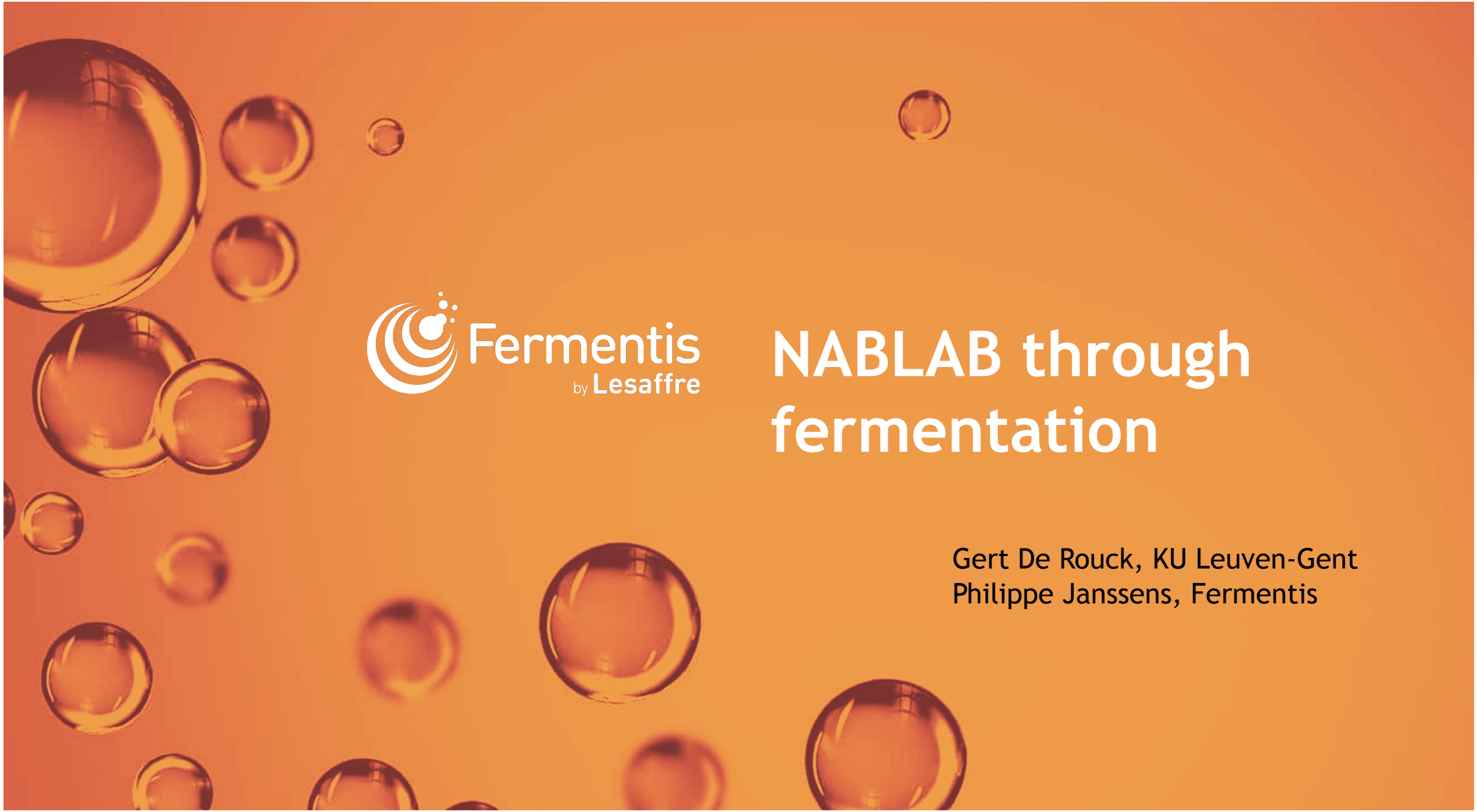
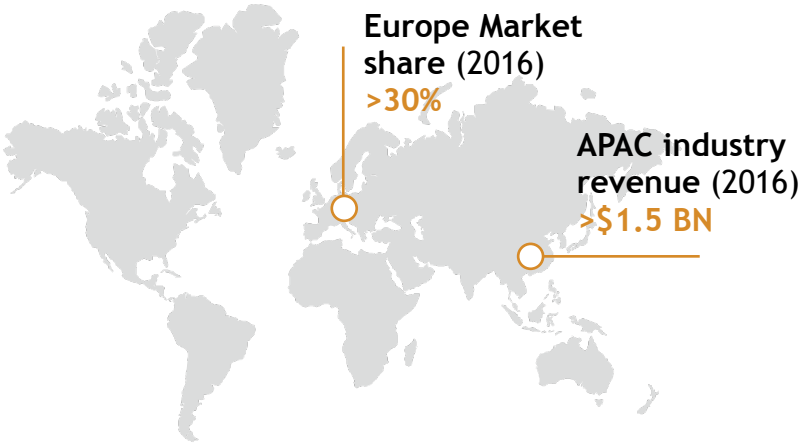


NABLAB through fermentation

Gert De Rouck, KU Leuven-Gent
Philippe Janssens, Fermentis

Markets
Non-alcoholic beer market



Hops market CAGR (2017-24): **>6.5%**

Enzymes industry share (2016): **>$550 MN**

Technology landscape:
Dealcoholisation technology share (2016): **>80%**

Restricted fermentation technology CAGR (2017-24): **>6%**

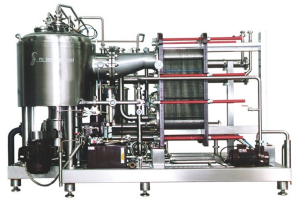
CAGR (2017-24) : >7.5%

2016 >$13 BN → 2017 → 2018 → 2019 → 2020 → 2021 → 2022 → 2023 → 2024

Methods
CAPEX - Biological

Equipment: CAPEX

Centrifugation



Rectification

Spinning Cone Column

Stopped fermentation

High-temp mashing and yeast

Specific micro-organisms

Non-saccharomyces organisms

Saccharomyces organisms

Objectives
FERMENTATION - Biological

Equipment: CAPEX

Centrifugation	Rectification	Spinning Cone Column
Stopped fermentation	High-temp mashing with regular yeasts for the production of LAB (<2,5% ABV)	Specific micro-organisms for the production of NAB (<0,5% ABV) *Non-saccharomyces* organisms *Saccharomyces* organisms

1st set of experiments
Experimental conditions

50l trials at the pilot plant

Objective:
2,5% ABV between OG 8-10°P

Composition
100%
pils malt

Wort filtration
Meura Filter
2001

Dilution & filtration
of beers

Brewing
75°C (167°F)
20min & bitterness of 20IBU

Maturation
7 days at 4°C
(39.2°F)

