CHARACKTERIZATION

of the

FERMENTATION PERFORMANCE

of Bottom Fermenting, Industrial Relevant

YEAST STEMS

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Introduction

Challenge

Numerous yeast strains are commercially available.

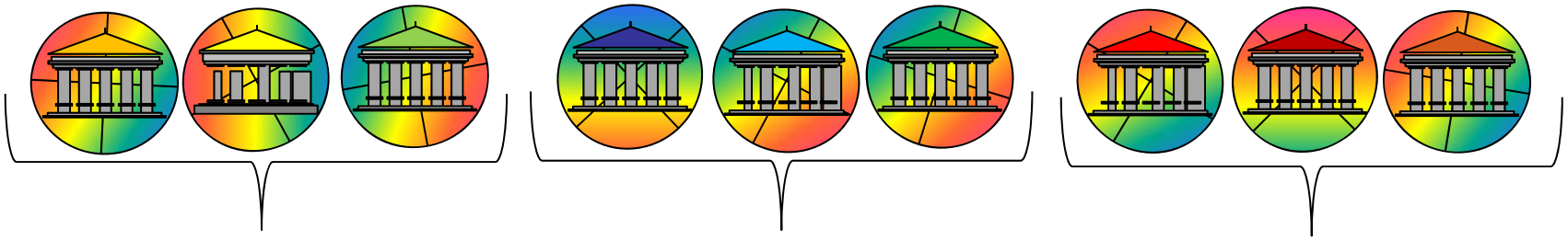
In addition, many breweries use exclusive strains.

Nevertheless, almost no statistically correct, reliable, and practice-oriented data can be found in which the organisms are compared adequately with each other

Reasons for Insufficient Characterization

Due to genetic differences, the yeast strains vary in their properties

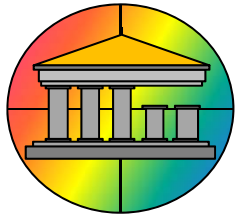
➔Identical conditions, result in different by-products🡐



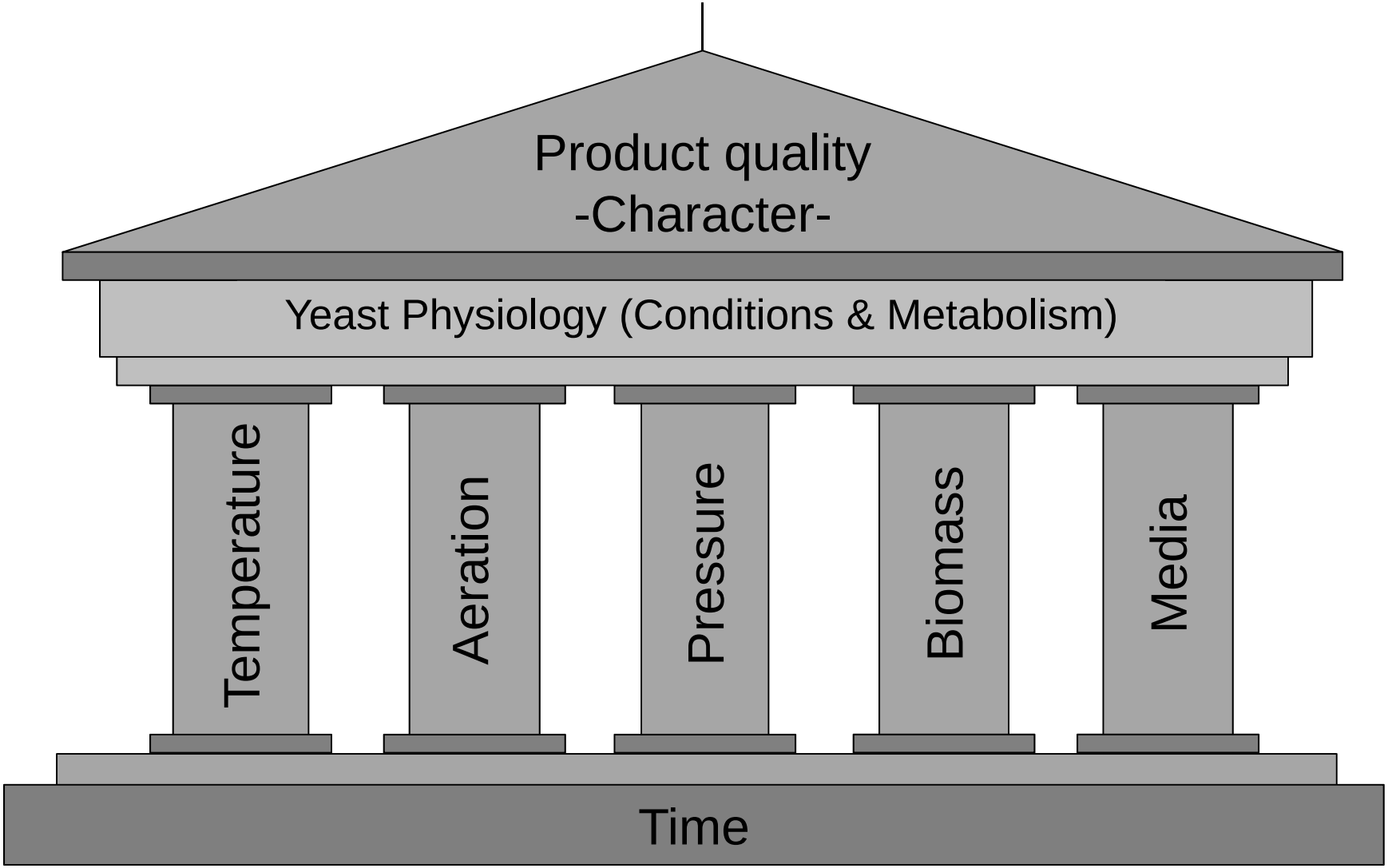
Phenolic | Fruity, Estery | Sulfury, Yeasty

