

OVERVIEW

- 1. Brewing NABLAB
- 2. A Better Approach with Fermentation
- 3. LalBrew LoNa™ Intro
- 4. Comparison with other Maltose Neg Strains
- 5. Trial Data
- 6. Key Fermentation Points & Summary







OVERVIEW OF LOW/NON ALCOHOLIC BEER PRODUCTION

Non Alcoholic Beer:

Defined as beer that is <0.05 - 0.5% ABV (dependent on your region)

Low Alcoholic Beer

- Defined as 0.5-1.5% ABV
- Non-alcoholic beers can be made in different ways



COMPARISON OF DIFFERENT NABLAB METHODS

PHYSICAL

Alcohol Removal

- 1. Thermal Processes
 - 1. Vacuum rectification plant
 - 2. Thin film evaporators
- 2. Membrane Processes
 - 1. Dialysis
 - 2. Reverse osmosis

BIOLOGICAL

Restricted Ethanol Formation

- 1. Alternative Mashing Process
- 2. Arrested/Limited Fermentation
- 3. Special Yeast
- 4. Continuous Fermentation



CHALLENGES — PHYSICAL METHODS

- 1. Costly equipment
- 2. Significant process optimization
- 3. Flavor loss by heating
- 4. Energy intensive
- 5. Thermal damage
- 6. Other licensing may be required



CHALLENGES — BIOLOGICAL METHODS

Other maltose negative strains

- Mostly Non-Sacch
- Almost exclusively wild yeast or food spoilage organism
- POF+
- Requires pasteurization

Maltotriose negative strains

• Requires very high mash temperatures

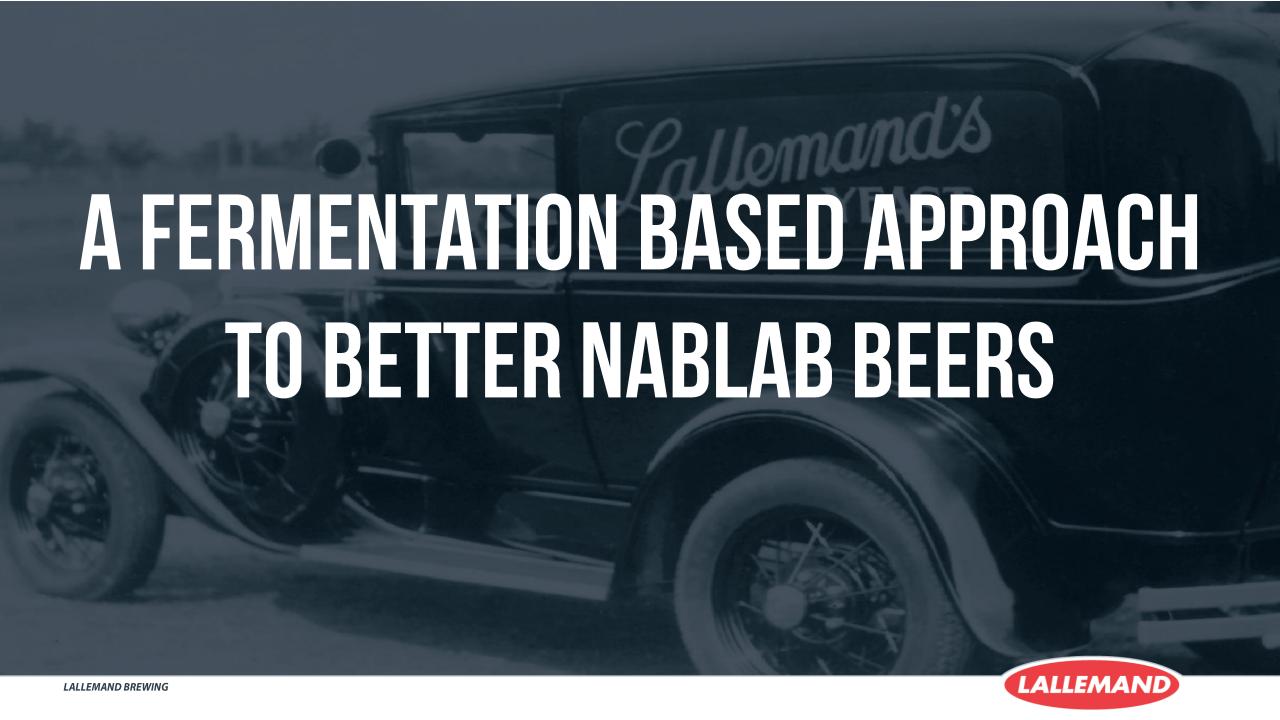
Arrested fermentation

- Requires close monitoring
- Inconsistent
- Worty flavors
- Risk of overattenuation

Cold Contact

Worty flavors





THE CHALLENGE: HOW TO MAKE A BETTER NALAB

Desired Traits:

- Fermentation solution (no ethanol removal equipment required)
- Cleaner profile
- No POF
- Reduced worty flavors
- Consistent performance
- Simple process
- Versatility for different styles



