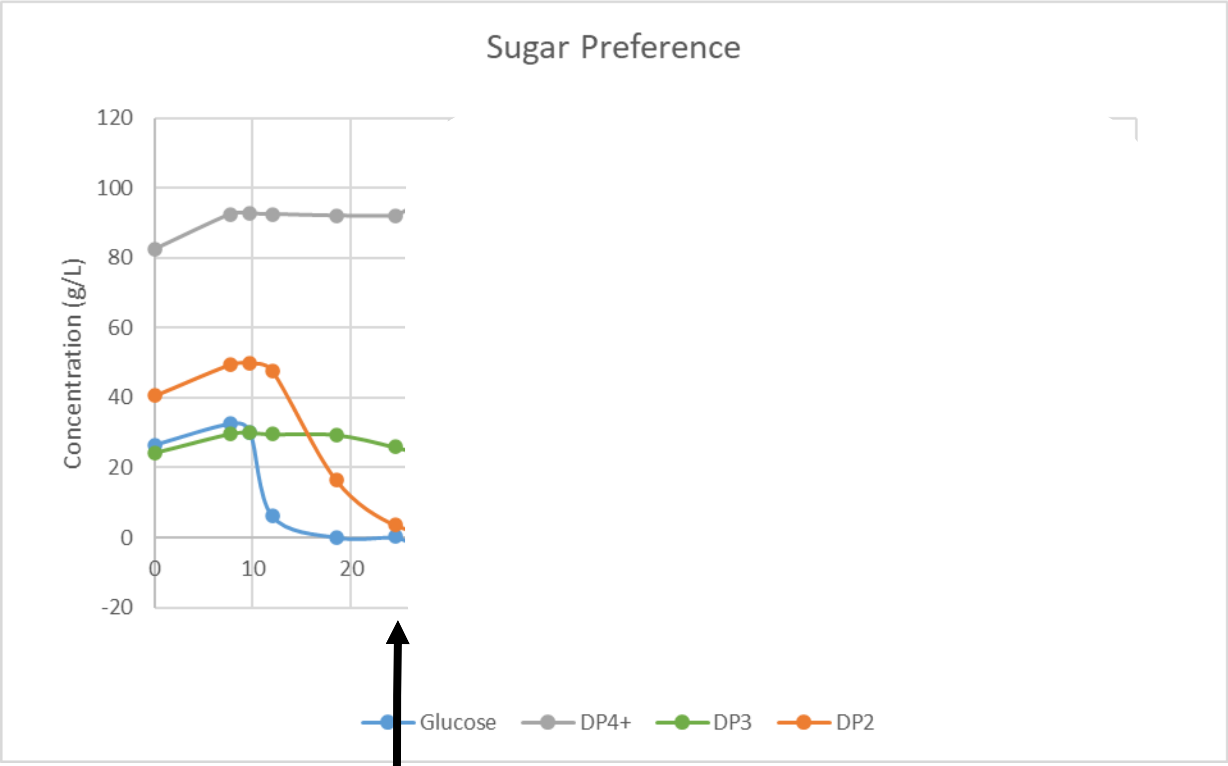
Yeast Sugar Preference: Bourbon Fermentation Example



Glucoamylase addition

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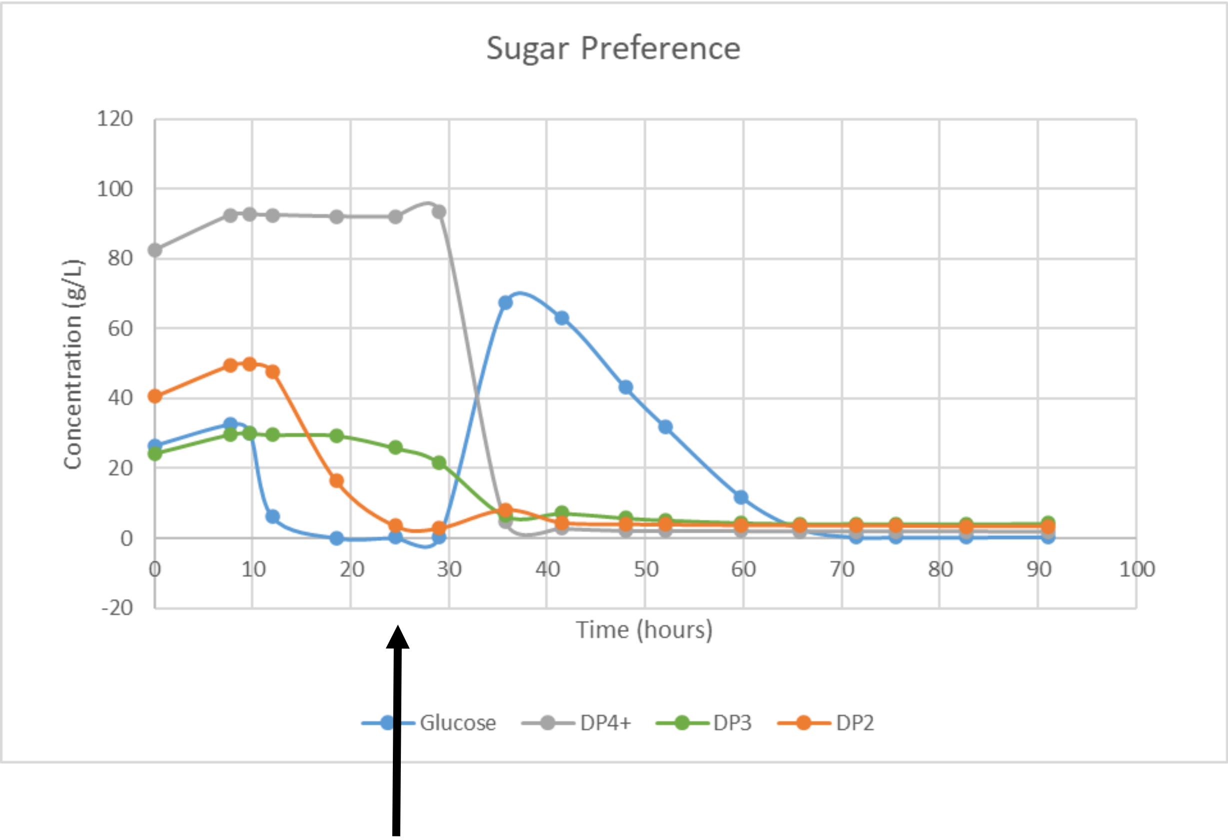
Yeast Sugar Preference: Bourbon Fermentation Example



Glucoamylase addition

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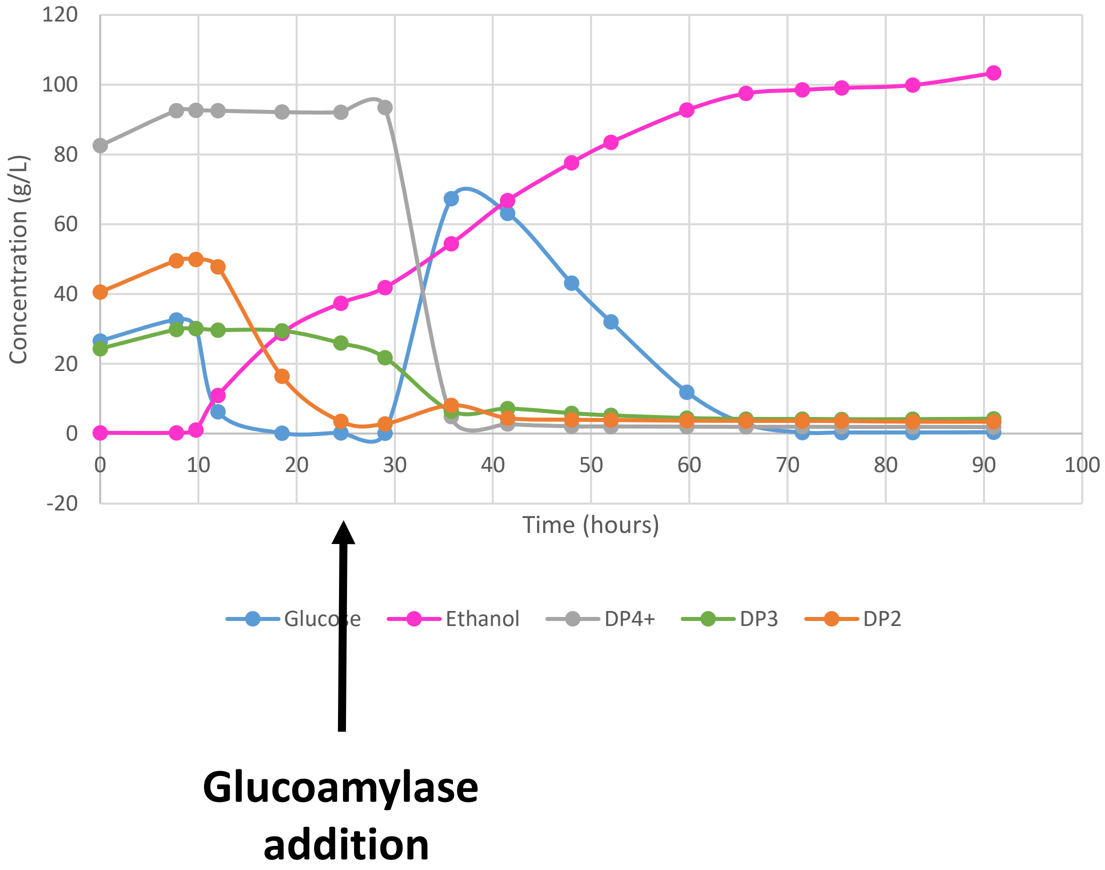
Yeast Sugar Preference: Bourbon Fermentation Example

- Sugar preference: Glucose > maltose > maltotriose
- GA addition converts sugars to glucose



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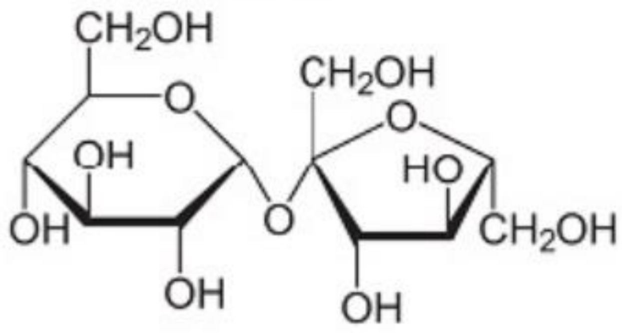
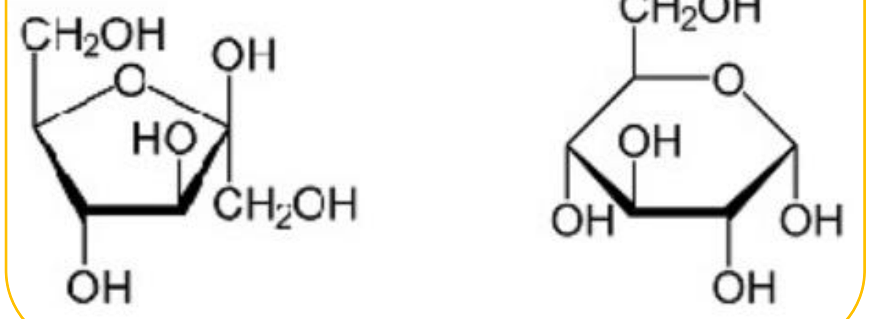
Sugar Feedstocks

Assimilable and fermentable!