Strains may react individually and distinctively to technological adaptions

Therefore, no precise data has been presented in the literature …



Reasons for Insufficient Characterization

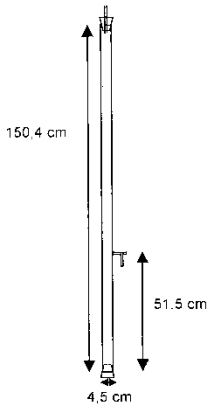
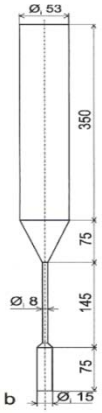


Scientific Approach…

Industrially relevant yeast characterization under standard conditions

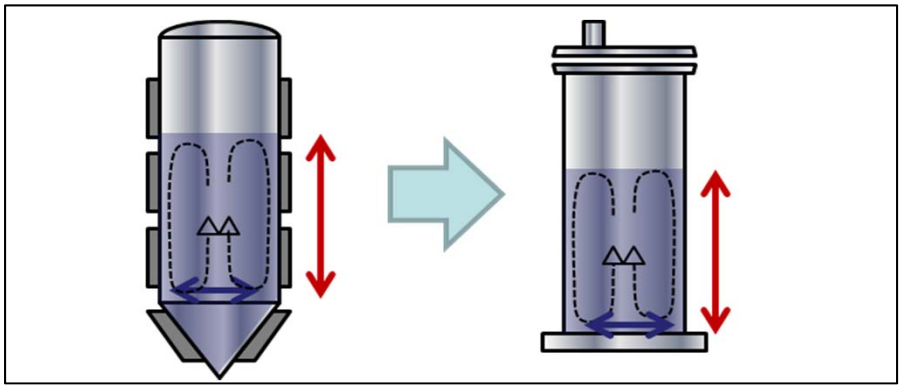
In order to generate results, which are also applicable on an industrial scale, the laboratory fermenters have to allow for the implementation of a diverse range of technological parameters

Standard equipment is therefore not suitable!