LOW OR NON ALCOHOLIC BEER PRODUCTION PROCEDURE

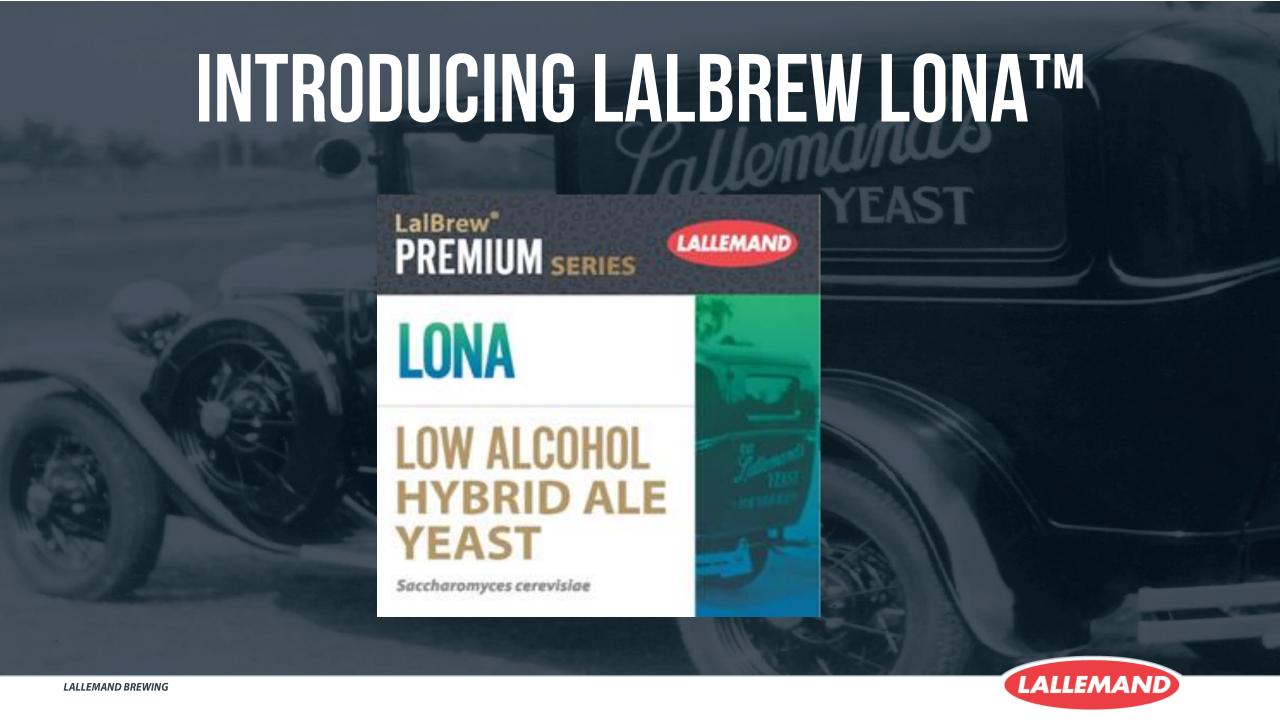
- 1. Mash a well modified Ale malt at a high mash temperature to limit glucose and maltose production
 - 1. A very high temperature mash can lead to a very hazy wort and processing problems
- 2. Target a low OG between 1.020 1.027 (5.1-6.8°P)
- 3. Lauter as normal but ensure pH and gravity remain within normal brewing levels. (5.1-5.4) Acid additions may be necessary.
- 4. Boil as normal and cool as normal, monitoring the pH
- 5. The pH should be adjusted to 4.6 prior to fermentation.



LOW OR NON ALCOHOLIC BEER PRODUCTION

- 6. Pitch LoNa™ hybrid maltose negative yeast
- 7. Final pH should be less than 4.4 and ideally between 3.9-4.1
- 8. Pasteurize to ensure product stability and safety





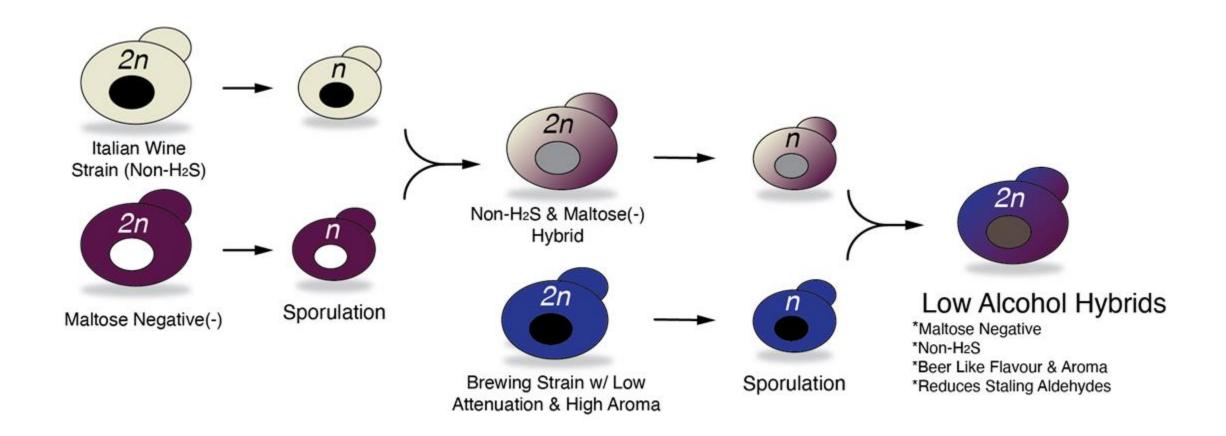




- Advanced non-GMO breeding methods were used to select a strain that does not consume maltose or maltotriose resulting in very low attenuation.
- LoNa™ is the first hybrid *Saccharomyces cerevisiae* strain that does not ferment maltose or maltotriose
- LoNa[™] does not produce H2S and uses a large proportion of staling aldehydes resulting in a low or non-alcoholic beer that tastes more like beer



BREEDING CHART — HOW IT'S MADE



All parents are Saccharomyces cerevisiae



FERMENTATION CHARACTERISTICS

Based on an 8°P wort



Pitch Rate: 50-100g/hl

Fermentation Temperature: 20-25°C (68-77°F)

Attenuation: 16-20% (lower with high mashing conditions)

Flocculation: Medium

Fermentation Completed in 3-4 days

Repitchability: Not repitchable

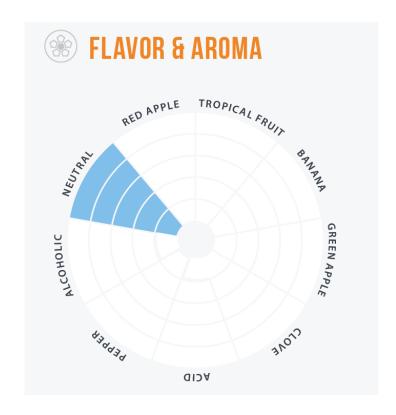


FLAVOUR CHARACTERISTICS



- Has a clean and neutral aroma
- Does not produce H2S and is POF-
- Reduction in aldehydes lends for reduced wort character, similar to a traditional beer fermentation

<u>Technical Data Sheet</u> (English)





NOTES ON USAGE - PASTEURIZATION REQUIRED

- It is recommended to adjust wort pH to ≤4.6 to limit growth of pathogenic bacteria.
- Repitching is not recommended
- Pasteurization is required when brewing with LalBrew[®] LoNa[™] to avoid refermentation after packaging and ensure beer stability