INVERTASE

- Periplasmic enzyme that hydrolyzes sucrose into glucose and fructose

HEXOSE UTILIZATION

- Glucose is generally the preferred sugar (sugar sensing)
- Accumulation of fructose in the presence of increasing concentration of ethanol is stressful for most yeast strains and leads to sluggish fermentations

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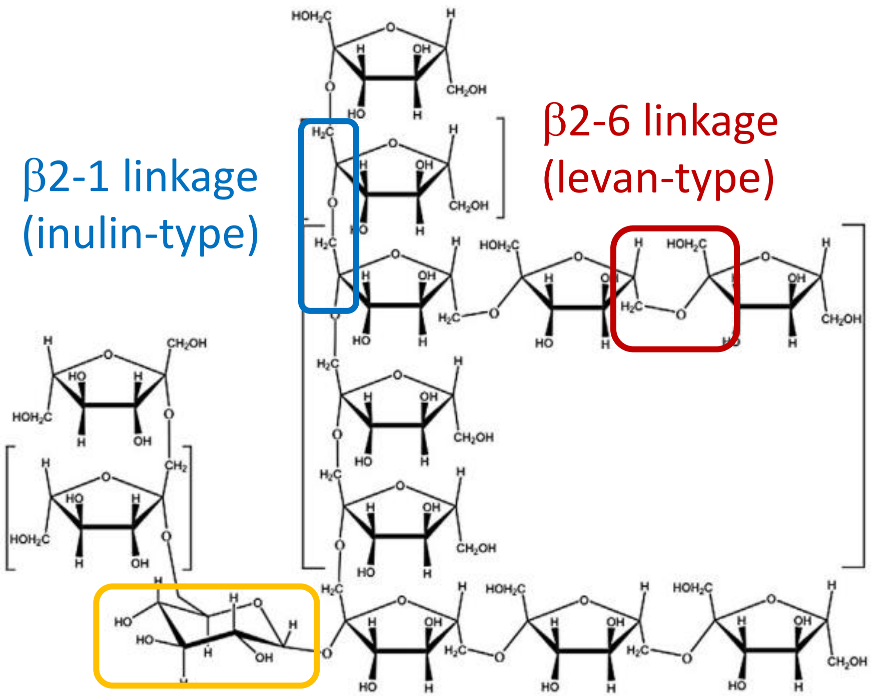
Sugar Feedstocks:
Fructans and Agave Juice Composition

Fructans



Source: Muñoz Gutierrez, *et al.*, 2009

Agave juice composition

	Juice 1	Juice 2	Juice 3
Extract (°Brix)	8.60	7.60	9.40
pH	4.48	4.33	4.22
FAN (mg/L)	2.30	3.1	4.0
DP2 (%w/v)	0.37	0.24	0.28
Glucose (%w/v)	0.76	0.62	1.00
Fructose (%w/v)	6.71	5.64	7.99

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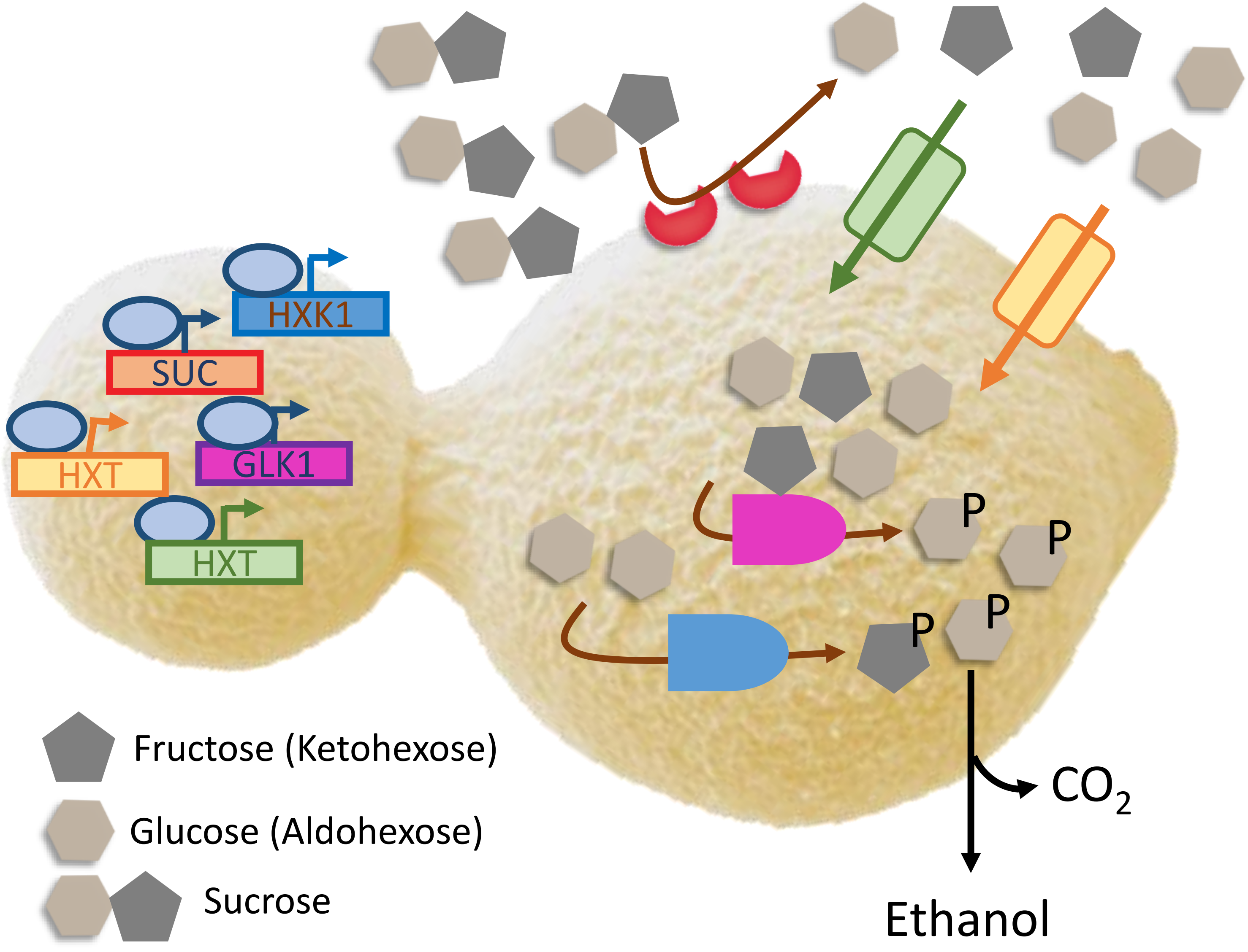
Sucrose, Fructose and Glucose Utilization



- Invertase (SUC1-5a and SUC7) Different strains express SUC genes differently
- Utilization of glucose and fructose:
  - ➢ Transporters (Hxt1-20): generally, have higher affinity for glucose
  - ➢ Glucokinase Glk1: specific for Glc
  - ➢ Hexokinase Hxk1 and Hxk2
- Fructophilic yeasts have mutations in HXT genes for improved fructose uptake (evolutionary adaptation)

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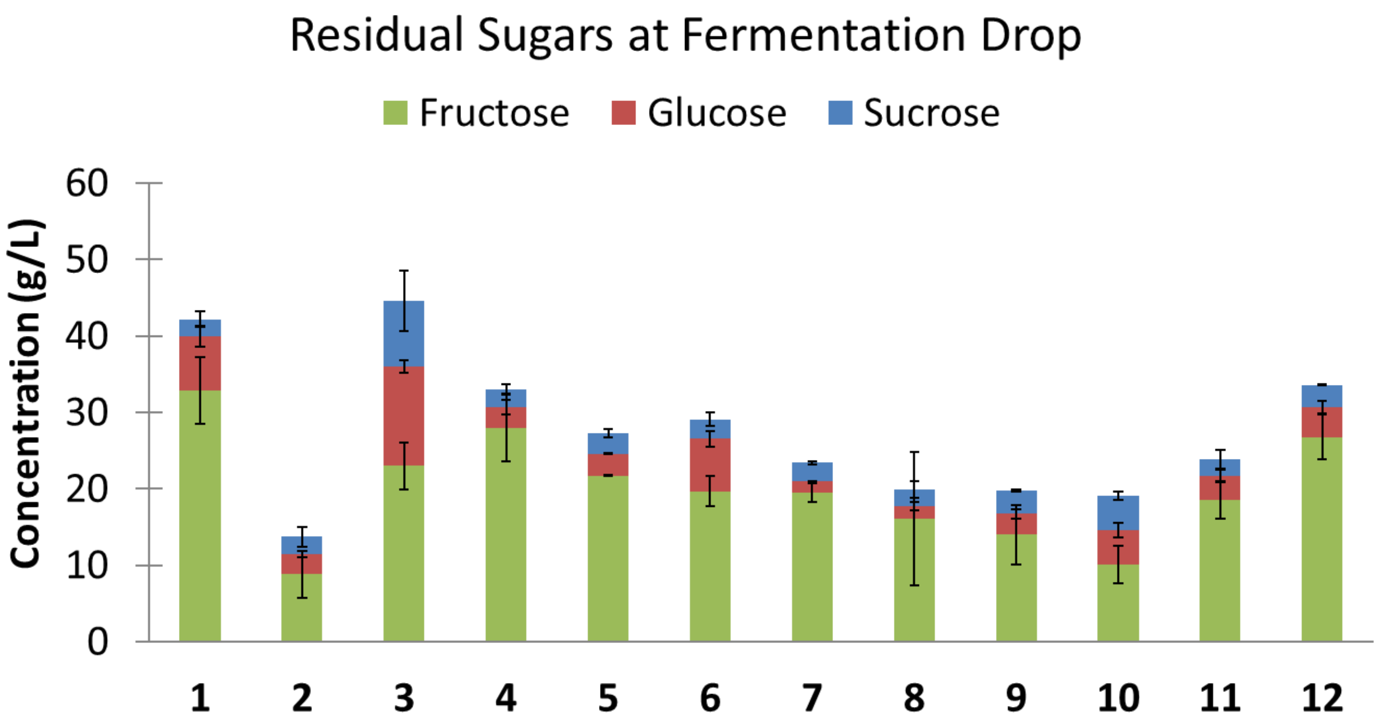
Yeast Screening for Sucrose and Fructose Utilization



Molasses fed-batch laboratory fermentations

- Most strains show invertase activity
- Not all strain can consume fructose completely!

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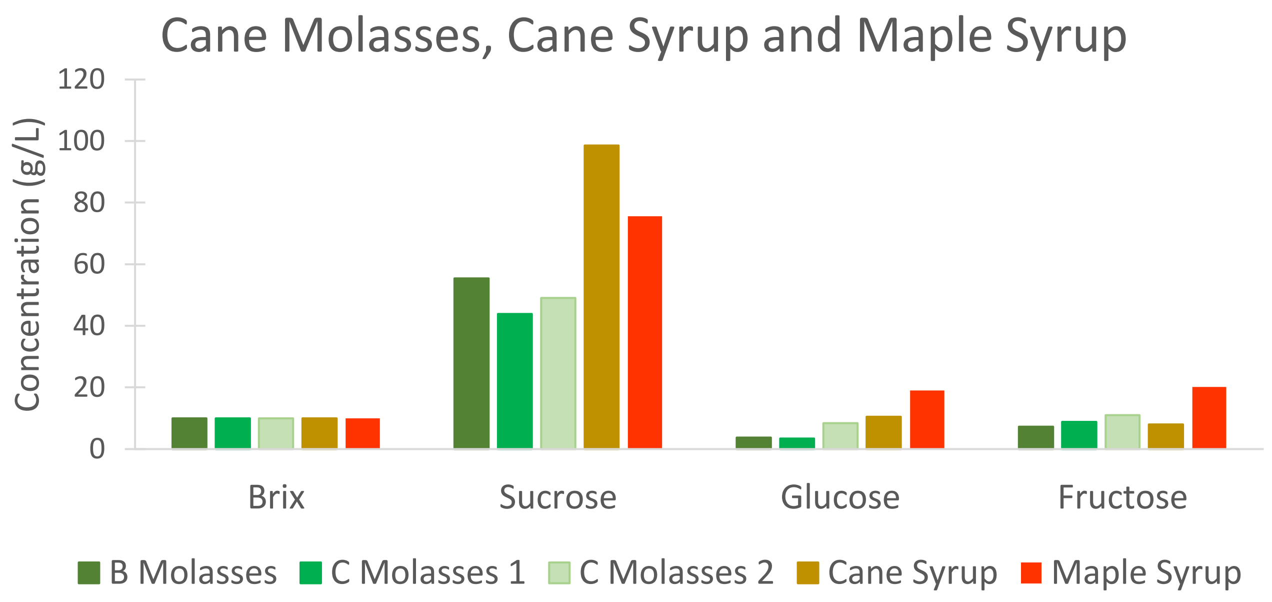
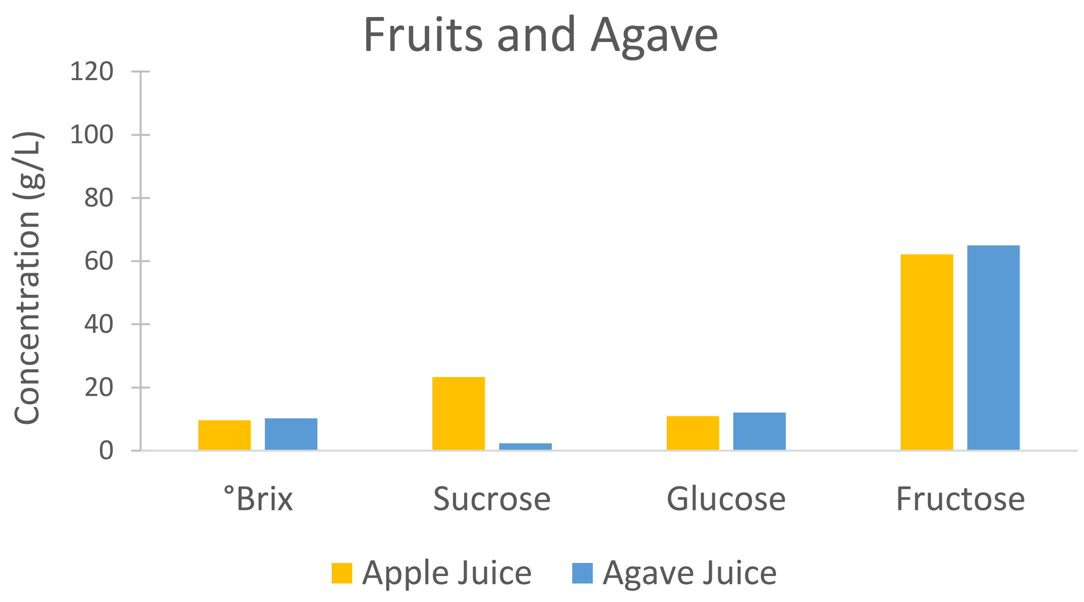
Sugar Feedstocks: Sugar Breakdown