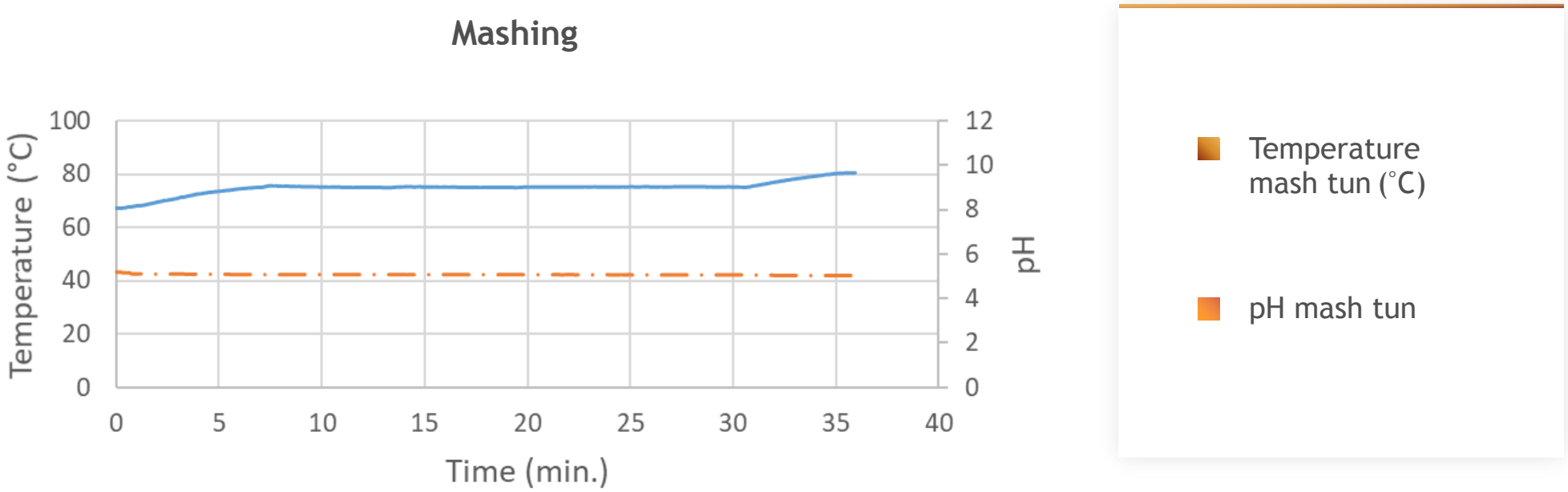
Wort gravity
10°P

Fermentation conditions

Lager yeasts:
SafLager™ S-23,
SafLager™ S-189,
SafLager™ W-34/70
at **14°C** (57.2°F)
Pitching rate: 100g/hl
(0.13 oz/gal)

Ale yeasts:
SafAle™ T-58,
SafAle™ S-33,
SafAle™ BE-256,
SafAle™ S-04,
SafAle™ K-97,
SafAle US-05™,
at **20°C** (57.2°F)
Pitching rate: 50g/hl
(0.06 oz/gal)

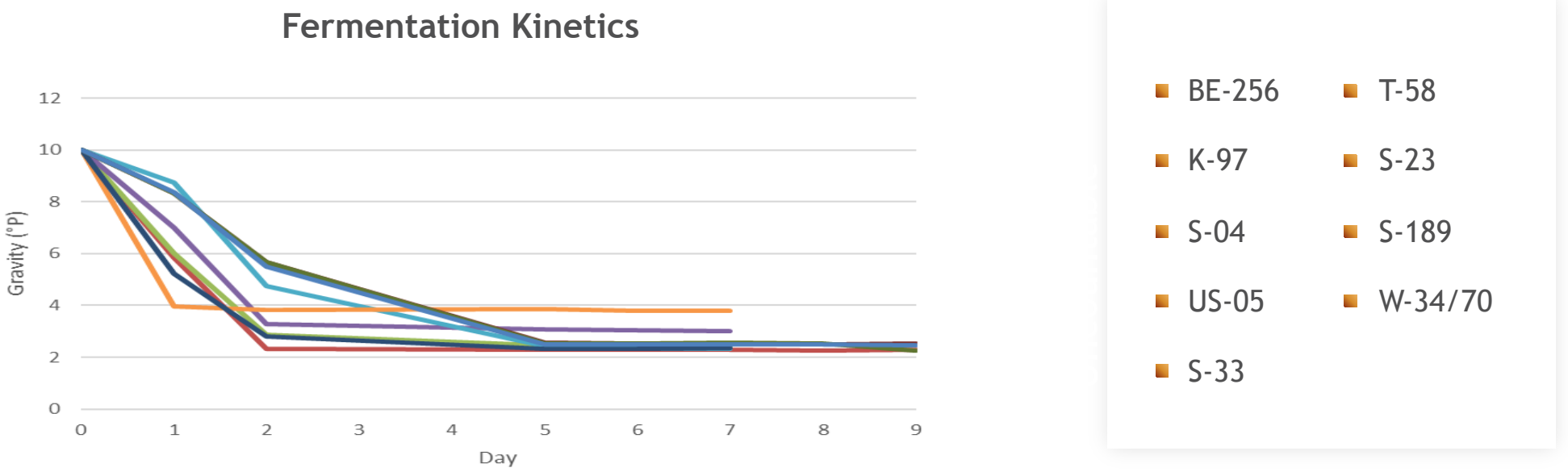
Experimental results
Brewing diagram



Starts below 75°C (167°F)

Total time of about 32 min

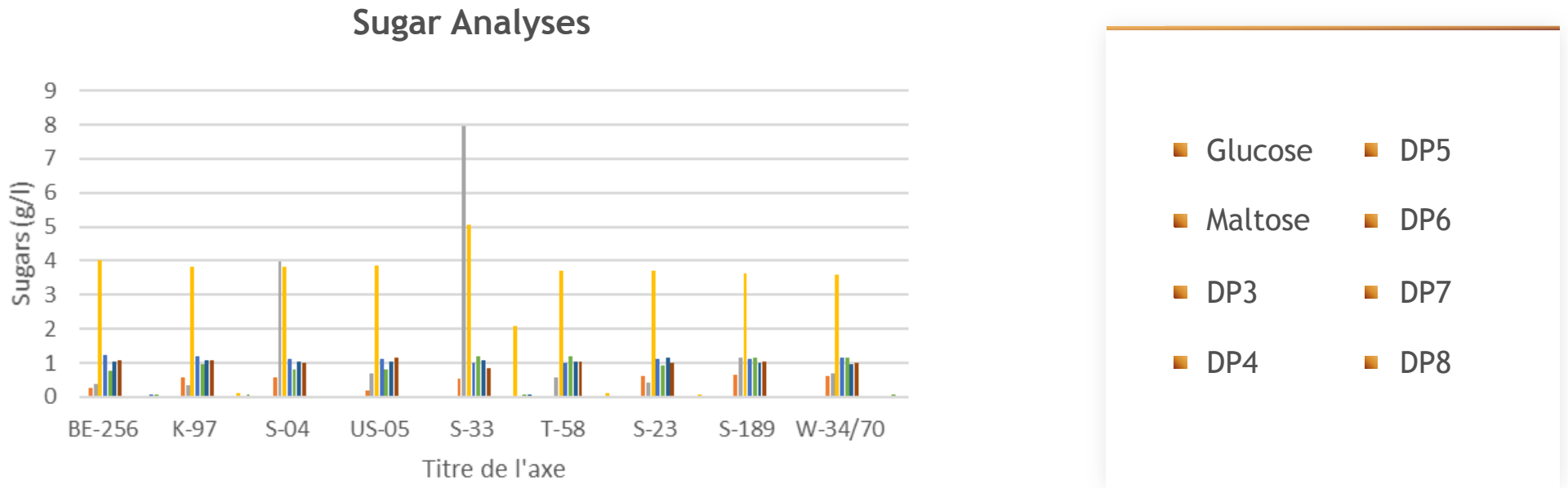
Experimental results
Ferm. Kinetics / limits