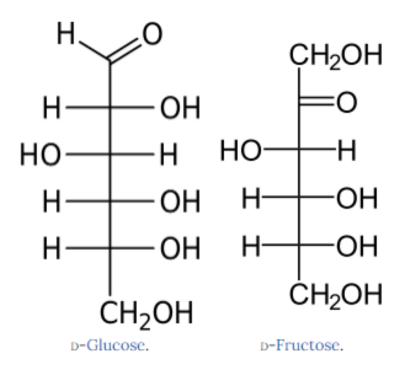
COMPARISON OF BEERS PRODUCED WITH MALTOSE NEGATIVE STRAINS

HPLC Data: Esters, Higher Alcohols, Aldehydes

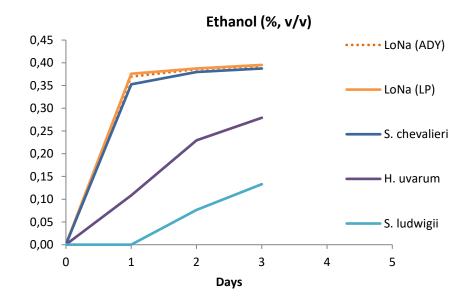


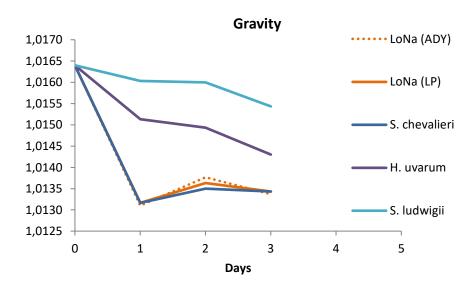
TYPICAL WORT PROFILE

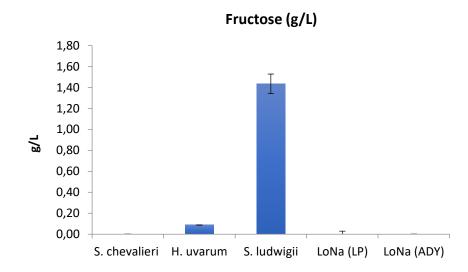
- Typical fermentable profile of wort:
 - 14% w/v monosaccharides (glucose, fructose, other hexoses)
 - 5% w/v sucrose (DP2)
 - 47% w/v maltose (DP2)
 - 14% w/v maltotriose (DP3)
 - 24% w/v "dextrins" (DP4+)
- DP = degree polymerization

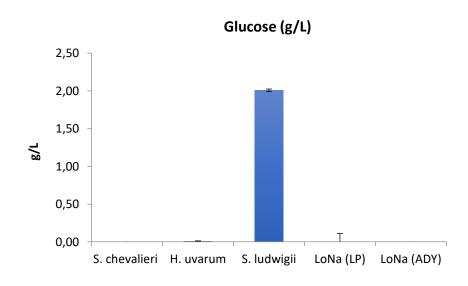




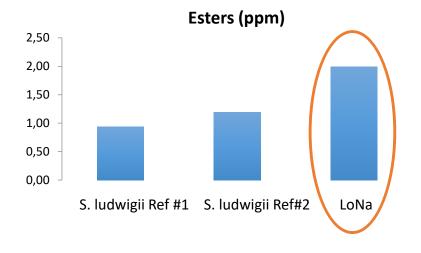


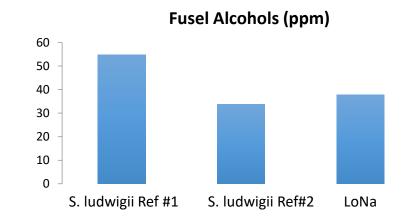


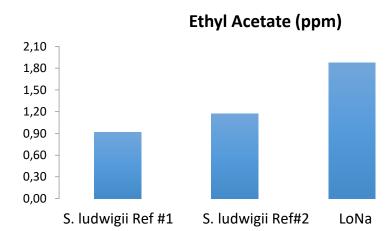


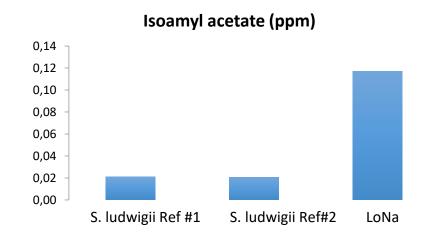


ESTERS AND FUSEL ALCOHOLS









• LoNaTM produces
a higher
proportion of
"beer-like" esters
and aromas
compared to the
reference
Saccharomycodes
ludwigii



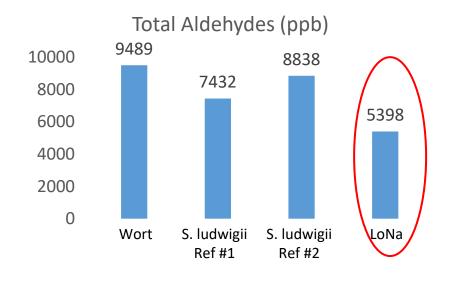
ALDEHYDES

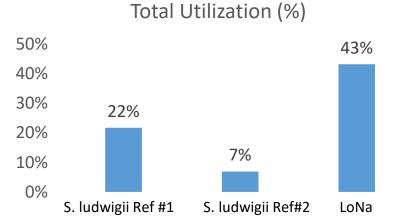
- Low and no alcohol beers suffer from flavor defects often described as sweet or worty.
- These flavors originate from flavor active aldehydes which are created in the mashing and boiling processes. The most abundant are 3-methyl butanal, 2-methylbutanal and methional.
- Ordinarily these aldehydes are reduced to their primary alcohols through the activity of yeast during fermentation, but in a limited or restricted fermentation this reduction may not happen to the same degree.
- LoNa™ exhibits good aldehyde reduction compared to other maltose negative strains