The choice of yeast will depend on the sugar substrate used

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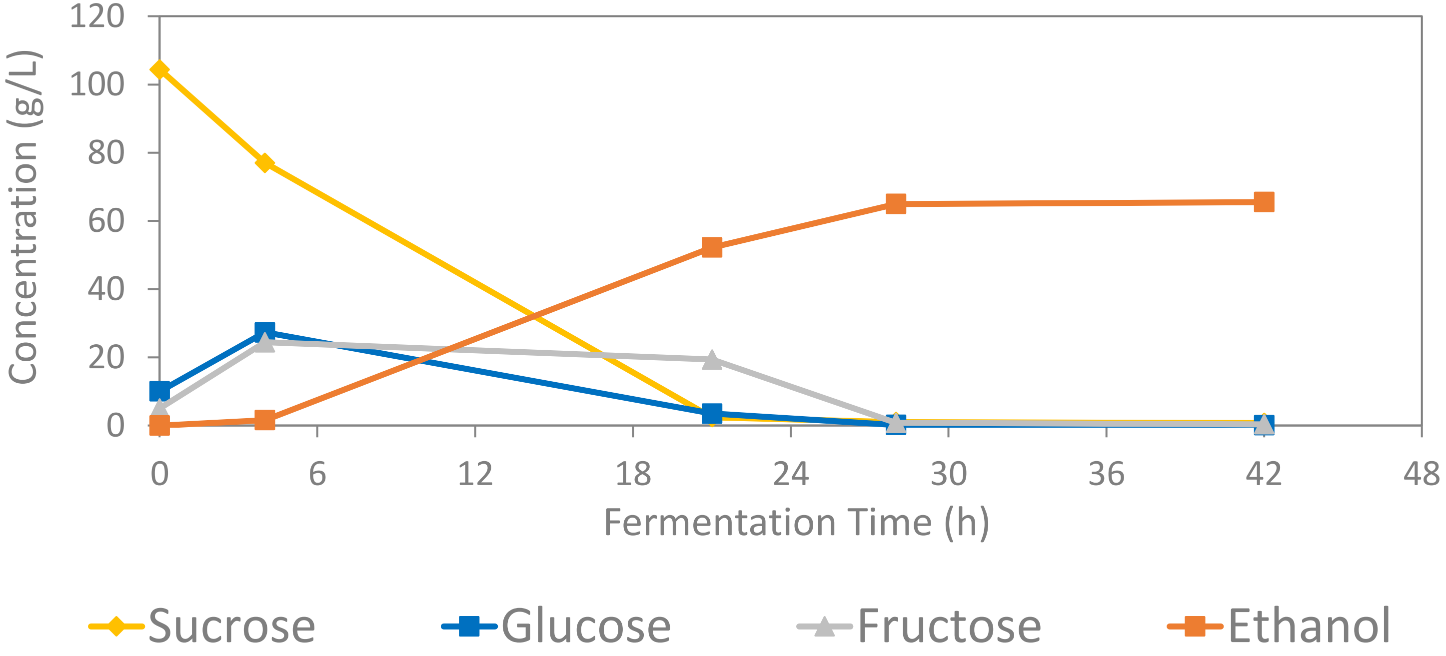
Yeast Sugar Preference

Cane Juice Fermentation

- Sucrose converted to glucose and fructose
- Glucose preferred over fructose



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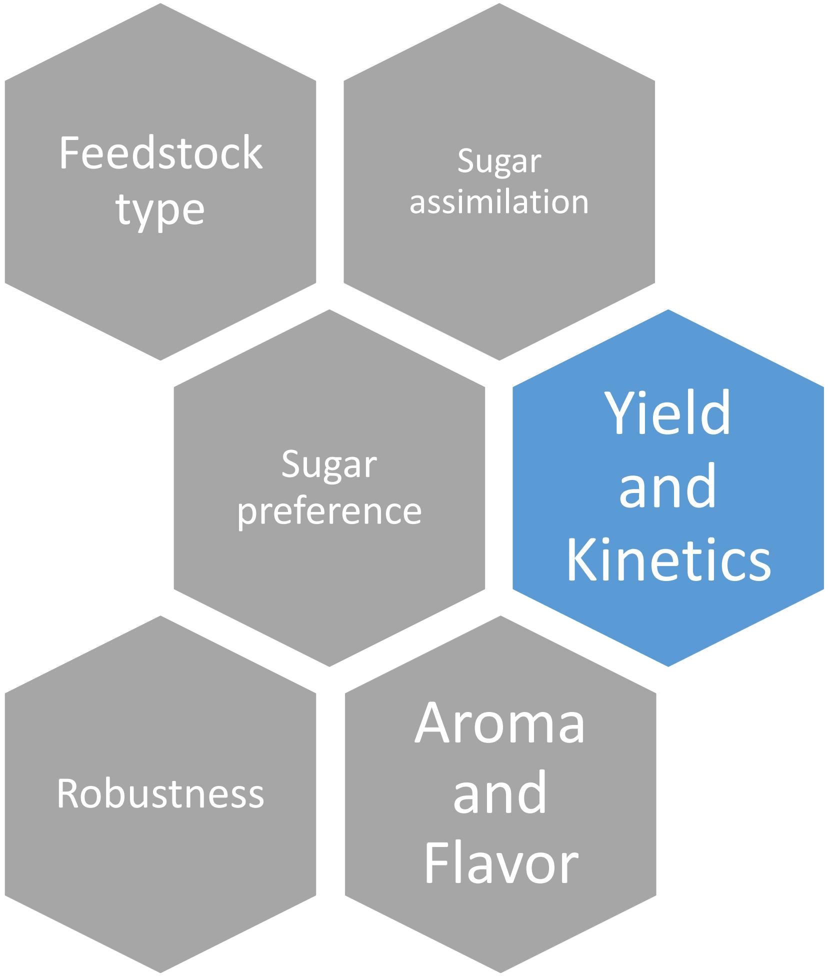
Strain Yield and Kinetics



- Yield & Kinetics
- Risks and challenges of co-inoculations

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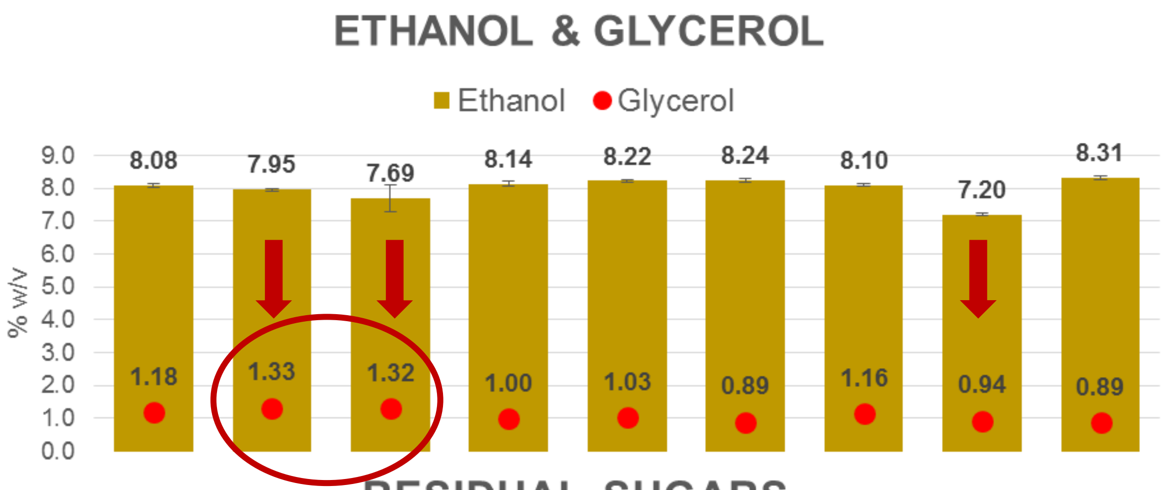
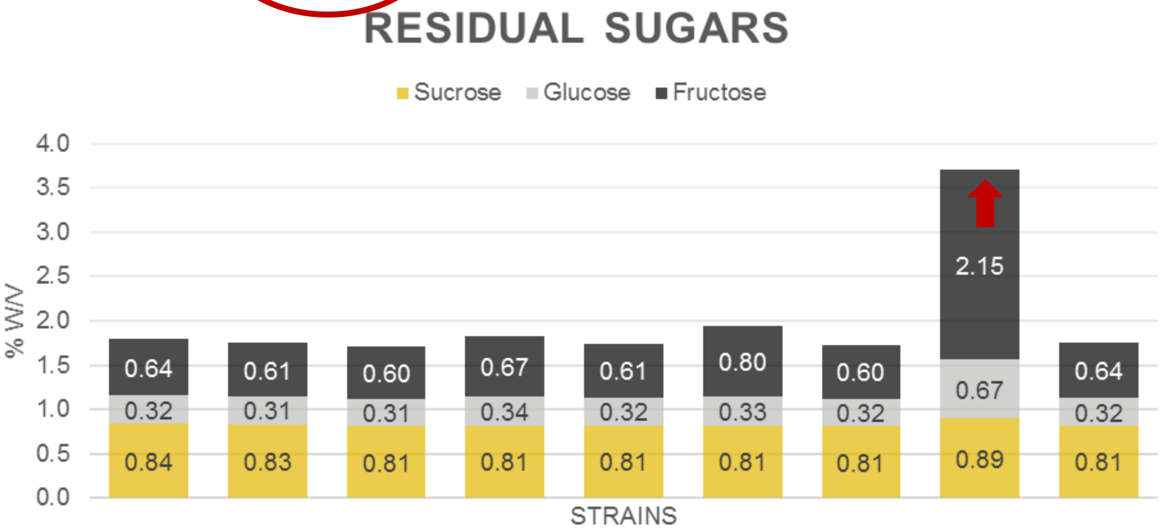
Screening for Yield on Cane Molasses