Limit attenuation close to 2°P (ADF~75%) for most of the yeasts

Higher limit for SafAleTM S-33 & SafAle™ S-04 (60% < ADF < 70%)

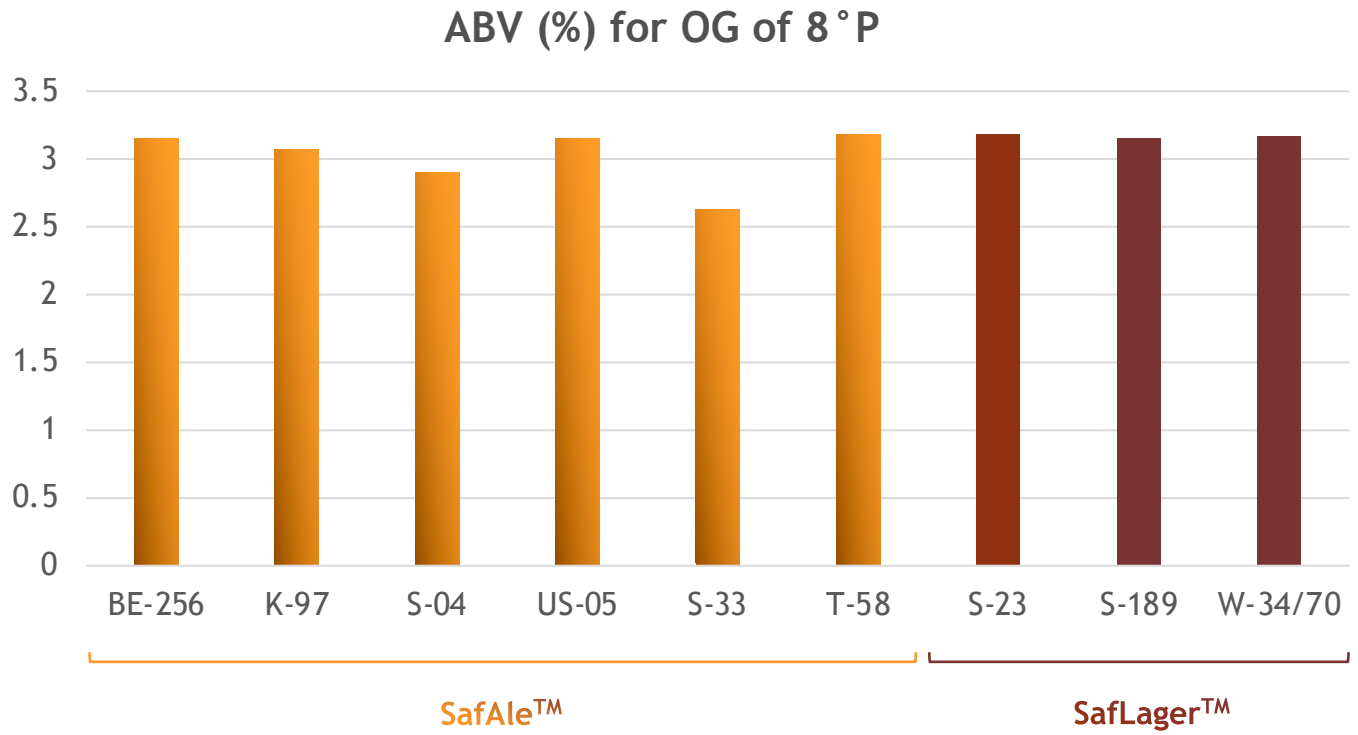
Experimental results
Sugar Analyses



Not much difference between the yeast

SafAle™ S-33 consumes no maltotriose

Experimental results
Dilution at 8°P (OG)



Except for SafAle™ S-33 all yeast produced higher level of alcohol than 2,5% ABV

Experimental results
Diacetyl

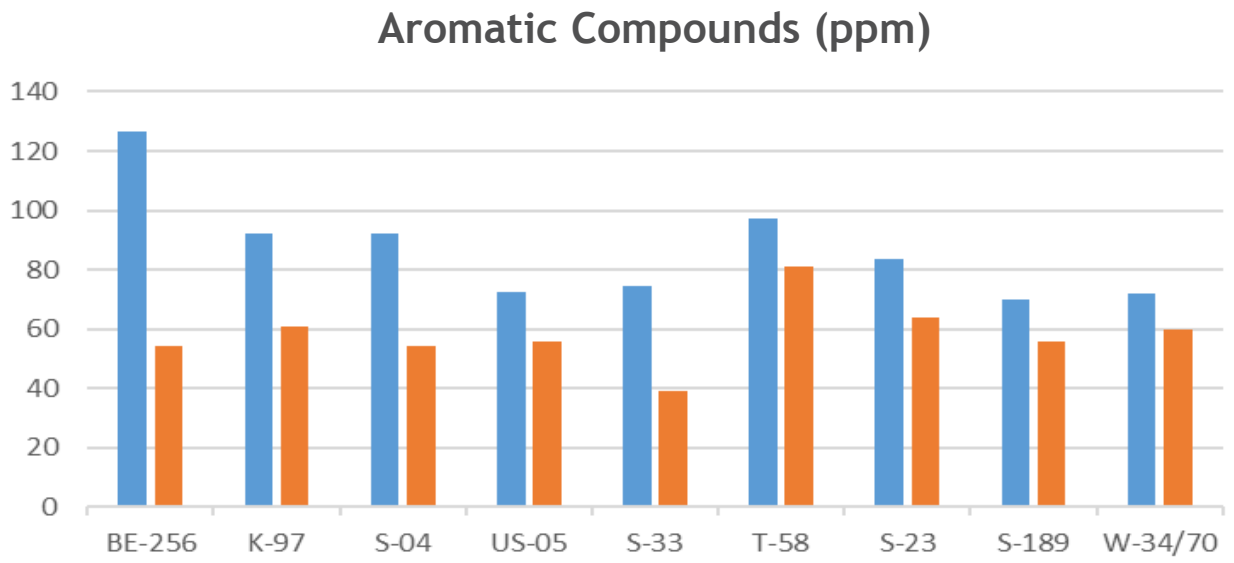
Scaling up at 50l at the pilot of KaHo

Large heterogeneity
in diacetyl reduction by yeast strains

Risk of presence
of diacetyl in beer for the 3 lagers

	Yeasts	Total diacetyl conc. after 7 days of fermentation (ppb)
SafAle™	BE-256	41,2
	K-97	2,1
	S-04	36,6
	US-05	4,1
	S-33	6,7
	T-58	3
SafLager™	S-23	**77,4**
	S-189	**58,7**
	W-34/70	**63,1**