UTILIZATION OF STALING ALDEHYDES

Aged at 42°C for 3 weeks, Total Aldehydes



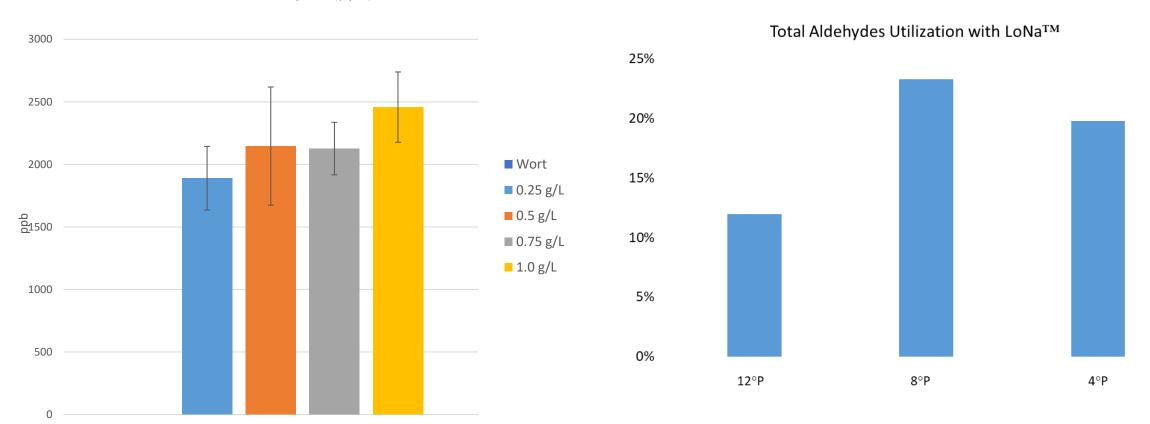


- Low alcohol beers are known to have a high level of staling aldehydes due to the limited nature of fermentation.
- LoNa™ strain utilize a higher proportion of aldehydes when compared to the reference maltose negative S. ludwigii strains.
- Low Alcohol beers fermented with LoNa™ taste fresher and cleaner than other maltose negative strains.



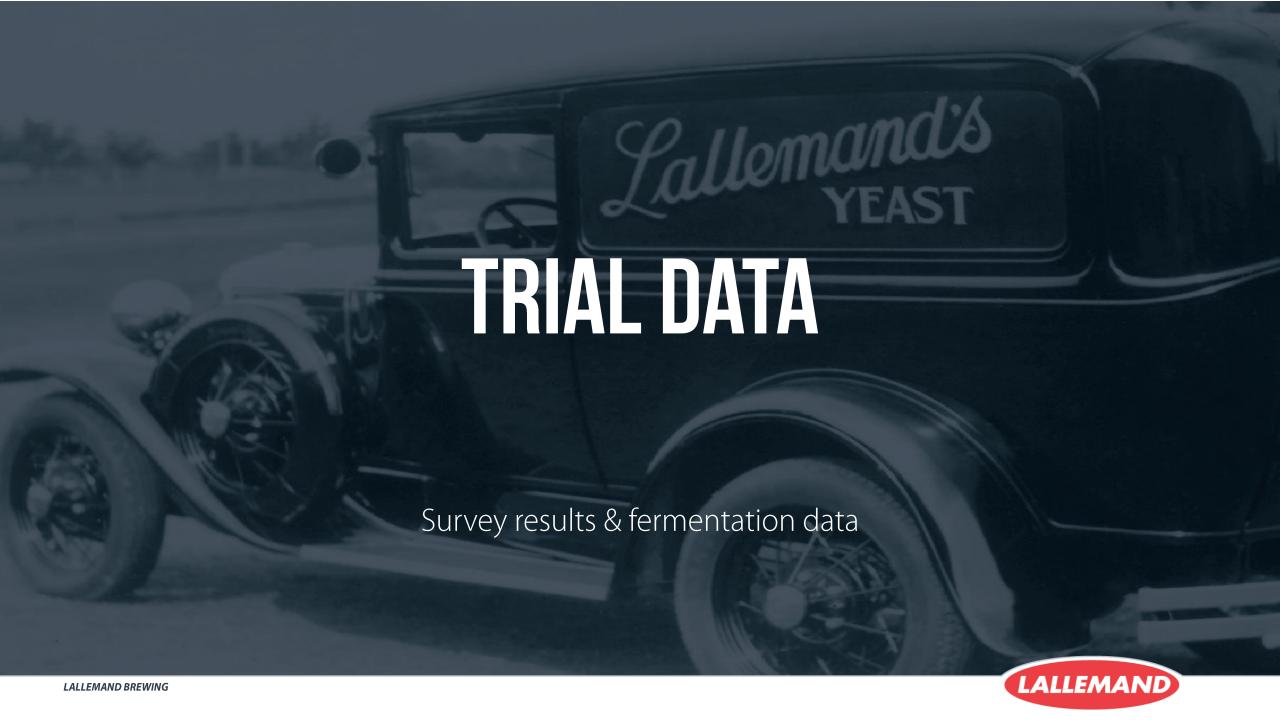
ALDEHYDES UTILIZATION COMPARISON

Total Aldehydes (ppb)



- Lower pitch rates yielded lower aldehydes
- More aldehydes were utilized at a lower original gravity





LALBREW LONATH TRIAL OVERVIEW



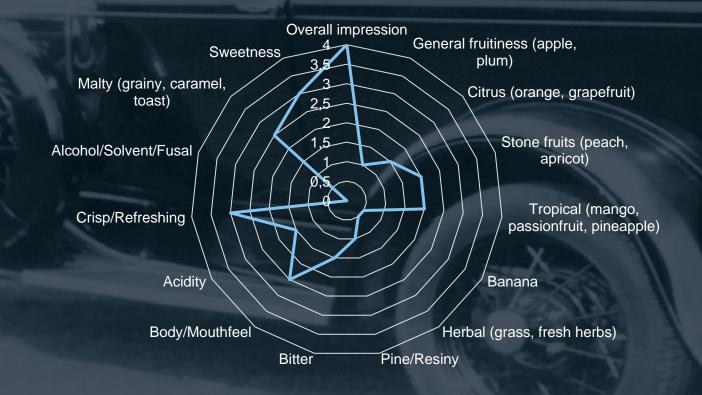
- Feedback is <u>VERY</u> positive
- Avg ABV: 0.5%
- Avg fermentation 3.2 days
- Sensory feedback: refreshing and clean with some fruitiness
 - Many tasters noted how similar to a traditional beer fermentation beers made with LoNa™ tasted
- Used for a variety of styles