EBC Fermentor	Lietz Glas-Fermentor	Lab.-Fermentor	Weinfurtner Fermentor
			

Scientific Approach…

Practice-orientated yeast characterization under standard conditions



Height-to-diameter ratio: 1: 2

40% headspace

Fill volume: 2 liters

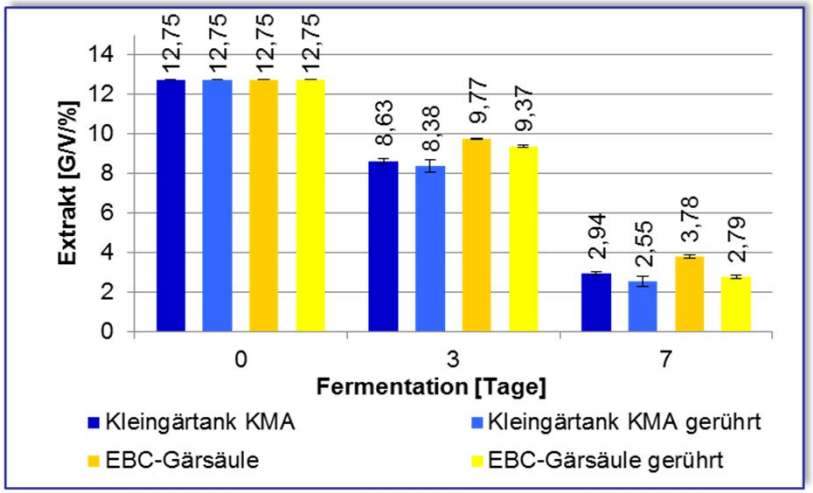
Pressure-tested

Easy to clean, open and handle

The fermentation was at least as quick as it was in the compared fermentors

Stirring did not have a significant influence
➔ sufficient convection

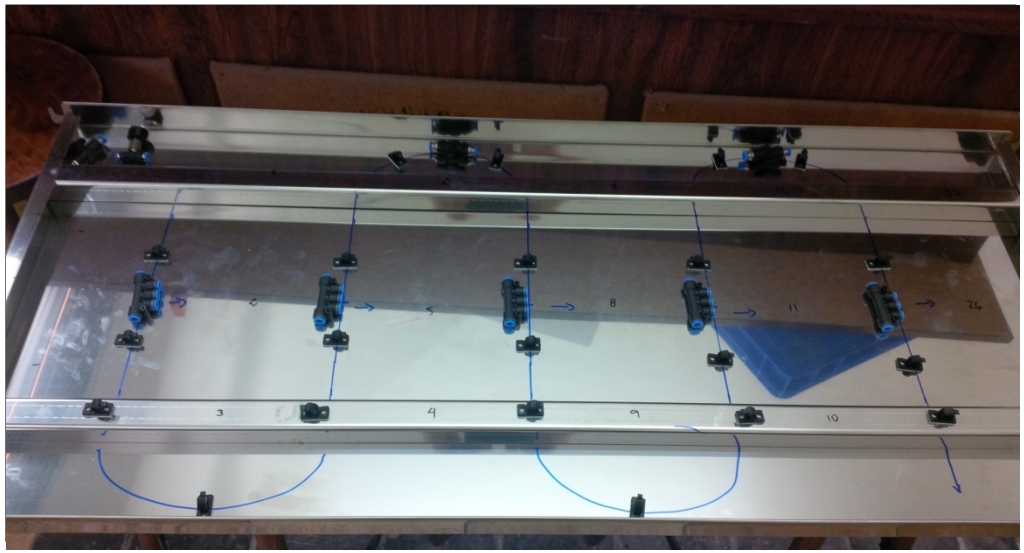
By generating a higher pressure throughout the process, a comparable fermentation – as it occurs in industrial tanks – could be achieved (scale-up!)



Scientific Approach…

Concept for Fermentors and the Facility

- Up to 30 identical fermentors were used simultaneously
- This produces a statistically relevant amount of data
- Instead of sampling out of single tanks, which may influence the remaining content, in this case individual tanks are simply removed, so that any influence on the other tanks can be omitted.