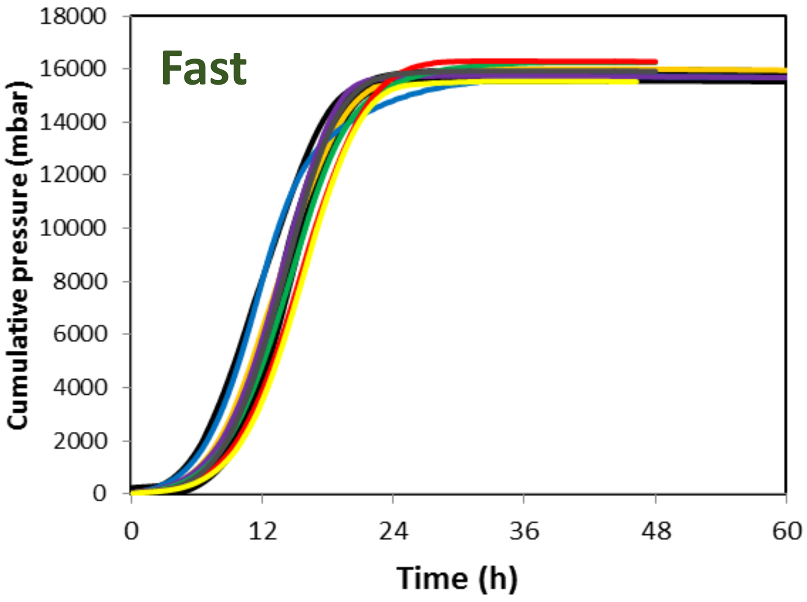


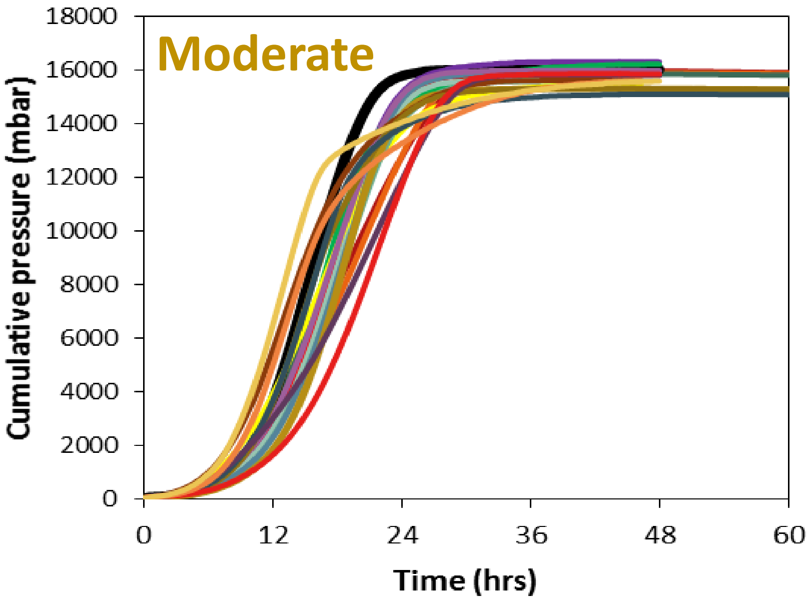


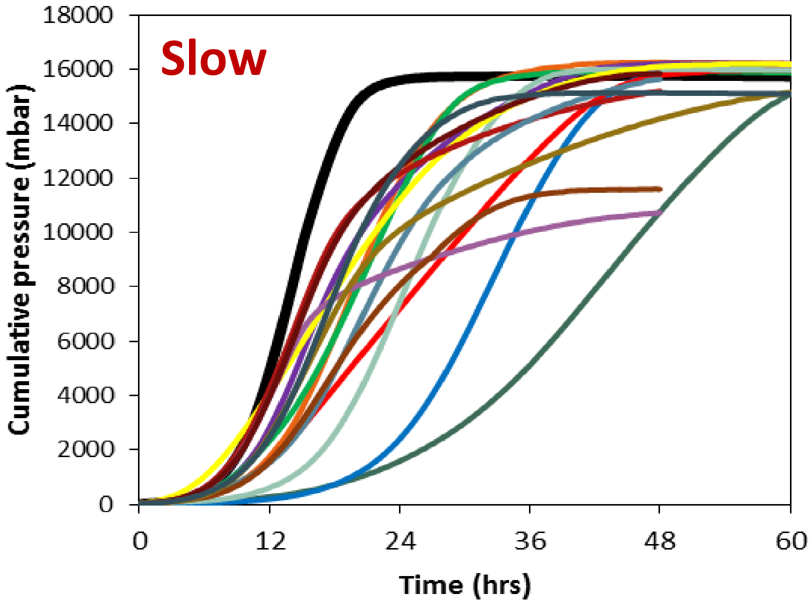
- Define the target yield
- Verify if the yeast of choice can reach the desired ethanol titer or higher in case you plan on increase original gravity

Molasses batch laboratory fermentations

Fermentation Kinetics on Cane Molasses







Molasses batch laboratory fermentations

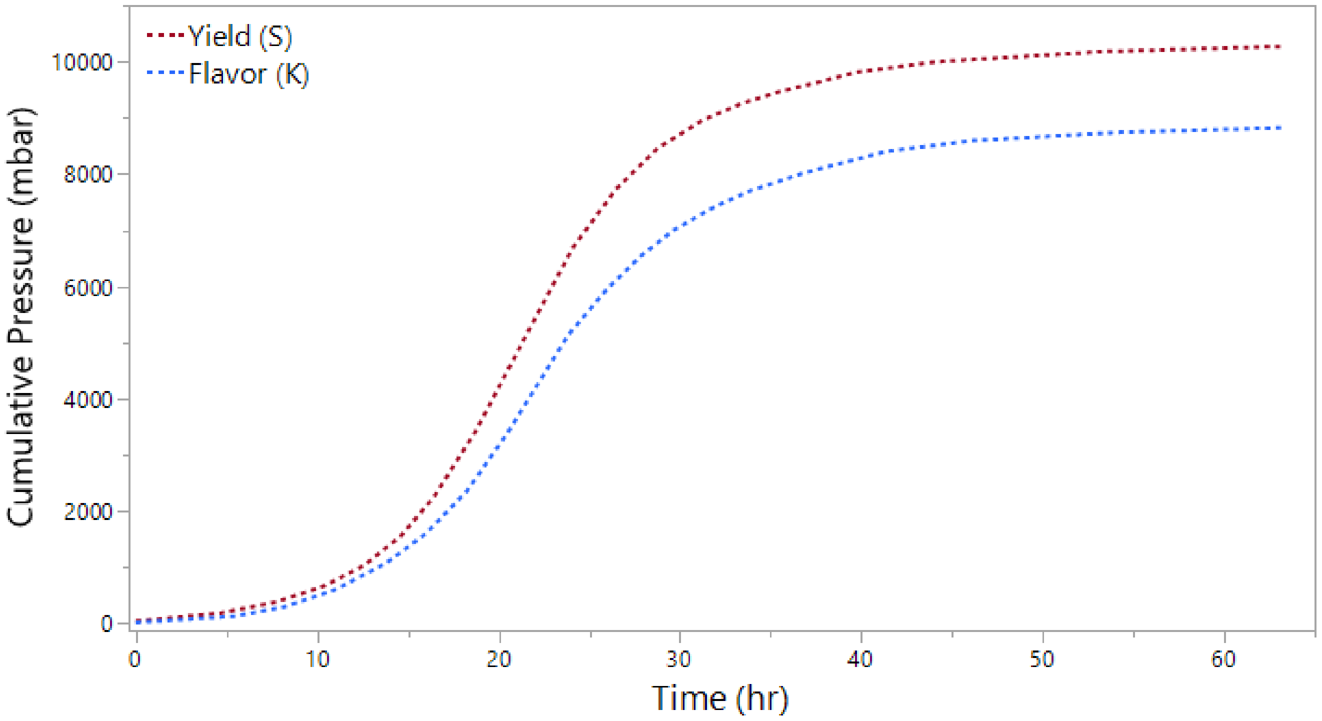
▬ *Control distilling yeast*

Certain strains ferment too slowly to be practical in fermentation: increased risk for contamination!

Co-inoculation

When using alternative strains, consider:

- Ability to ferment all sugars in substrate
- Killer factor status
- Pitch rate
- Time of inoculation
- Kinetics



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Co-inoculation

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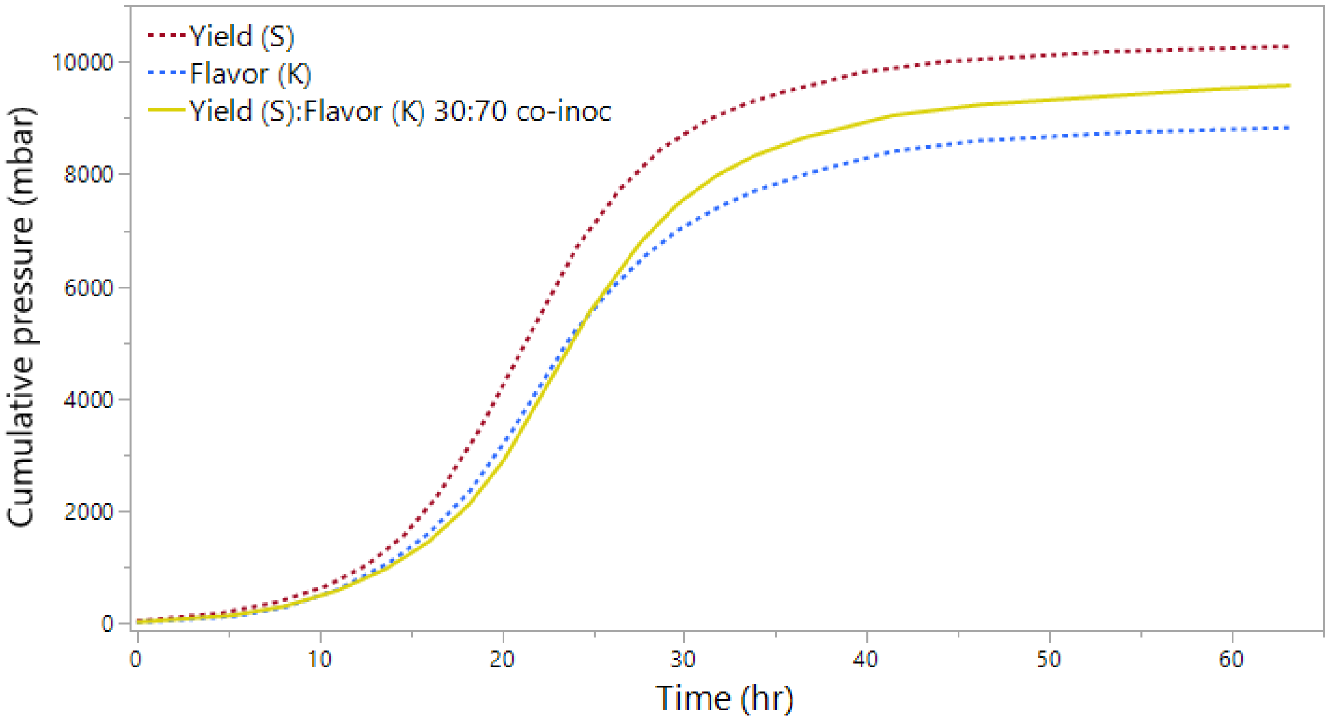