AVERAGE TRIAL SPECS



the War	Apparent Attenuation	ABV	Start pH	End pH	pH drop	Fermenation Time (Days)	Flocculation
Averages	13.2	0.5	4.8	4.5	0.3	3.2	3.3 / 10





*Survey results from 7 commercial trials

MASHING

1. What was your mash temperature?

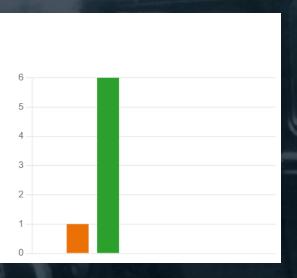
More Details

85-89'C

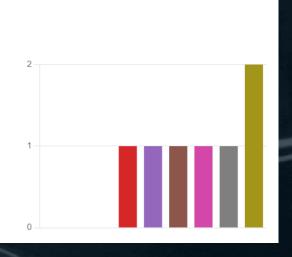


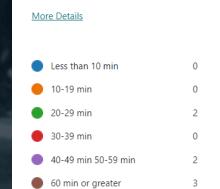


- 91-94'C 0
- 95'C or greater









3. How long was your mash?



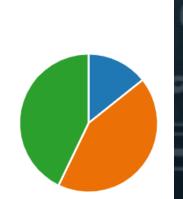


ATTENUATION AND ABV

4. What attenuation was achieved using LoNa™?



- 5% or less
- 6-10%
- 11-15%
- 16-20%
- 21% or greater







6. How did the actual ABV achieved compare to your target ABV?

5

0

More Details

- Much lower
- Slightly lower
- On target
- Slightly higher
- Much higher





FLAVOR

7. How would you describe the body/mouthfeel?

More Details

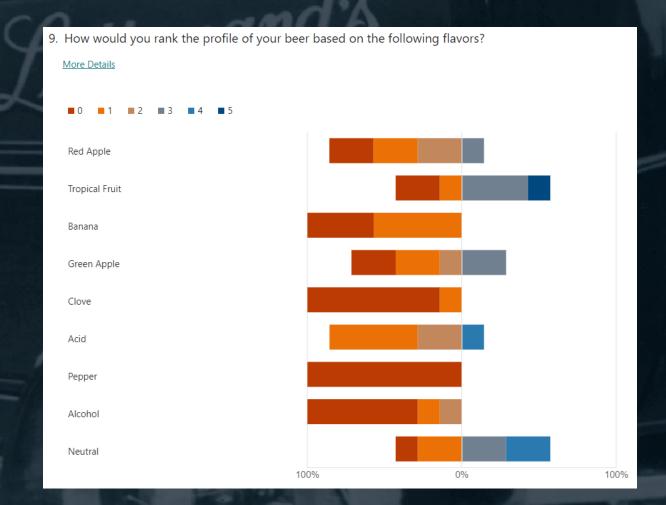
	Very low	1
	Somewhat low	3
•	Medium	3
•	Medium-High	0
	High	0



8. Do you have any additional comments about body/mouthfeel?

7 Responses

ID ↑	Name	Responses	
1	anonymous	it could get higher but its not so low bodie as we expected.	
2	anonymous	Is a light beer, no very sweet.	
3	anonymous	The sample was not carbonated so it was a bit tough to tell	
4	anonymous	Body and mouthfeel is appropriate for many of our core NA beers.	
5	anonymous	Clean and on target for what we were brewing with nothing that was an outlier.	
6	anonymous	N/A	
Higher ov	Higher overall body compared to current strain, but less than full strength beer.		



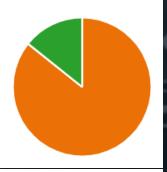


OFF-FLAVORS

13. Was there any wort-like character remaining in the finished beer?

More Details

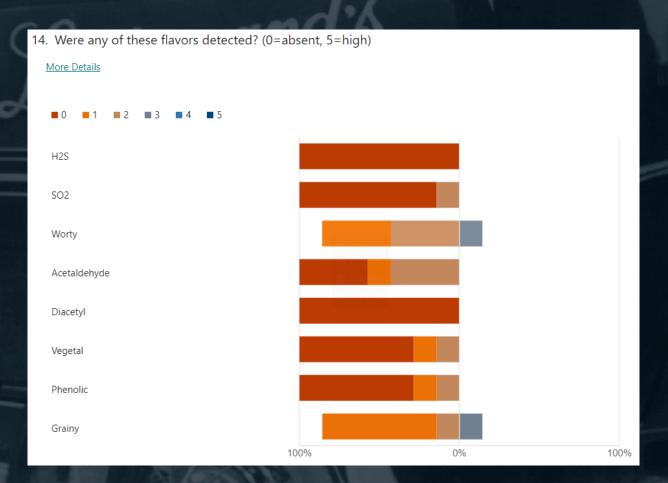
- None
- Slight wort character
- Moderate wort character
- Strong wort character 0



15. Was there anything in particular that you did not like ab LoNa™? 4?

7 Responses

ID ↑	Name	Responses
1	anonymous	Nothing particular, maybe the dosification ratio for the nutrient it depends a lot of the type of wort.
2	anonymous	No. But I would like to try with differents recipe to have more conclusion
3	anonymous	Not at this point!
4	anonymous	Cleaner and does not mask flavors as much as our current strain.
5	anonymous	Nothing.
6	anonymous	N/A





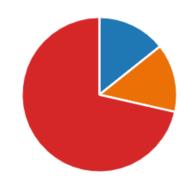
*Survey results from 7 commercial trials

CLARIFICATION

10. What method did you use to clarify the beer?

More Details

- Finings
- Centrifuge 1
- Filtration
- No clarification



11. Were you satisfied with the clarity of the finished product?

More Details

- Yes
- No





COMPETITOR COMPARISON

16. Have you used other maltose negative strains for non-alcoholic or low-alcohol applications?

More Details

Yes



17. How does LoNa™ compare to other maltose negative strains you have used?

6 Responses

$ID \uparrow$	Name	Responses
1	anonymous	it's better because of the fruity flavours and the absence of off-flavors
2	anonymous	It's by far the winner in terms of ease of use and quality of the finished product.
3	anonymous	Much cleaner flavour/aroma and a significantly faster fermentation at comparable pitch temperatures (18-20C). LoNa™ was 24 hrs faster than current strain.
4	anonymous	Far and above exceeds other commercial strains.
5	anonymous	N/A
6	anonymous	Same performance (ferm time and attenuation), much cleaner, would need flavour assistance to bring some character back, even if the desired outcome is a clean NA lager