Experimental results
Aromatic compounds

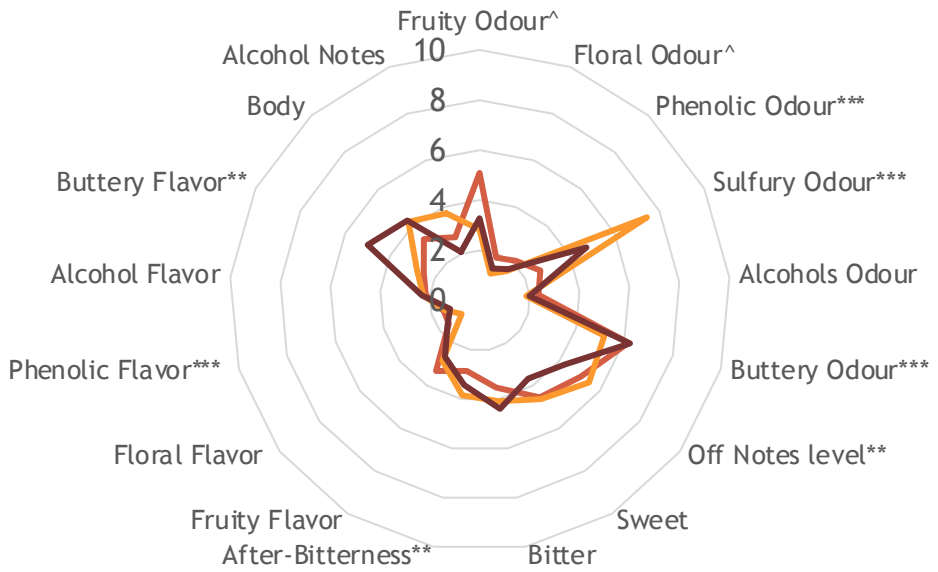


***HA**: n-propanol, Iso-butanol, 2+3-methyl-butanol and phenylethylalcohol*
***Esters**: acetate, butanoate, hexanoate, octanoate and decanoate*

Scaling up at 50l at the pilot of KUL-Gent

- Significant amount of aromatic fraction
- Higher HA level with SafeAle™ BE-256
- Higher Esters level with SafeAle™ T-58

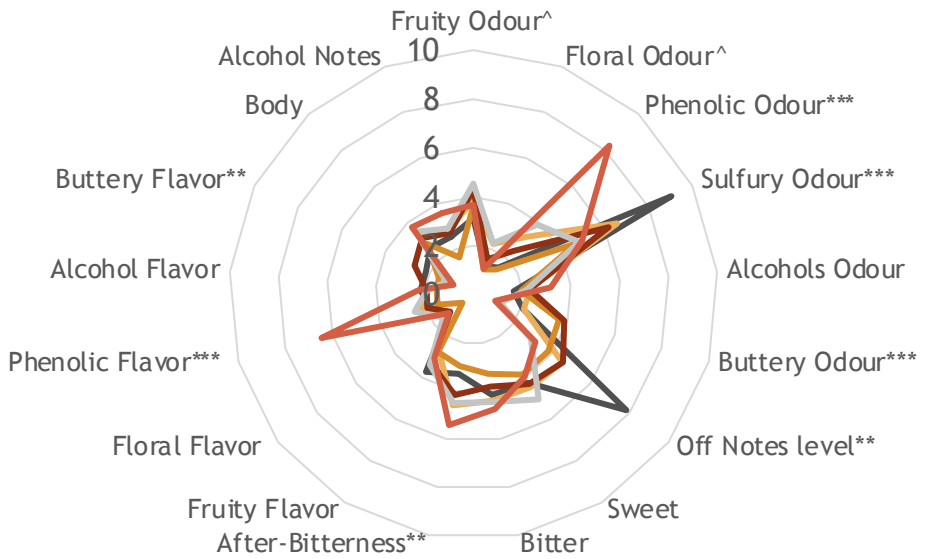
Fermentis Academy
Taste panel results



Lager beer yeasts

- SafLager™ S-23 presents more fruitiness, no off-flavor and is the lager best solution

Fermentis Academy
Taste panel results



Ale beer yeasts

- SafAle™ S-33 is the non-phenolic Ale solution
- SafAle™ T-58 is the phenolic Ale solution

Conclusions & recommendations

Meura Filtrer 2001 with fine coarse seems to increase enzymatic activities during the brewing step!

Objective: 2,5% ABV between OG 8-10 °P

Composition

100% pils malt

Brewing

≥ 80°C (167°F)

According to the malt gelatinization point

F2001

Reduced contact time of about 10-15min max

Lauter tun

Contact time between 15-20min

Yeast strains

SafAle™ S-33 non phenolic Ale solution

SafAle™ T-58 phenolic Ale solution

SafLager™ S-23 Lager solution

Selection of a new strain to brew non-alcoholic beers

Characteristics of the micro -organisms:

- Maltose negative
- Presence of aromatic fractions
- Higher alcohol/esters production
- Phenol production 4-VG

MO & experimental protocol
Additional trials in flask

External NCYC collection

Comparison of micro-organisms

- *S.Chevalieri* : Lesaffre collection
- *Pichia kluyveri*
- *Saccharomycodes ludwigii*
- *Torulaspora delbrueckii*
- *Zygosaccharomyces rouxii*

Malt composition
100 pils

Mash diagram for a wort at 12°P and 25BU*
65°C (149°F) 50mn
73°C (163.4°F) 10mn

Temperature of fermentation
22/10°C (71.6/50°F)
S.chevalieri

Dilution
8°P for about 16BU to get 0.5 ABV

*Mosaic Hop Pellets

Wort composition
Maltose – Micro-organisms

Should present some important differences compared to *Saccharomyces cerevisiae* in the way **this yeast assimilates the sugars present in a wort**.