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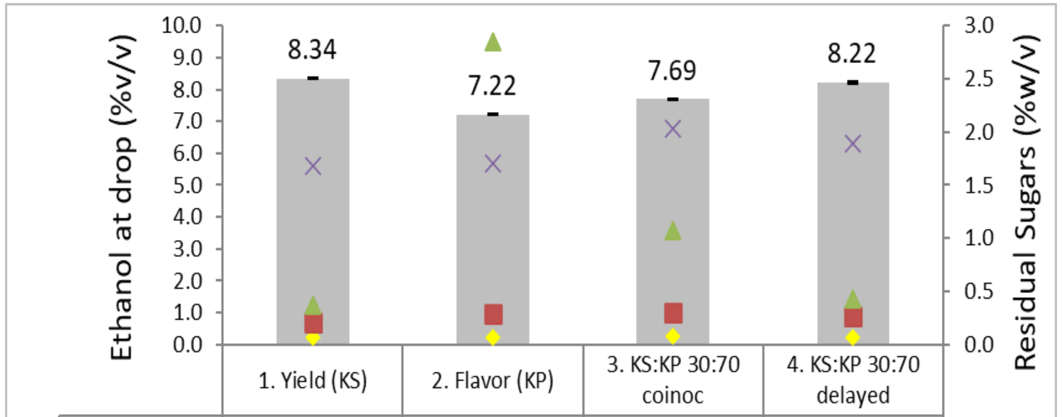
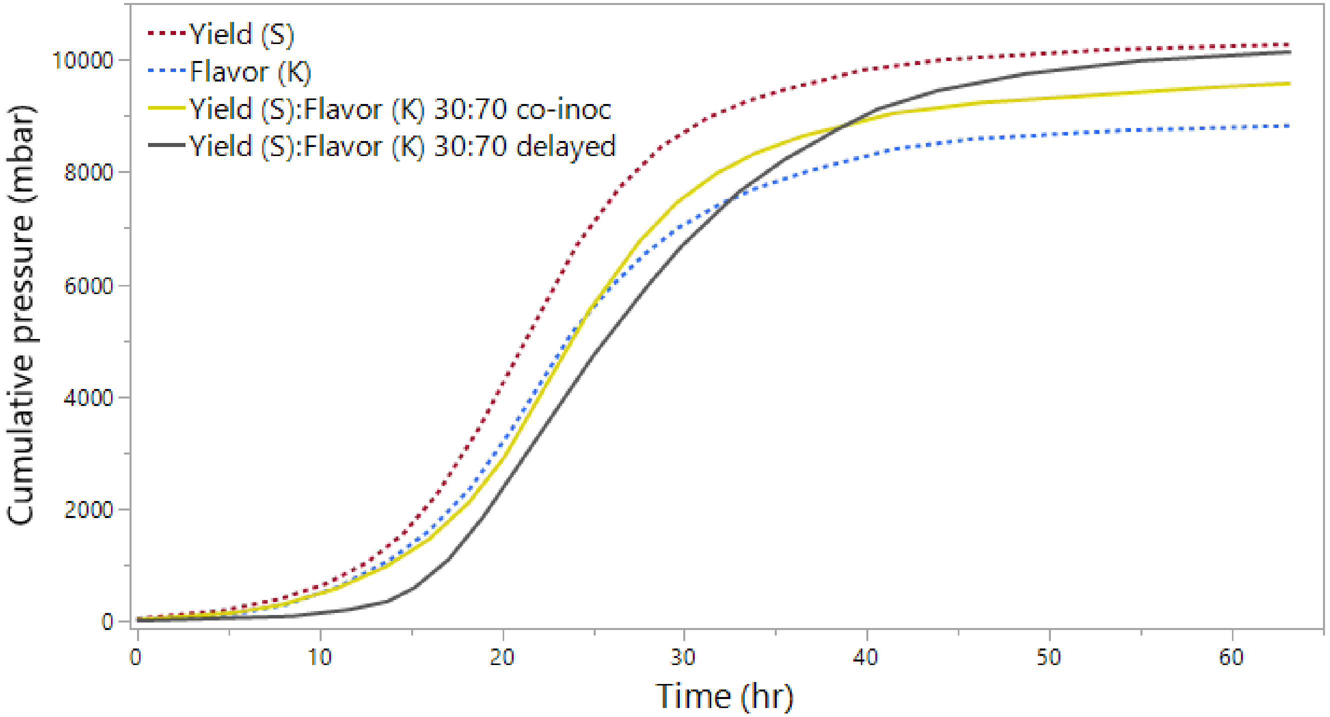
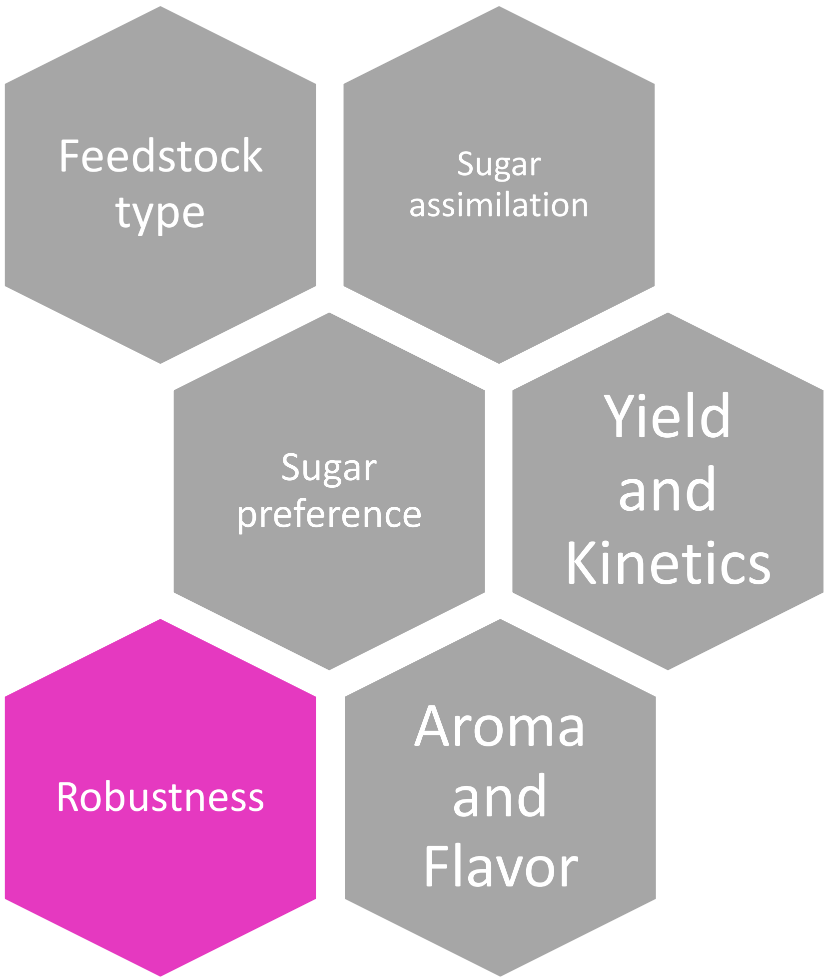
Co-inoculation



	1. Yield (KS)	2. Flavor (KP)	3. KS:KP 30:70 coinoc	4. KS:KP 30:70 delayed
Ethanol(%v/v)	8.34	7.22	7.69	8.22
Glucose	0.07	0.06	0.07	0.07
Maltose	0.21	0.29	0.30	0.26
DP3	0.37	2.85	1.07	0.43
DP4	1.68	1.70	2.04	1.89



Yeast Stress Tolerance



What are the stresses related to the specific process?

- High temperature
- High gravity / high ethanol
- Osmotic stress
- Bacterial contamination
- Low nutrient levels

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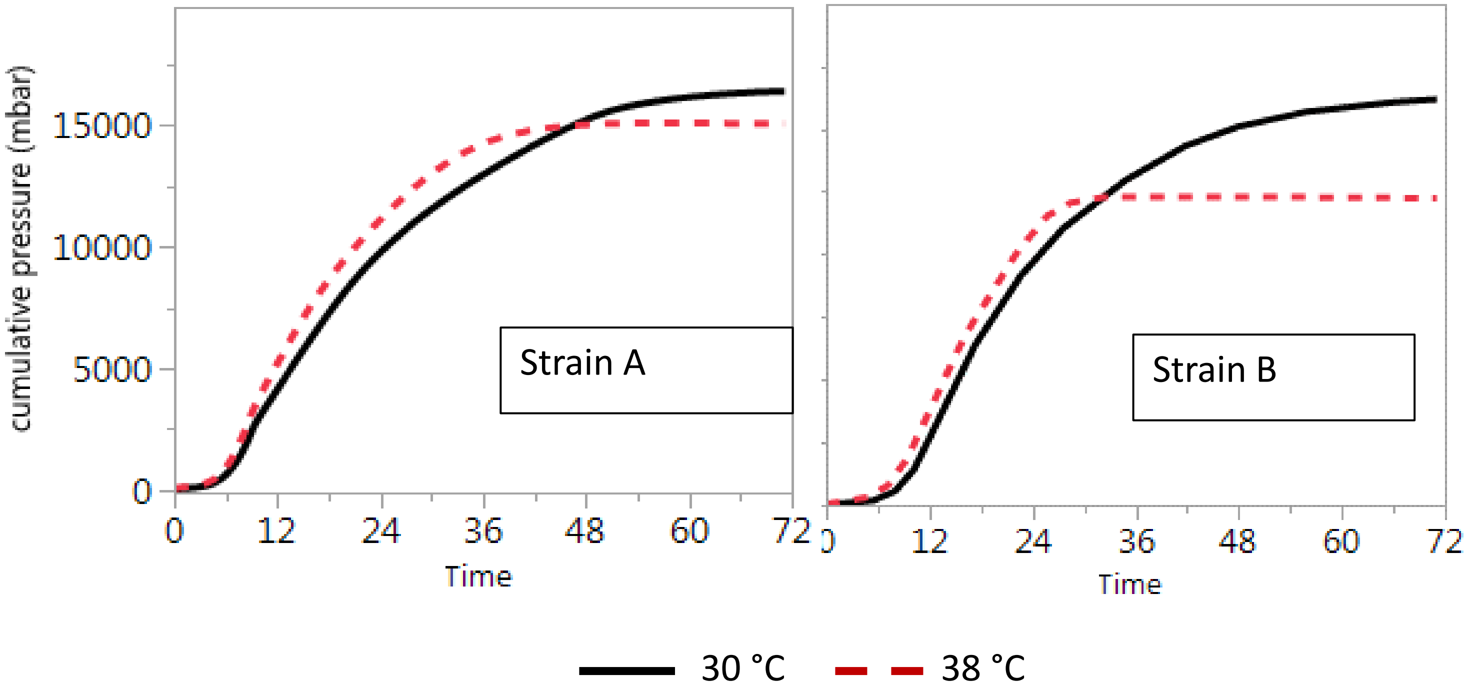
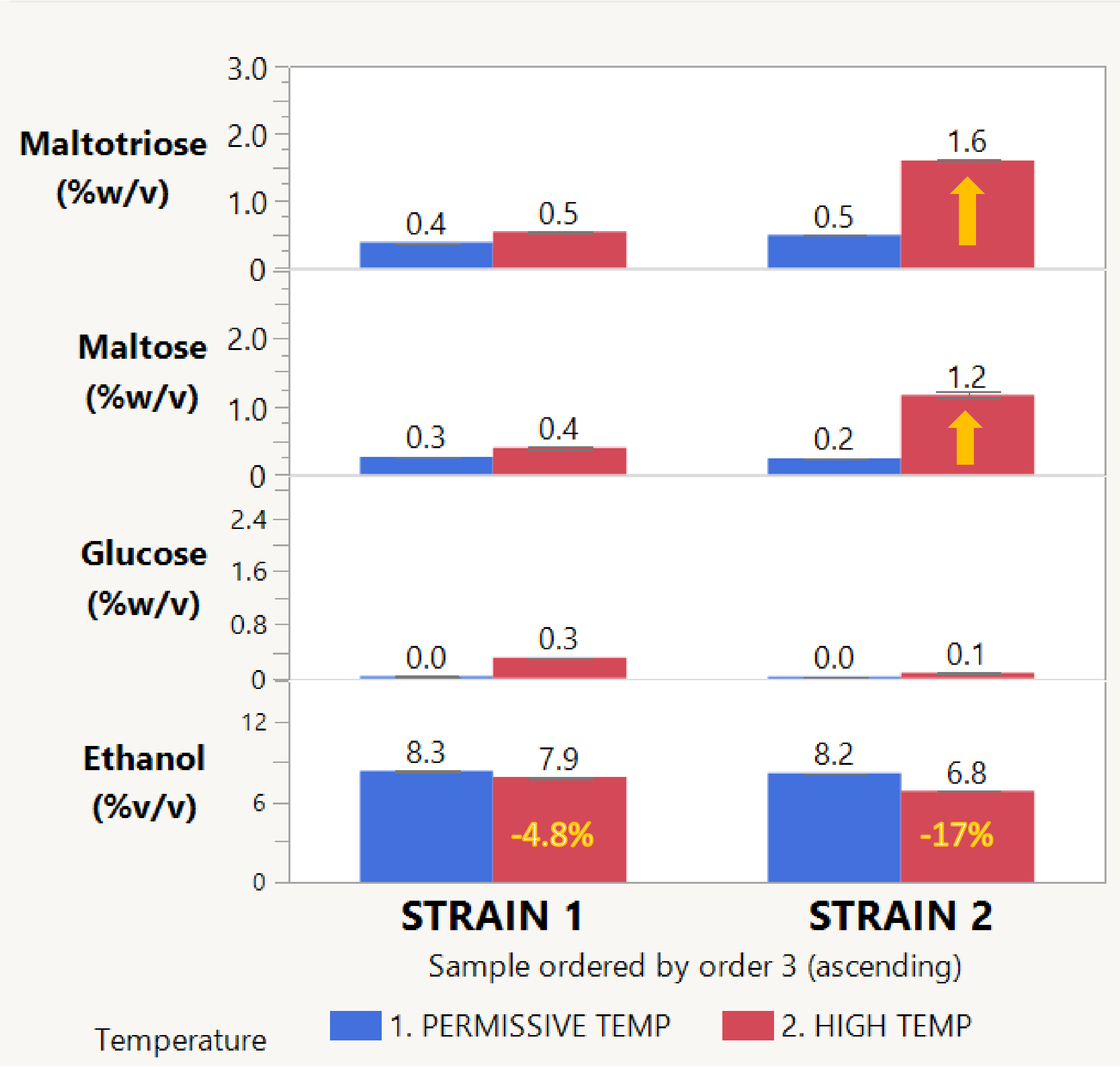
Temperature Stress: Effect on Yield and Kinetics