PASTEURIZATION

18. Please describe briefly your method for pasteurization (including pasteurization units achieved).

7 Responses

$ID \uparrow$	Name	Responses
1	anonymous	we pasteurize at an industrial paster achieving 55 UP (60 min at 60°C more or less).
2	anonymous	We send the bottle to Factory and the paster bottle to 55-60UP (1hour - 60°C)
3	anonymous	No pasteurization - testing preservatives instead.
4	anonymous	We used a tunnel pasteuriser and applied about 60-80 PUs on the packaged product. The actual PU units achieved was 62 PU.
5	anonymous	Tunnel - 40pu
6	anonymous	N/A
7	anonymous	tunnel pasteurization (50-110 PU spec) on our commercial products, currently using LA-01 strain



GENERAL IMPRESSION



More Details

- Yes, for core brands
- Yes, for occasional seasonal beers 1
- Maybe, not sure
- No

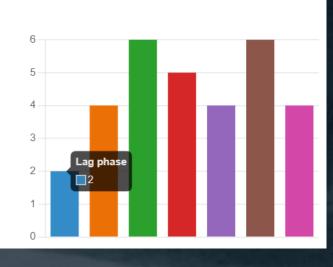


12. What did you like about LoNa™?

More Details

- Lag phase
- Fermentation speed 4
- Flavor profile 6

 Absence of off flavors 5
- Dry yeast format
- Ease of use 6
- Species (S. cerevisiae and not "w... 4



- 20. Do you have any other general comments about this strain?
- **7** Responses

$ID \uparrow$	Name	Responses
1	anonymous	Very useful yeast for low alcohol beers, the profile its not as citrics as others.
2	anonymous	I think is a good strain for low alcohol IPA's and hazy IPA's. For pilsen, I think is a very aromatic strain yeast.
3	anonymous	We're excited to keep working with it!
4	anonymous	Easy to use, fast, clean, and excellent quality.
5	anonymous	Nothing else
6	anonymous	N/A
7	anonymous	Clean was the most common comment on our panel of 6 tasters. Can provide individual tasting sheets upon request, scores and comments provided above are averages.



HAZY IPA

Trial details:

- Batch size: 3.9BBL
- Mash temp: 80°C
- Mash: 100% two row pale
 - Limited sparge top up kettle with liquor
 - 15BU hop charge plus 3lb/bbl Idaho 7 in WP
- OG: 5.2 plato
- Starting pH: 5.2 (adjusted with citric)
- Pitch rate: 109g/HL (very high)
- Fermentation & package:
 - Crashed to 1.6°C for 7 days, carbonated to 2.6 vol/vol and kegged for consumption on site only



HAZY IPA

Trial feedback:

- Final alcohol of 0.23% as measured by alcolyzer
- Final pH 4.62 (not adjusted)
- Attenuation of 9.61% (impact of high mash potentially)
- Very good hop character
- Flavour described as overwhelmingly positive





AMBER ALE

Trial details:

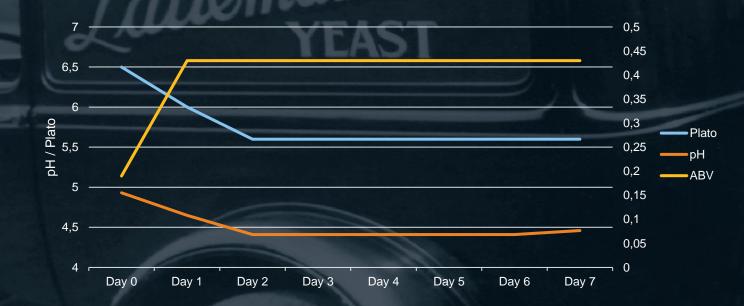
- Mash temp: 77°C
- Pitch rate: 33g/HL
- Mash:
 - Pale with small quantities of chocolate and crystal
 - Full sparge, pH maximum of pH 5.7
- OG: 6.5 plato
- Starting pH of 4.93 treated with an acid product
- Fermentation & Package:
 - Target dry hop
 - Crashed to 1 for five days, centrifuge, carbonate to 2.4vol and bottle



AMBER ALE

Trial feedback:

- Final alcohol of 0.43% as measured by alcolyzer
- Final pH 4.46 (not adjusted)
- Attenuation of 13.8%
- Flavour reasonably good although a little thin and less hop character than desired.
 They will try upping the dry hop. Suggest inactivated specialized yeast for body.





PALE ALE

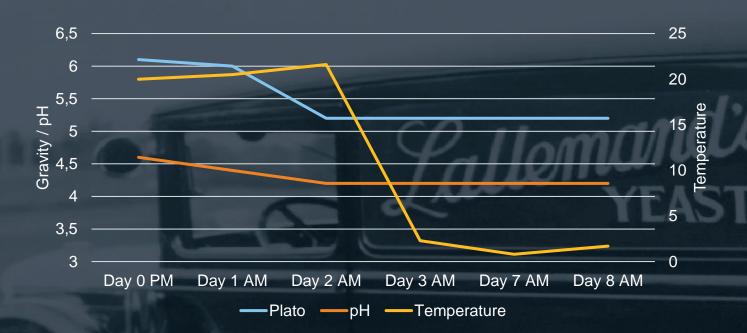
Trial Overview:

- 28HL
- Mash:
 - 64% pilsner and 36% malted oats.
- 72°C mash for 45mins before rising to 78°C for mash out
- 60 min boil with 12 IBU bittering addition
- Lactic acid was added to the boil boil to adjust the pH to 4.6
- Pitch rate: 35g/HL
- Fermentation: 20-21°C for three days

Trial results:

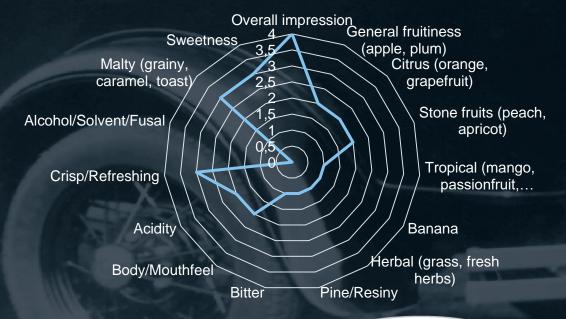
- Terminal gravity reached in 3 days
- Apparent attenuation of 14.8%
- Final pH of 4.2, drop of 0.4 point
- Flocculation rated 2/10





"No off flavours. The resulting beer didn't have the phenolic/plastic aromas that we detect with other strains. Resultantly, the cleanliness of the yeast flavour led to more worty/malty aromas in the finished product."