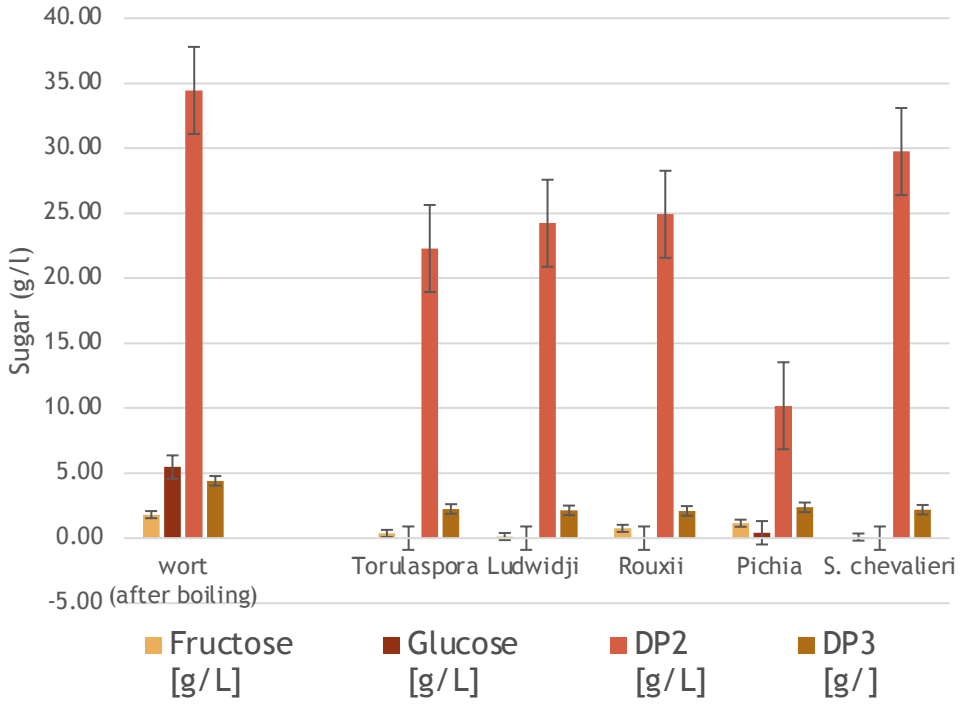
Sugar composition in % w/w of typical wort

Glucose	Maltotriose
10-15%	10-20%
Maltose	Dextrins
50-60%	15-20%

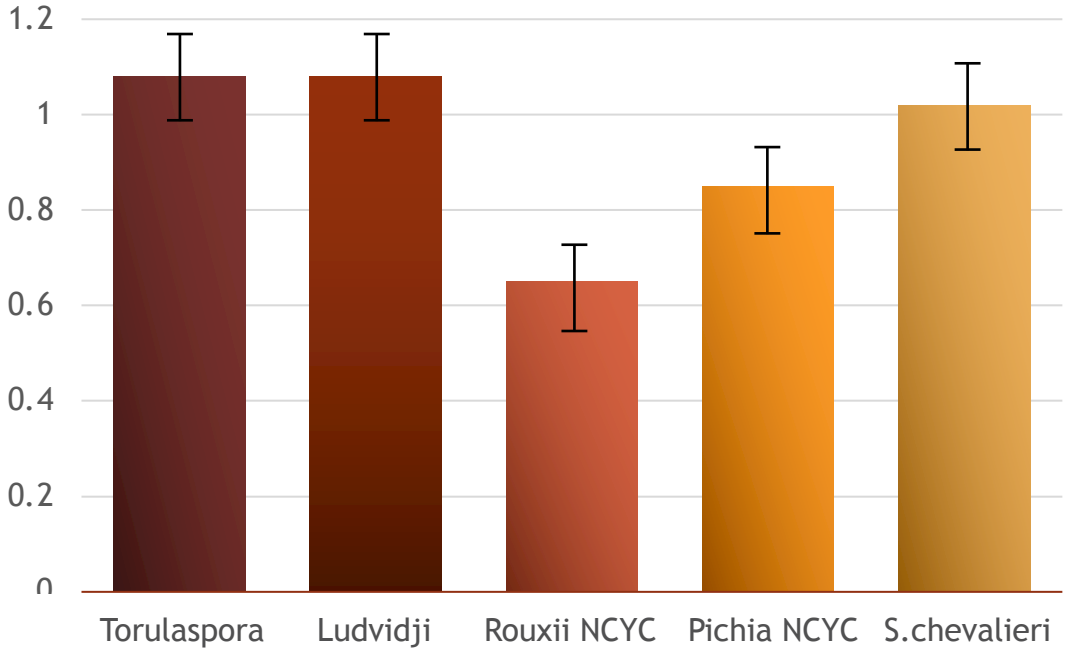
Results and discussion

OG of 12°P

Sugar Analyses



Ethanol production (T°=22°C / 71.6°F)



Results and discussion
Fermentation results

Heterogeneity between the Microorganisms

Some *non-Saccharomyces MO* are **maltose +** even very slow with a **risk of over attenuation / carbonation**

S.chevalieri is **maltose-** stops after consumption of simple sugars with **less risk of over attenuation**

Results and discussion
Testing results

Torulaspora delbrueckii
produces a neutral **malty and worty beer**

Saccharomycodes ludwigii
produces a **well balanced and fruity beer**, with slight touch of DMS (plastic)

Zygosaccharomyces rouxii
produces a beer with **a lot of off flavors**

Pichia kluyveri
produces a very **fruity beer** with high level of IAA and poor drinkability

Sacharomyces chevalieri
produces good beers **slightly phenolic (POF+)** without defect

SafBrew™ LA-01 has been selected!

Flask tests
Experimental protocol effect of gravity & temperature

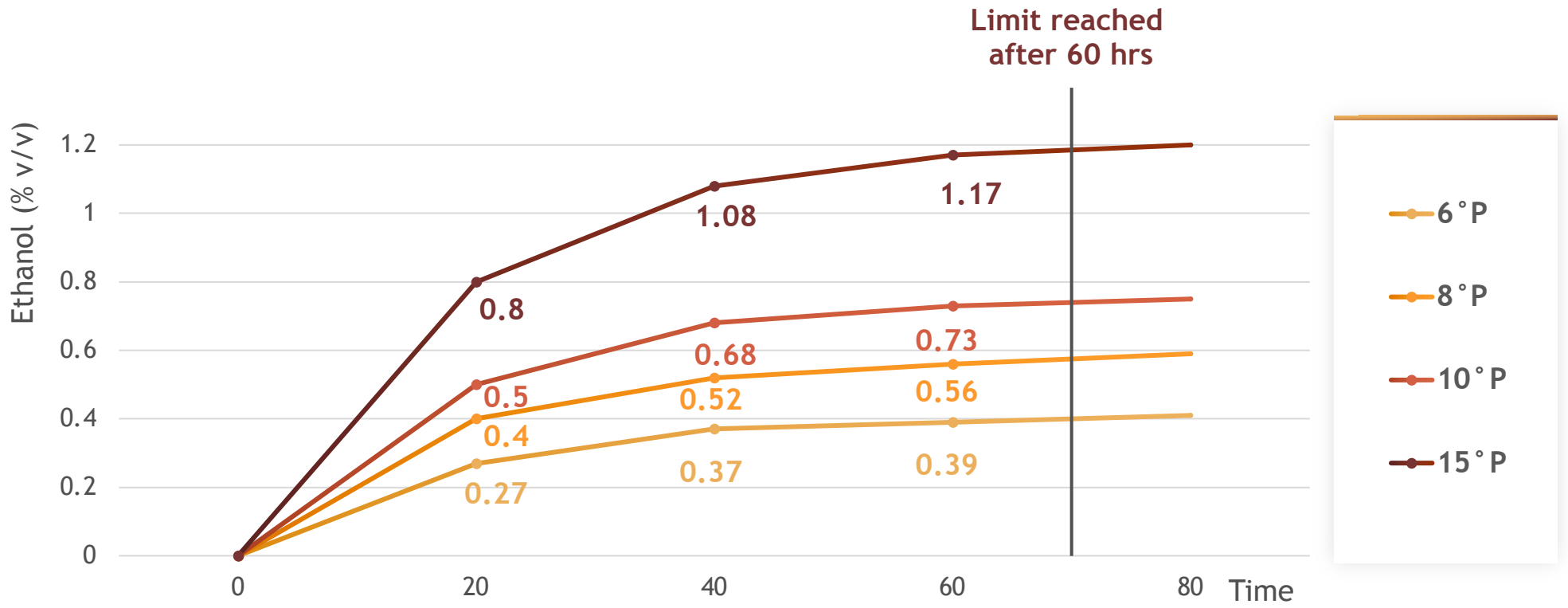
Flask tests were performed under the following conditions:

SafBrew™ LA-01
(*Sc. Var. chevalieri*)
50g/hl
(0.06 oz/gal)

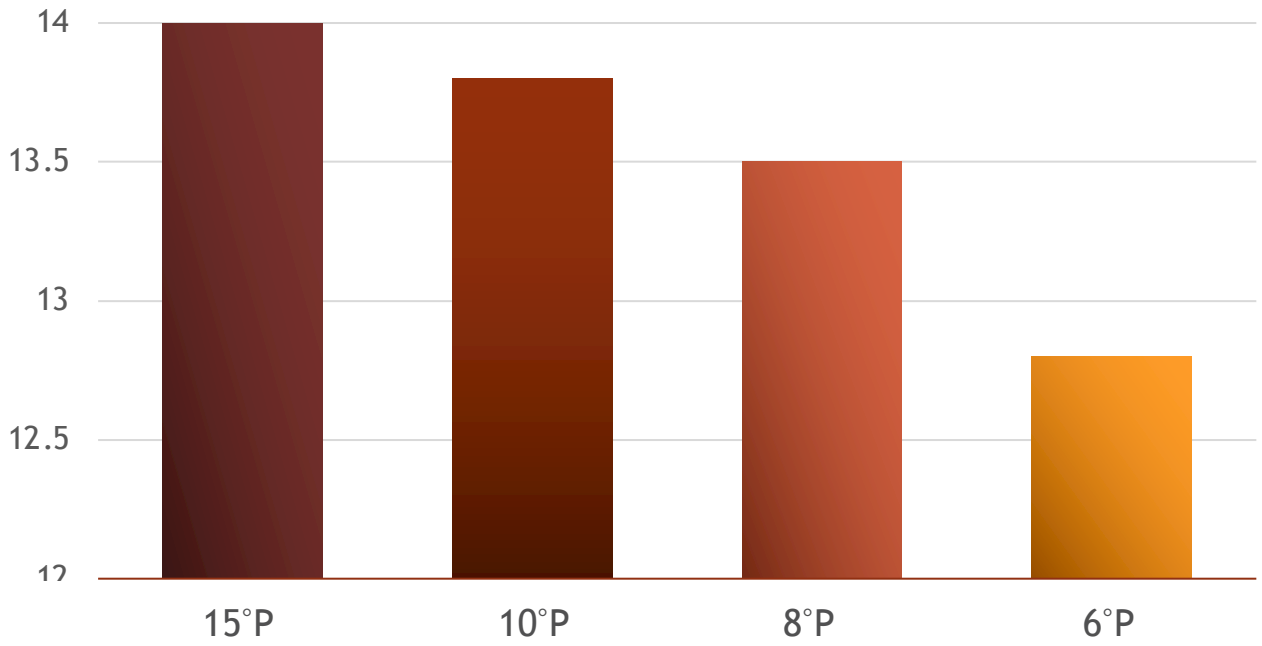
Weyerman ®
wort at
15-10-8-6 °P

Fermentation
temperature of
22 & 10°C
(71.6 & 50 °F)

Effect of gravity
Fermentation Kinetics



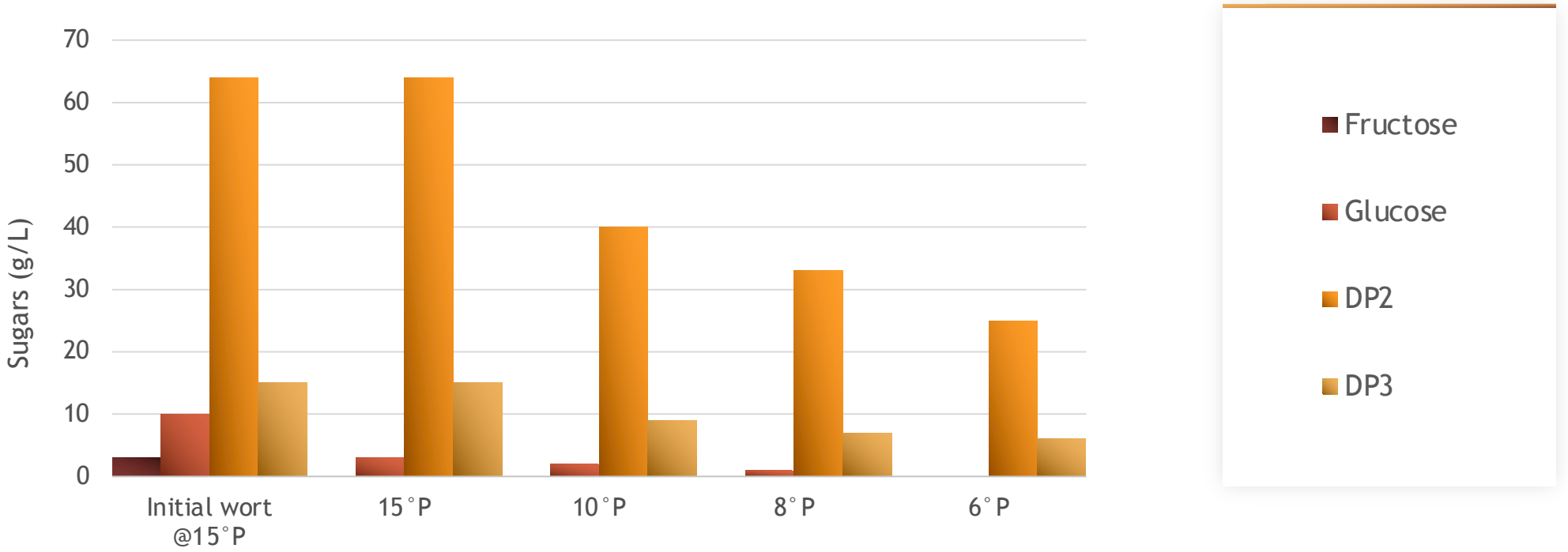
Effect of gravity
Apparent Degree of Fermentation (ADF%)