- Strain B is more affected by high temperature stress than strain A

Temperature Stress: Effect on Sugar Assimilation



- Temperature stress often results in reduced capacity to assimilate least preferred sugars
- The effect of temperature stress is different on different strains

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Fermentation Temperature and Gravity

Alcoholic fermentation is an exothermic reaction

$$C_6H_{12}O_6 \xrightarrow[\Delta H \approx 1217\ \mathrm{kJ/kg}]{\Delta} 2C_2H_5OH + 2CO_2$$

- The overall reaction is driven by the formation of gaseous carbon dioxide, which gives a positive entropy change
- Most commercial distilling strains of *S. cerevisiae* can ferment up to 35°C without significant issues
- High-gravity fermentations allow to reduce water demand during mashing and distillation (condensers) and to increase overall distillery throughput
- Increasing gravity increases temperature generation during fermentation
- Higher temperature combined and high ethanol are cumulative stresses

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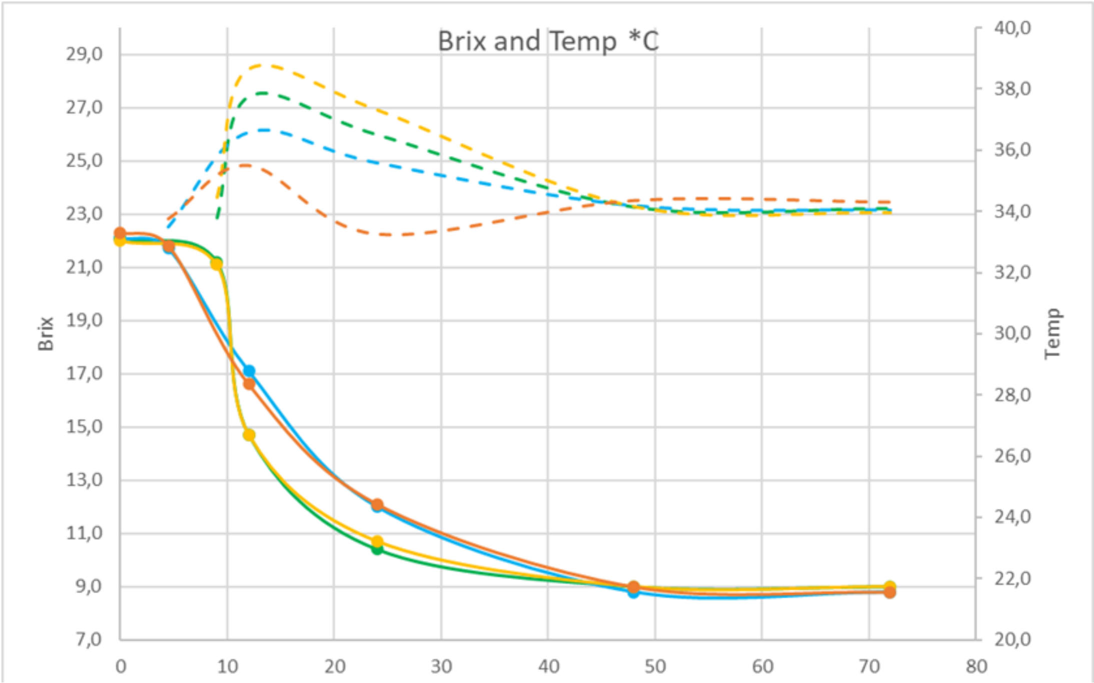
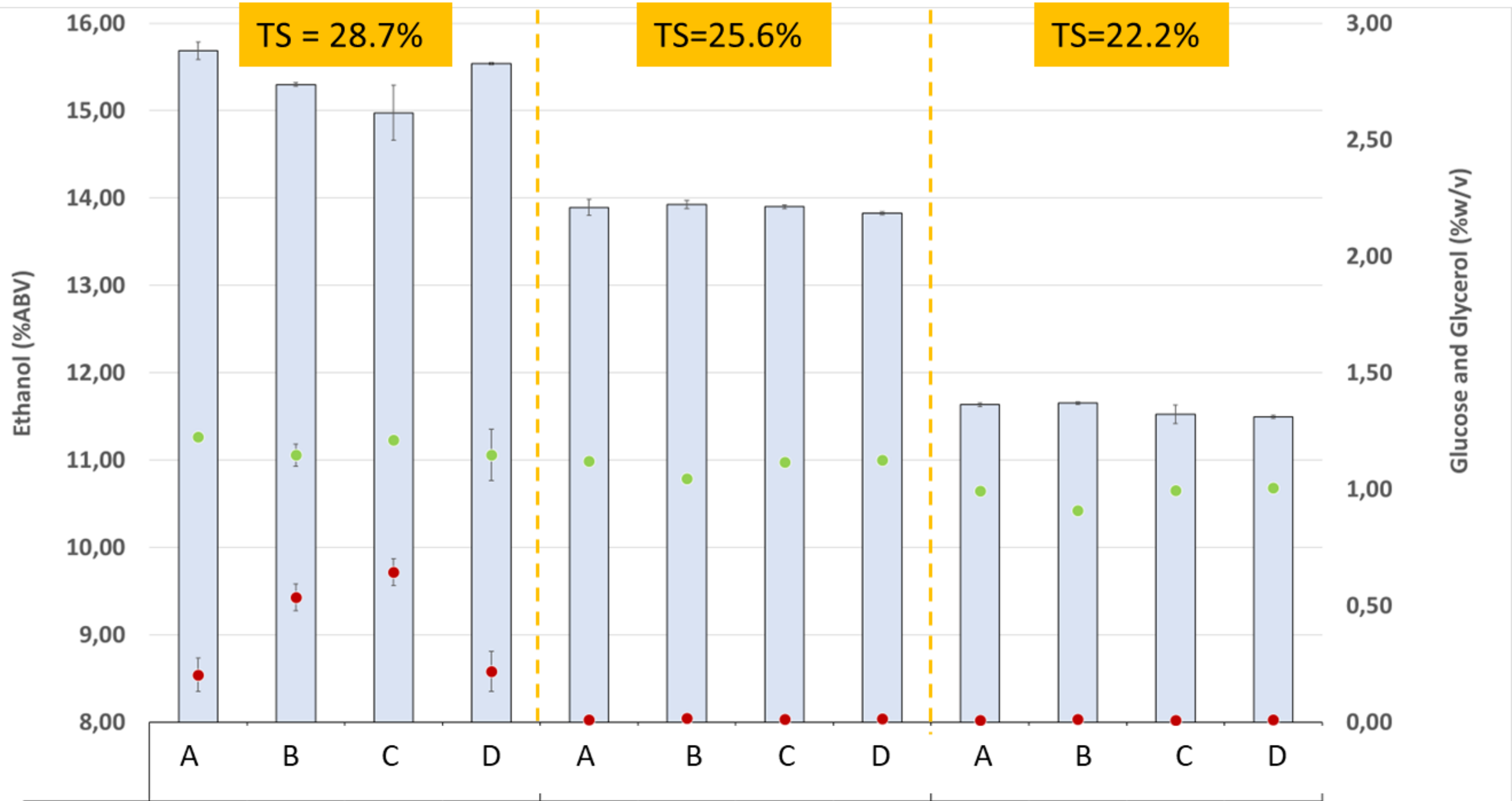
Industrial Fermentations: Temperatures Spikes



- Yeast pitched into fermentation at an optimal temperature
- Initial temperature spike due to fermentative activity, then the temperature is brought back to 34°C

2 strains, 4 different fermentations
Dotted lines: temperature profile
Solid lines: °Brix

Fermentation Completion:
Combined effect of High Temperature and High Ethanol



	A	B	C	D	A	B	C	D	A	B	C	D
Ethanol	15,69	15,30	14,98	15,54	13,89	13,93	13,90	13,83	11,64	11,65	11,53	11,49
Glucose	0,20	0,54	0,64	0,22	0,01	0,02	0,01	0,01	0,01	0,01	0,01	0,01
Glycerol	1,23	1,15	1,21	1,15	1,12	1,05	1,12	1,13	0,99	0,91	0,99	1,01

Strain screening at different gravities under temperature spike

- 4 strains tested in 3 corn mash SSF at different %TS
- Initial temp spike (40°C-34°C)
- Drop time: 54 hr

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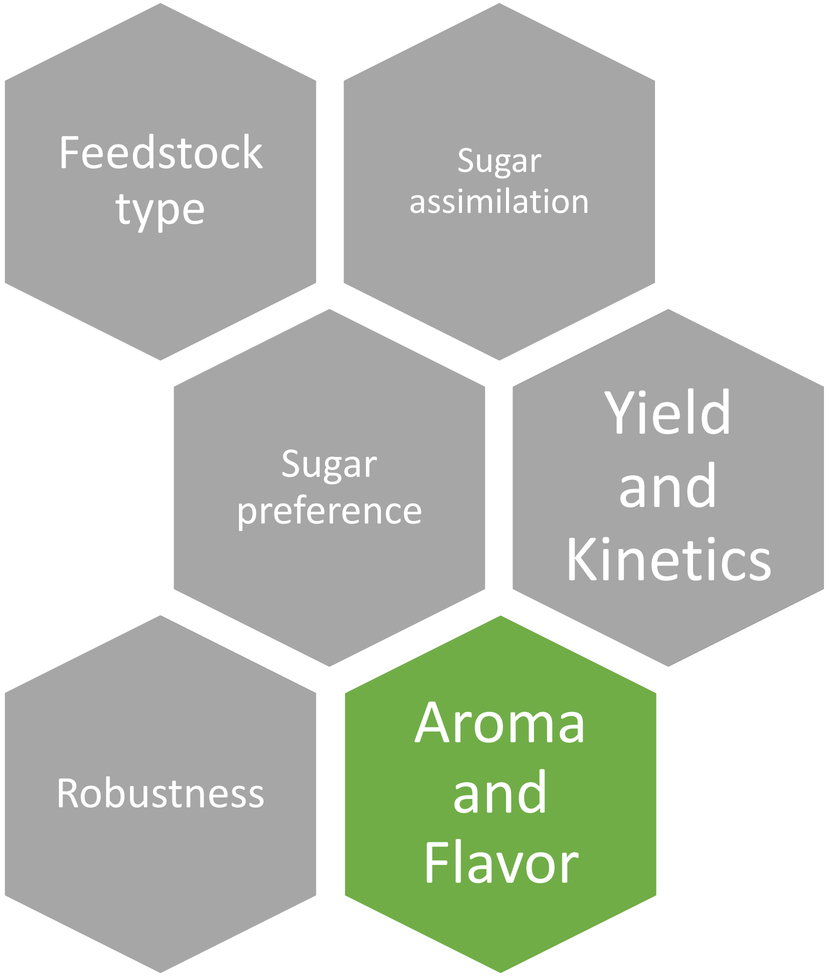
Aroma and Flavour



- **Consistency**: Reproducible congener profile under the same conditions
- **Variability**:
  - Different strains: Different aromatic profiles
  - Different conditions: Different effect on congener production