"Overall, we're really pleased with the results and this trial was a great opportunity and experience for us. We are looking forward to working with you in the future if there are more opportunities."





IPA & STOUT

Trial Overview

- 6 batches including five different IPAs and one Stout
- 10-13 BBL size
- All pitched at 43g/hL with a similar mash regime.
 - 60°C Steep 70°C mill (wet mill) 71°C mash
- Boil times 30mins simmer 130mins boil
- Oxygenated at 10ppm



Trial results:

- Average pH drop of 0.132
- Somewhat variable apparent attenuation with an average of 12.48.
- Average alcohol by volume of 0.42 v/v

Beer	Apparent Attenuation	ABV	Start pH	End pH	pH drop
IPA Citrus Wheat	6.7	0.4	5.11	4.6	0.51
IPA Blonde	12.5	0.4	4.65	4.4	0.25
IPA M01	19.2	0.45	4.49	4.62	-0.13
IPA M12	9.1	0.44	4.63	4.6	0.03
Stout	14.9	0.45	4.64	4.64	0

No acid malt in the citrus wheat likely contributing to the greater change in pH

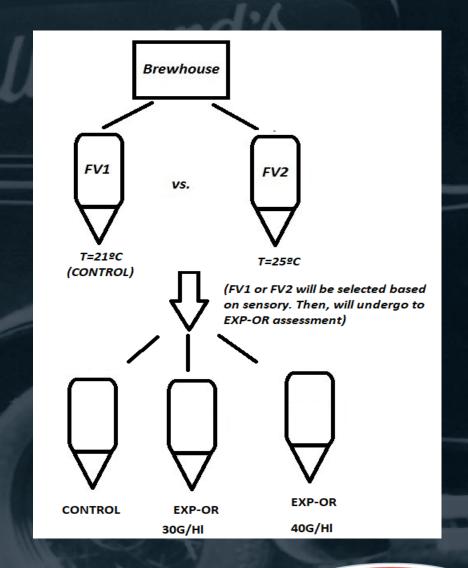


PILSNER TRIAL WITH INACTIVATED SPECIALIZED YEAST (ISY)

Trial detail:

Double trial looking both at LoNa™ and inactivated specialized yeast product.

- Mashing of pilsner malt took place at 74°C for 1 hour
- Mash pH was corrected to 5.48
- Starting gravity was 8.91 plato down from a desired 10 plato due to a leaky valve. pH was 5.28
- LoNa[™] was pitched at 50g/HL
- Initial fermentation was completed at 21°C for three days
- Post fermentation the beer was split three ways and dosed with the inactivated specialized yeast product before sensory analysis



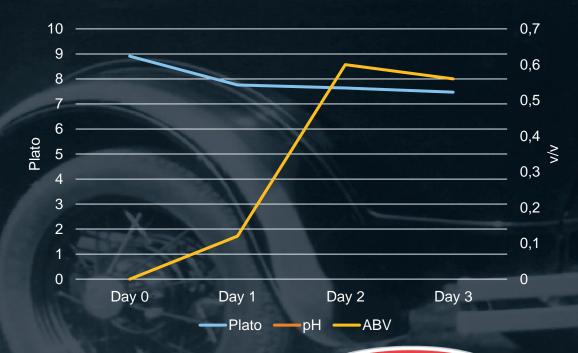


Date	Day 1	Day 2	Day 3
Original extract (ºP)	8,01	8,78	8,58
Extracto real (%w/w)	7,81	7,87	7,69
Apparent extract (%w/w)	7,76	7,64	7,47
Alcohol (%v/v)	0,12	0,6	0,58
Kcal/100ml	28,85	33,02	30,9
рН			4,84
EBC			5,34
Remarks	Measured in the micro	Measured in the micro	Measured at the factory (more reliable data)

Day 0 not included in table. Start OG of 8.91, pH 5.28

Fermentation results:

- Final gravity reached in three days at 21°C
- pH drop of 0.4 units
- No pH correction after fermentation





Volatile analysis:

Diacetilo	2-3 pentanodiona	Acetaldehido	DMS	Ethyl acetate	Isoamyl acetate	Higher alcohols
(μg/L)	(µg/L)	(mg/l)	(µg/L)	(mg/l)	(mg/l)	(mg/l)
12,9	4,9	2,2	35	0,08	0,008	24,8

All volatiles are at or below flavour threshold in regular beer.

Tasting analysis with Inactivated Specialized Yeast Product

1	2	3
No dosage	20g/hl	35g/hl
Refreshing, citrus and very light non-alcoholic beer	Refreshing non-alcoholic beer, citrus and with some softness in the mouth. Increased palatability. *Preferred dose rate	Beer without alcohol, with much more body. Slightly salty flavor.



PALE ALE

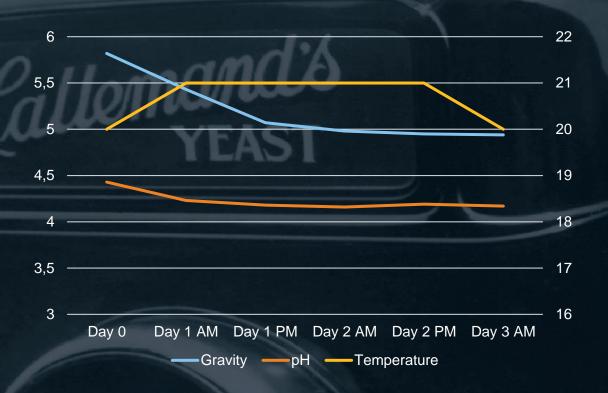
Trial Overview:

- OG: 5.9 plato
- Mash:
 - 55% ale malt, 27% Wheat and 18% dextrapils
 - 70°C mash for 20mins
- 90 min boil with 9 IBU bittering addition
- Phosphoric acid was added post boil to adjust the pH to 4.4
- Pitch rate: 50g/HL
- Fermentation: 20-12°C for three days



Trial results:

- Terminal gravity reached in three days
- Final gravity of 4.17 plato and pH of 4.17.
- pH drop of 0.23 units
- Flocculation rated 7/10
- Apparent attenuation of 15%





PALE ALE

Trial detail:

Detailed side by side trials with competitor strain but from different worts

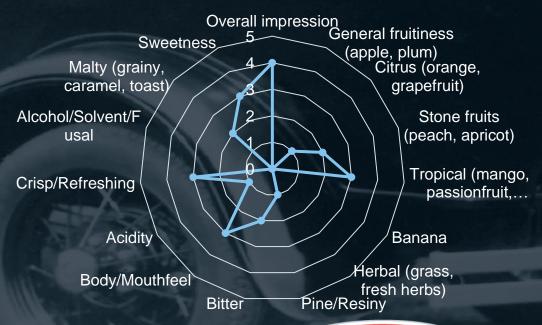
- OG: 6.5 plato
- Mash temp: 68 °C rising to 78°C
- Fermentation at 21°C
- Immediate chill to 2^{0C}



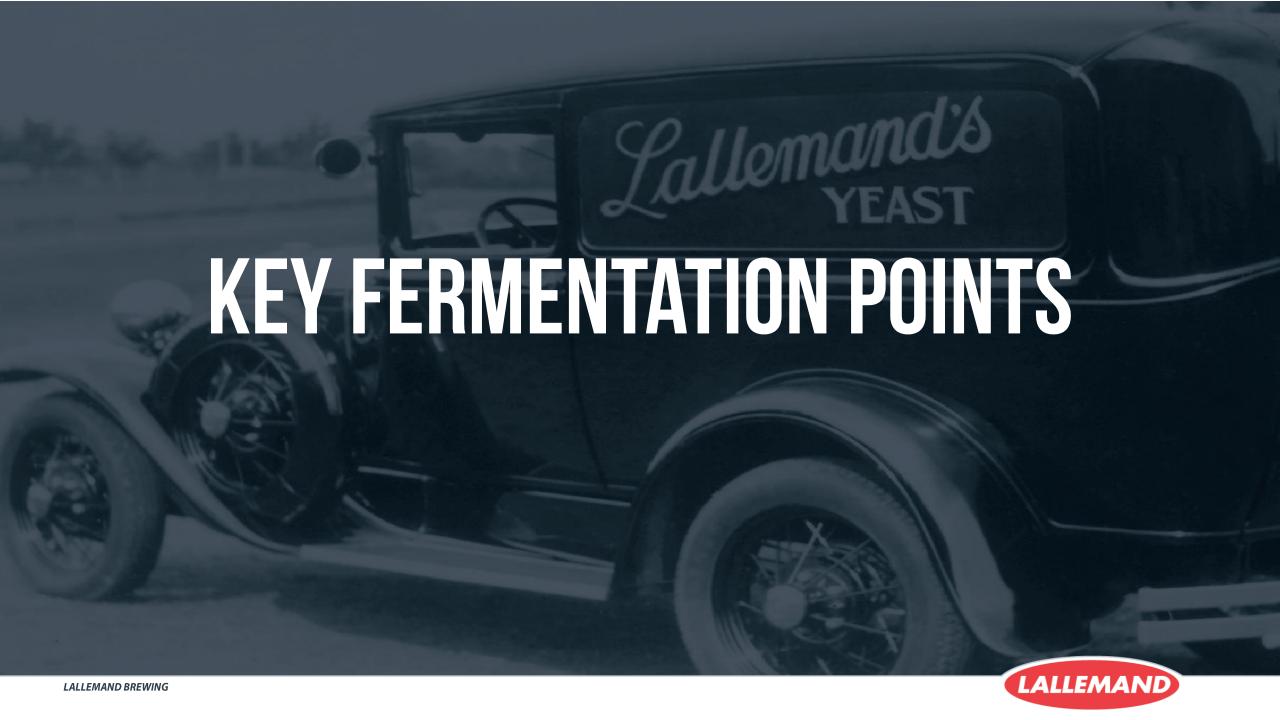
Results

- Fermentation in four days
- Apparent attenuation of 9.23%
- A slight difference in final gravity between the two strains
- So far the flavor and aroma of the Lallemand yeast seams more aromatic, cleaner, and less POF compared to current maltose –ve strain.
- Flocculation rated as 1













- **LoNa™** is a *Saccharomyces cerevisiae* yeast strain
- 1. Does not ferment maltose
- 2. Efficiently reduces wort aldehydes, resulting in a beer with reduced wort/vegetable flavours
- 3. Does not produce H2S and is POF-
- 4. Expresses a clean and neutral ale aroma
- 5. Fermentation completed in 3-4 days
- 6. Able to achieve 0.5% ABV



FOOD SAFETY

- NABLAB beers are prone to microbiological spoilage due to high pH, low ethanol content, and high sugar content
- Stabilization by pasteurization is recommended when producing these beers to prevent food pathogens
 - Chemical stabilizers may not be enough for complete stabilization
- It is recommended not to serve these beers on draft
- Master Brewers Association Food Safety Page
- Brewers Association Food Safety Resource Hub
- Brewers Association Non Alcoholic Beer Overview



FAQS

1. What acids do you suggest to use for pH adjustment?

1. Different acids have different flavor profiles, but all should be food grade when added.

2. How does LoNa™ compare with other maltose negative yeast strains?

- 1. LoNa[™] is a hybrid *Saccharomyces cerevisiae* strain
- 2. LoNa[™] is POF- (unlike other maltose negative yeast strains), so the resulting beer is cleaner tasting
- 3. Overall brewers have found beers fermented with LoNa™ to have a refreshing taste and a fruity aroma

3. If you do not ferment maltose, does the resulting beer taste sweet?

1. Maltose is relatively less sweet than other sugars (larger sugar molecules are less sweet than smaller ones)

4. Why is pasteurization required?

1. Remaining maltose is fermentable by other brewing strains, wild yeast, and bacteria. Pasteurization stabilizes the product and prevents microbial activity in the packaged product.