ADF is around

13-14%

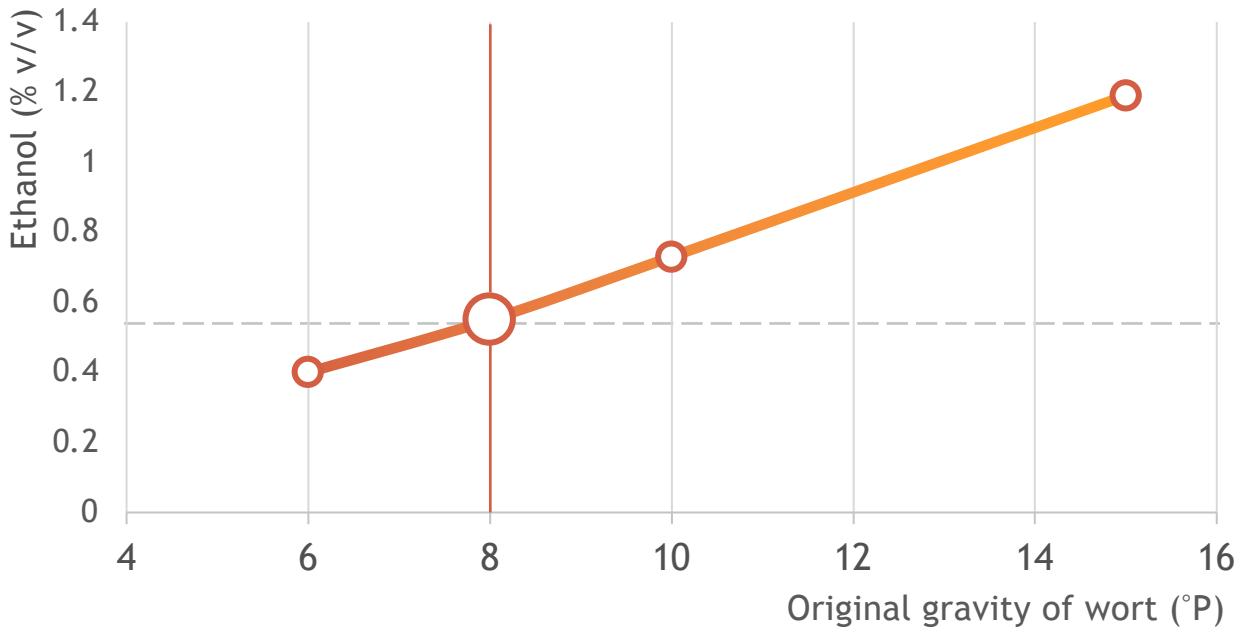
Effect of gravity
Sugar consumption



DP1 totally consumed during fermentation

DP2 & higher not fermented

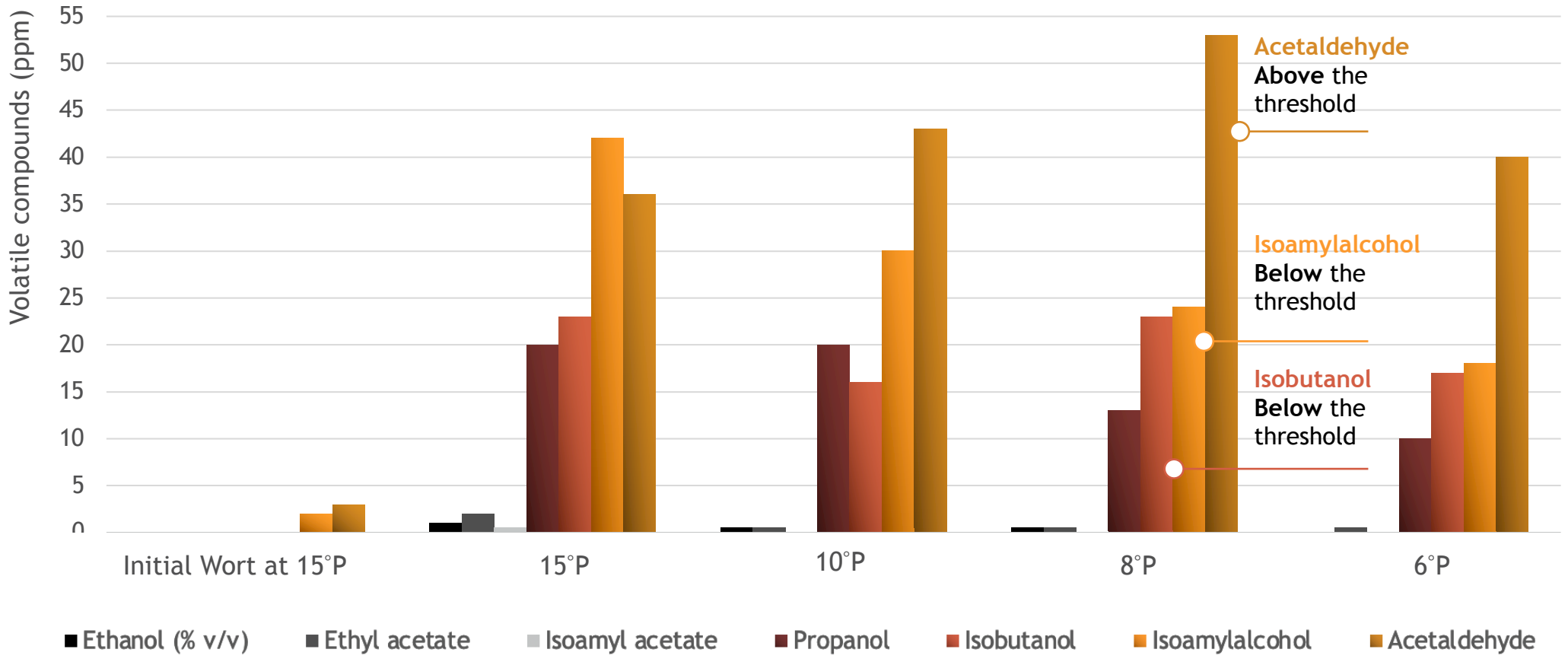
Effect of gravity
Ethanol production VS sugar consumption