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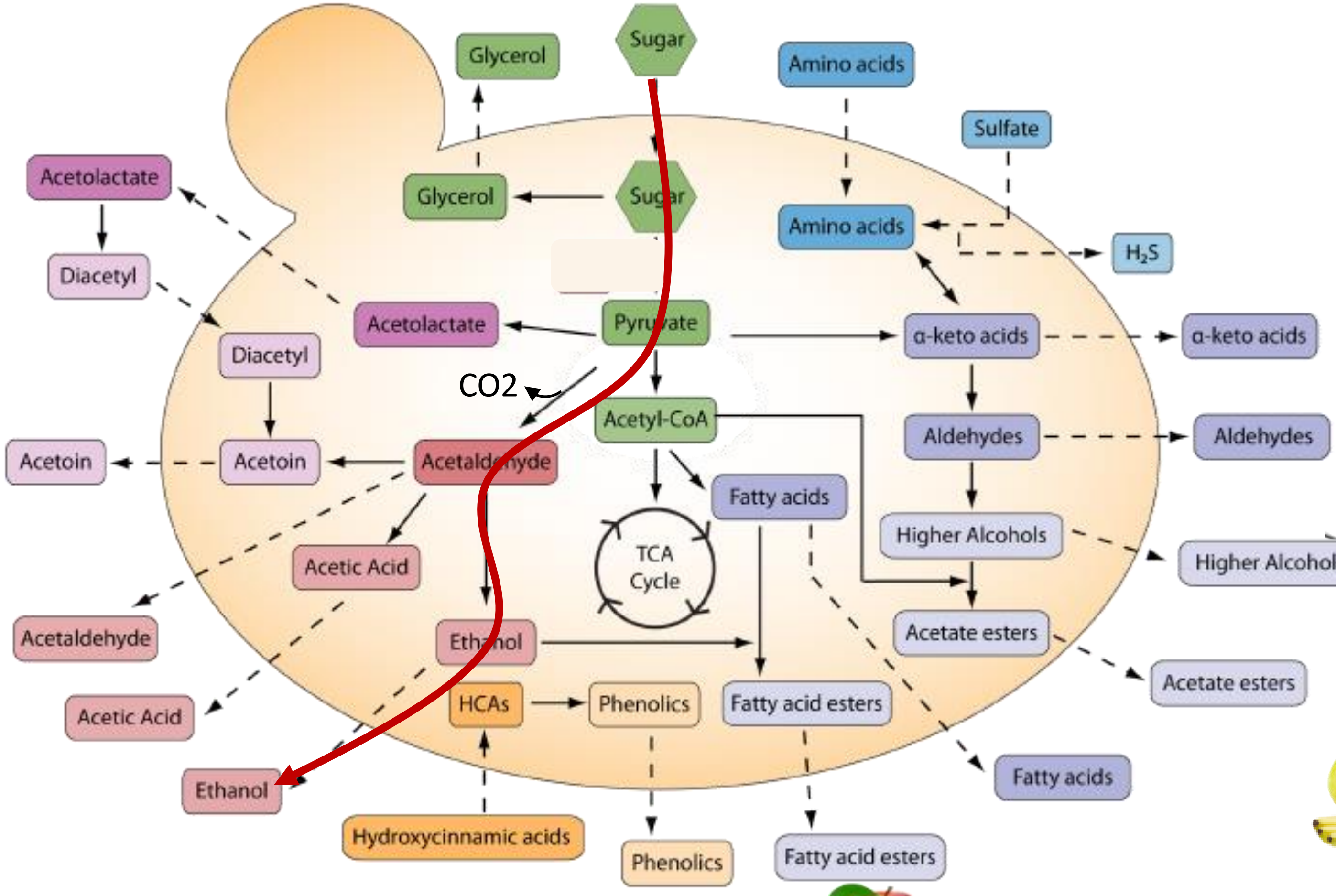
Spirit Quality: Aroma and Flavour



Dzialo M.C. *et al.* 2017. FEMS Microbiology Reviews

- Fermentation is key for the synthesis of flavor and aroma compounds and precursors
- Yeast genetics affect enzyme activity and therefore congener production

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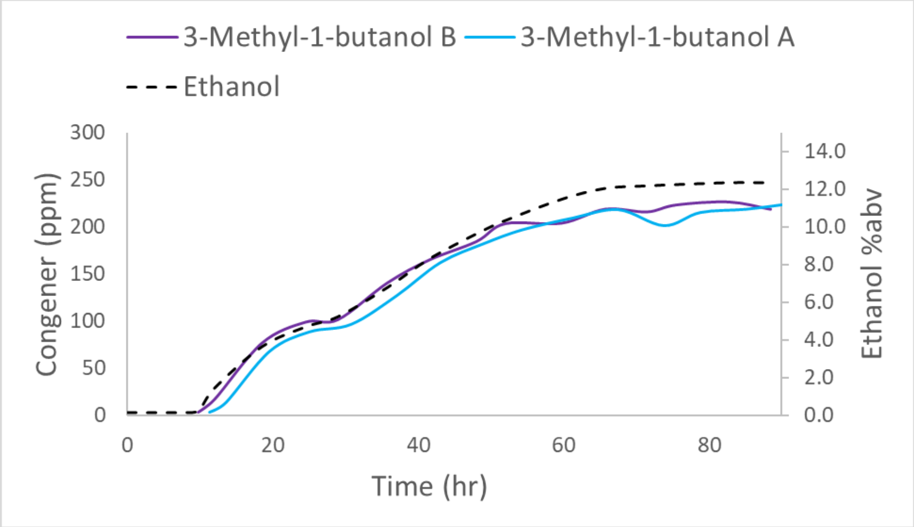
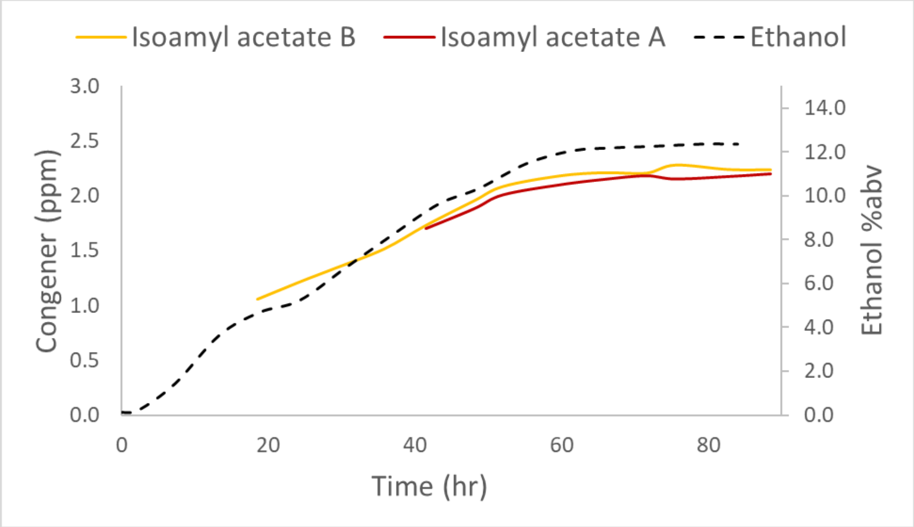
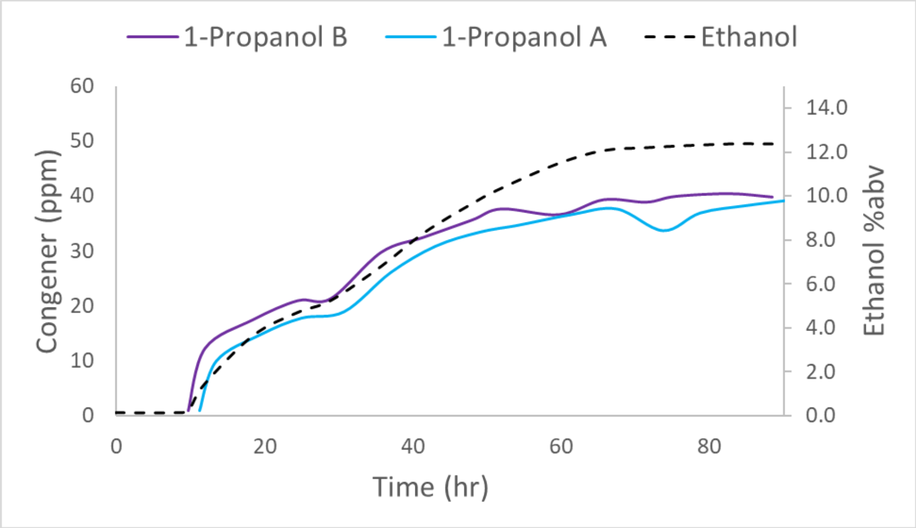
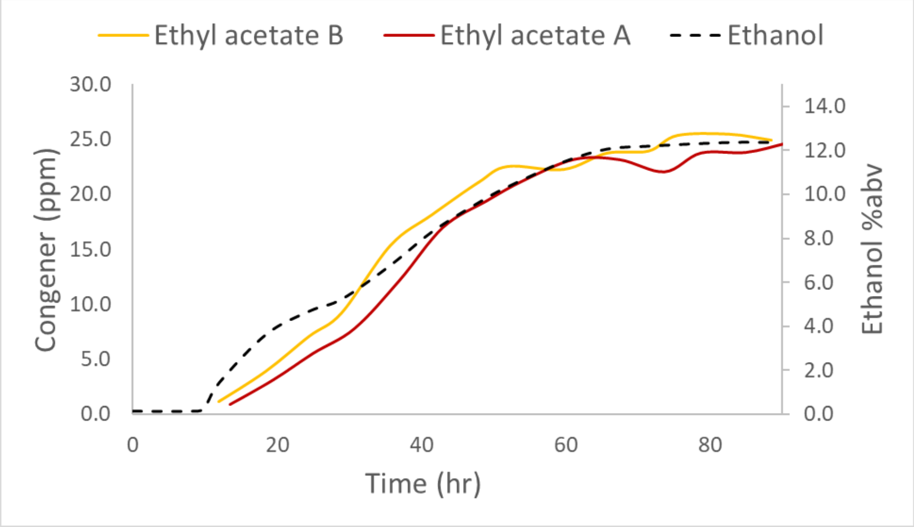
When are Congeners Produced During Fermentation?









- Distillery Study
  - ✓ Bourbon Whiskey fermentation
  - ✓ 2 sequential trials
- Both higher alcohols and acetate ester synthesis follows ethanol production
- Controlled fermentation is necessary to obtain a consistent congener profile

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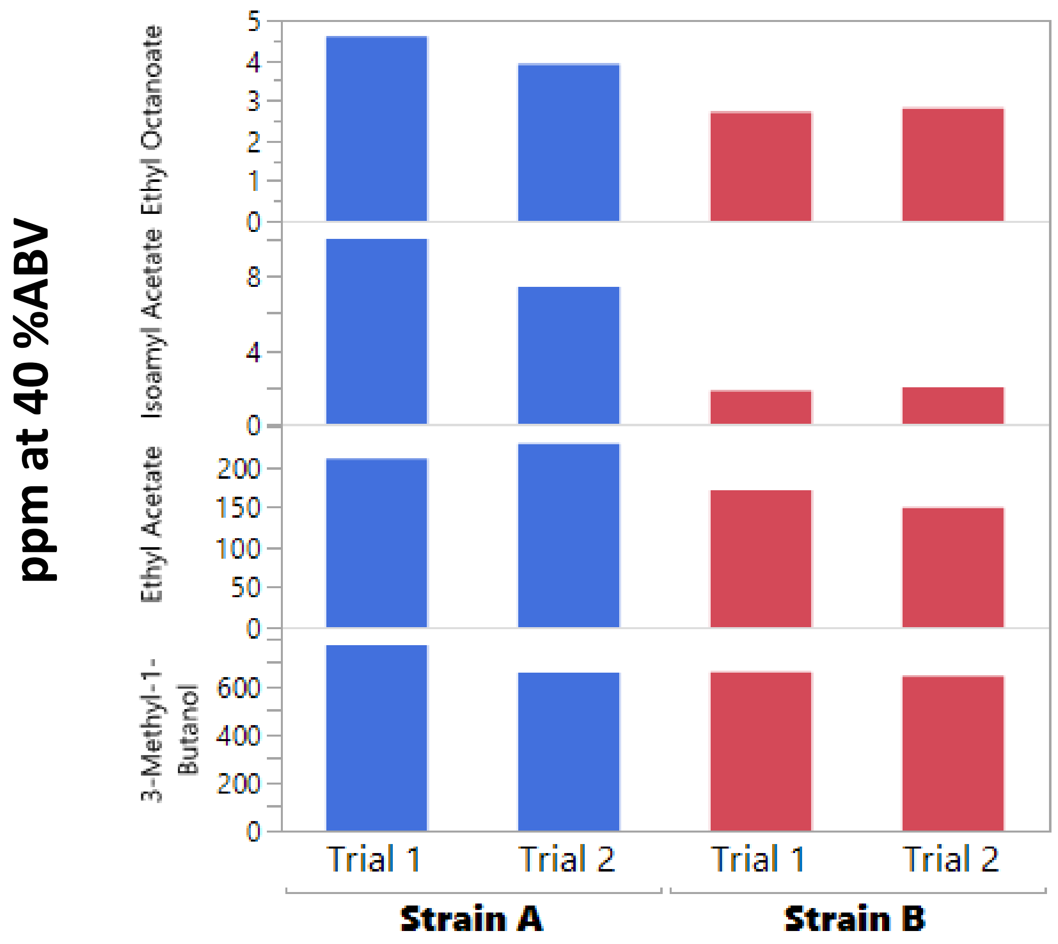
Consistency: Similar Results in the Same Conditions