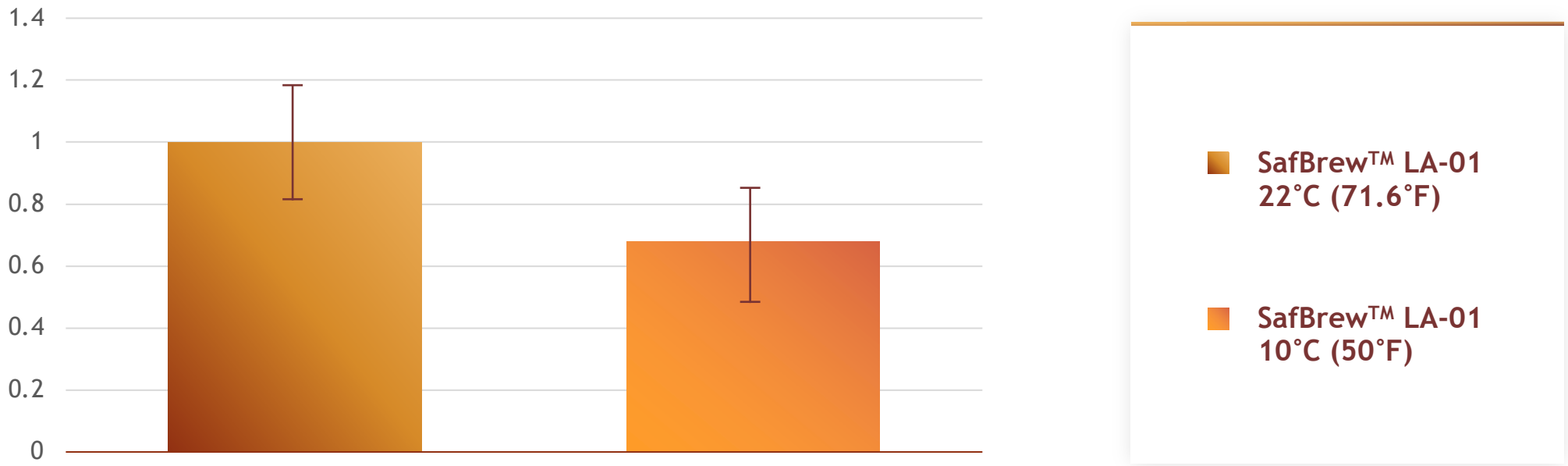
Linear regression
between the ABV (%)
and the OG (°P)

0.5% ABV
is reached
with a wort of about 7-8°P

Effect of gravity
Aromatic compounds production



Flask test results
Effect of temperature



SafBrew™ LA-01 at 10°C (50°F) shows:

- slow fermentation with a risk of non complete fermentation with residual fermentable sugars
- No significant aromatic differences

Experimental Trials First Conclusions

SafBrew™ LA-01 produces well balance beers

slightly phenolic (POF+) without defect and preferably fermented above 10°C (50°F)

Contains residual sugars

Therefore the beer is fragile in regard to contaminations; that can be dangerous for beer stability

« Cold Crashing » ≤ 4 °C (39,2 °F)

after assimilation of simple sugar (60hrs) is highly recommended

Pasteurization is mandatory

to stabilize the beer

Pasteurization

Wort fermentation with SafBrew™ LA-01

at 25°C (77°F)

Centrifugation

Cross-contamination with SafAle™ T-58

0 cfu/ml
10^3 cfu/ml
10^4 cfu/ml
10^5 cfu/ml

Pasteurization at 63°C (145.4°F)

time:
PU/1.393$^{(63-60)}$

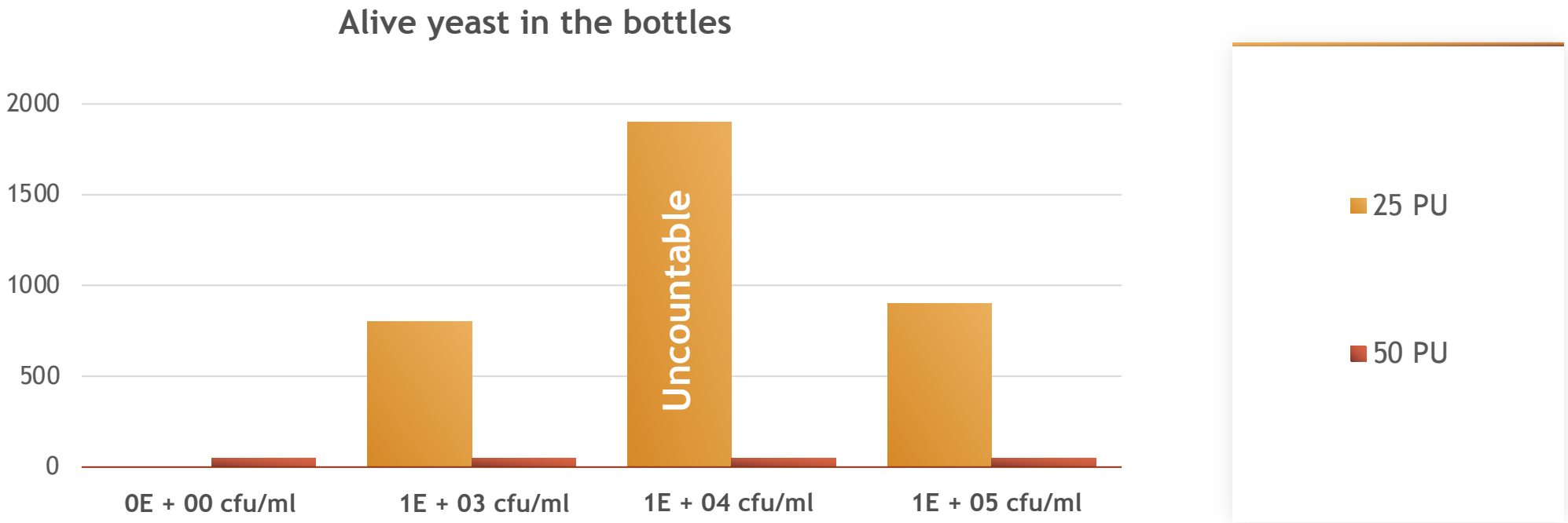
Sequence with no latency

Contamination

Pasteurization
25 PU, 50PU, 75PU and 100 PU

Plating
with YPD media

Pasteurization - Results



Presence of alive yeast at 25 PU:
SafBrew™ LA-01 or SafAle™ T-58

Absence of yeast:
from 50 PU and above

Mandatory:
Min 50 PU

SafBrew™ LA-01 Conclusions

Pitching rate

50g/hl

(0.06 oz/gal)

Fermentation

15-25°C

(59-138.2°F)

Apparent degree of fermentation

15%*

Alcohol produced

0.5 ABV**

for 7-8°P

Cold crashing when limit is reached

+/- 60hrs

Off-flavors

None

Pasteurization

50-100 PU

Repitching

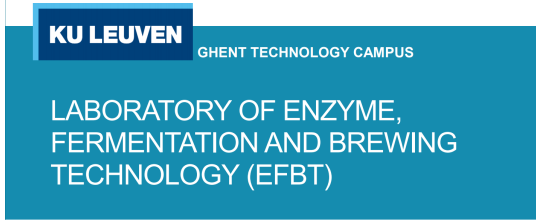
None

Refermentation

None

*Traditional brewing diagram +/-15% ADF, High T°mashing or shorter time: <15% ADF / **pH is around 4.5

ACKNOWLEDGMENTS



Fermentis' technical team

Thank you for your attention!

Fermentis by Lesaffre

www.fermentis.com