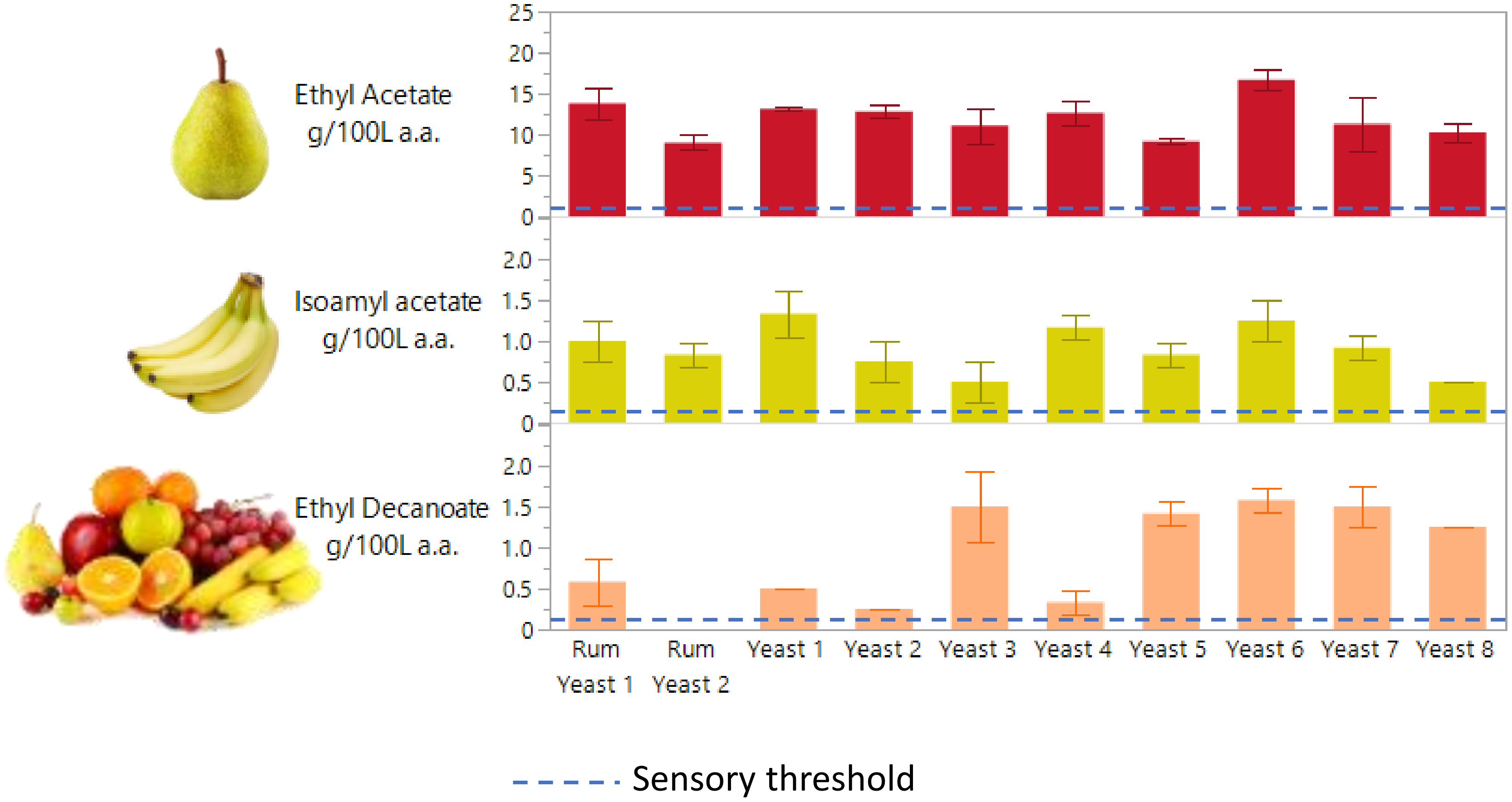


- Low wine congener profile of Strain A and B tested under the same fermentation conditions
- When conditions are consistent, a same strain gives a reproducible congener profile
- Controlled fermentation is necessary to obtain a consistent congener profiles

Flavour Diversity: Same Process Conditions, Different Yeast Strains

Blackstrap cane molasses fermentations with different yeast strains

Low wine distillates (no cuts)



- Different strains tested under the same conditions give different congener profiles
- Why? **Yeast genetic diversity**

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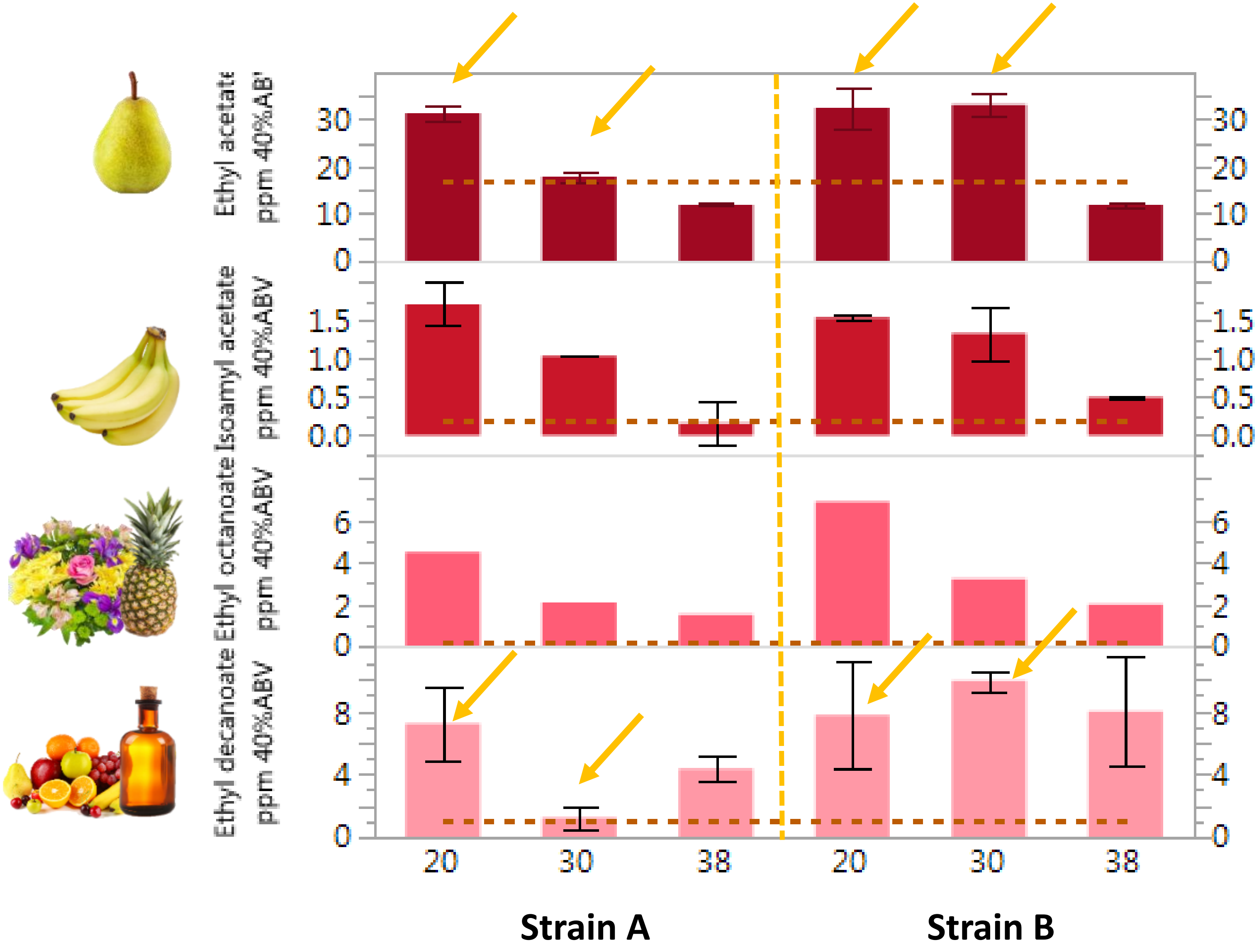
Variability: Effect of Process Conditions on Two Different Yeast Strains



- Low wines congeners are shown
- Strain B is much less affected by temperature than Strain A at 20°C and 30°C
- Process temperature should be considered when choosing a yeast strain
- At high temperature, ester production is generally lower

- - - - Sensory threshold

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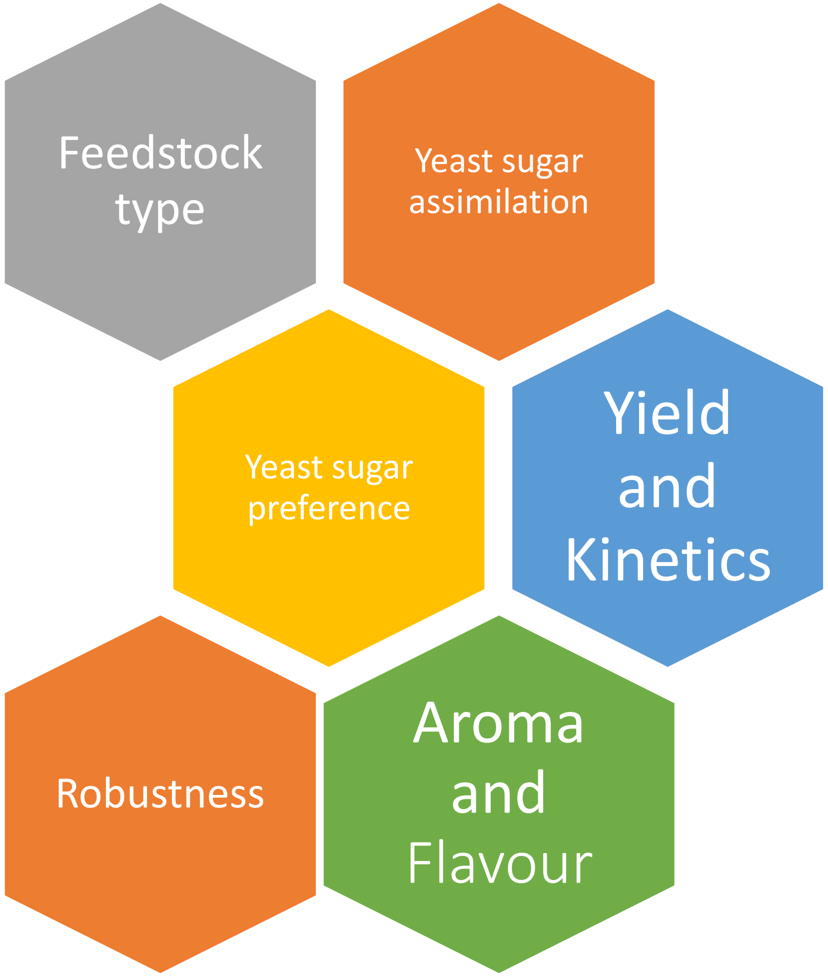
Conclusions



Selecting an effective yeast strain for your feestock will impact

- Profitability
- Spirit profile and quality

A DEDICATED STRAIN TO

A DEDICATED FEEDSTOCK TO

A DEDICATED FINAL PRODUCT

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Thanks for Your Attention!
Any Questions?

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