Scientific Approach…

ANALYSIS PARAMETERS

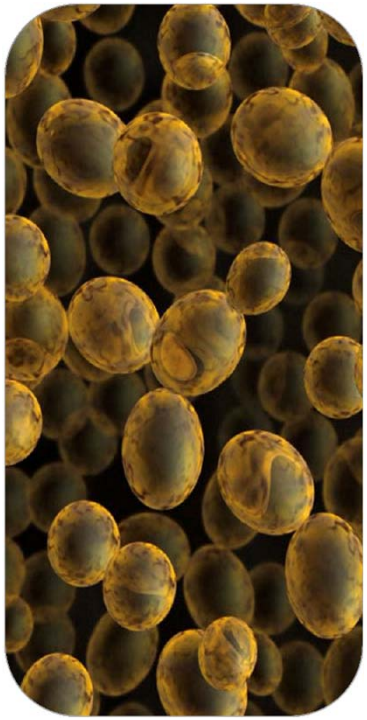
Parameter	Conditions/Specifications for the Trials
Adjusted, overpressure	0.5 bar
Fermentation media	Lager beer wort (11.8 ° P)
Propagation	Standardized and parallel
Cell count for inoculation	30 x 10^6 Cells/ml
Fermentation temperature	15 ° C
Fermentation volume	2000 ml/fermentor
Number of fermentors	30
Homogenization	using N_2
Yeast used as control strain	TUM 34/70
Number of yeast strains evaluated in one trial	2 (here: always two commercially relevant, readily available strains)
Resulting evaluation time	Fermentation: 4 days / Storage: 7 days

Scientific Approach…

Control yeast strain TUM 34/70 (bottom-fermenting, flocculent yeast)

= the most widely employed and best described bottom-fermenting beer yeast of the world

- Short lag phase
- High level of acid formation
- Good reduction capacity
- Rapid fermentation leaving very little residual sugars
- Highly flocculent (late flocculation)
- Effective diacetyl reduction
- Creates fine, mild beers of excellent quality
- Suitable for high gravity & fermentation under pressure
- Moderate production of higher alcohols
- Good ester production
- SO_2 formation is temperature and gravity dependent?!?

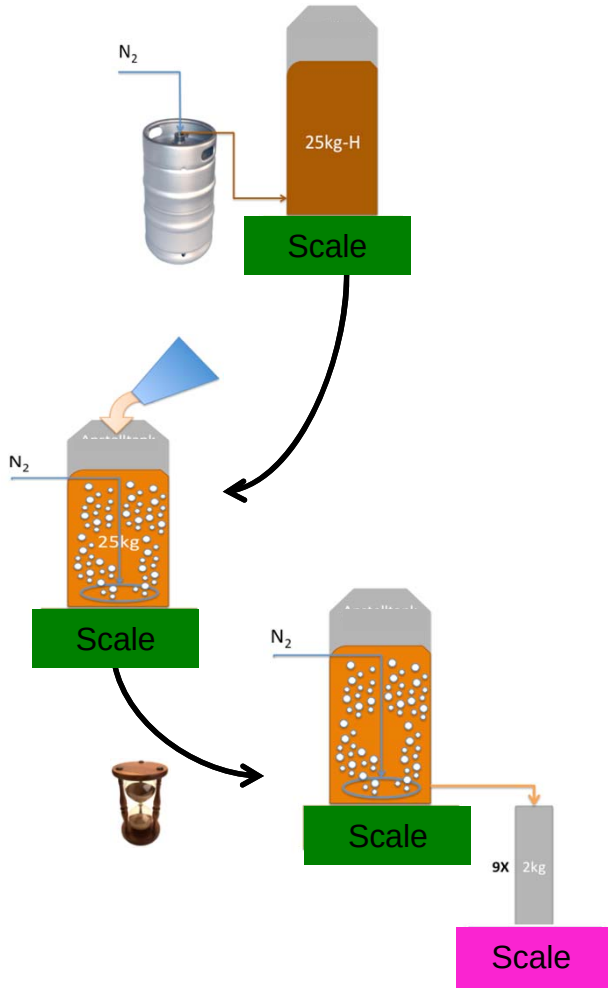


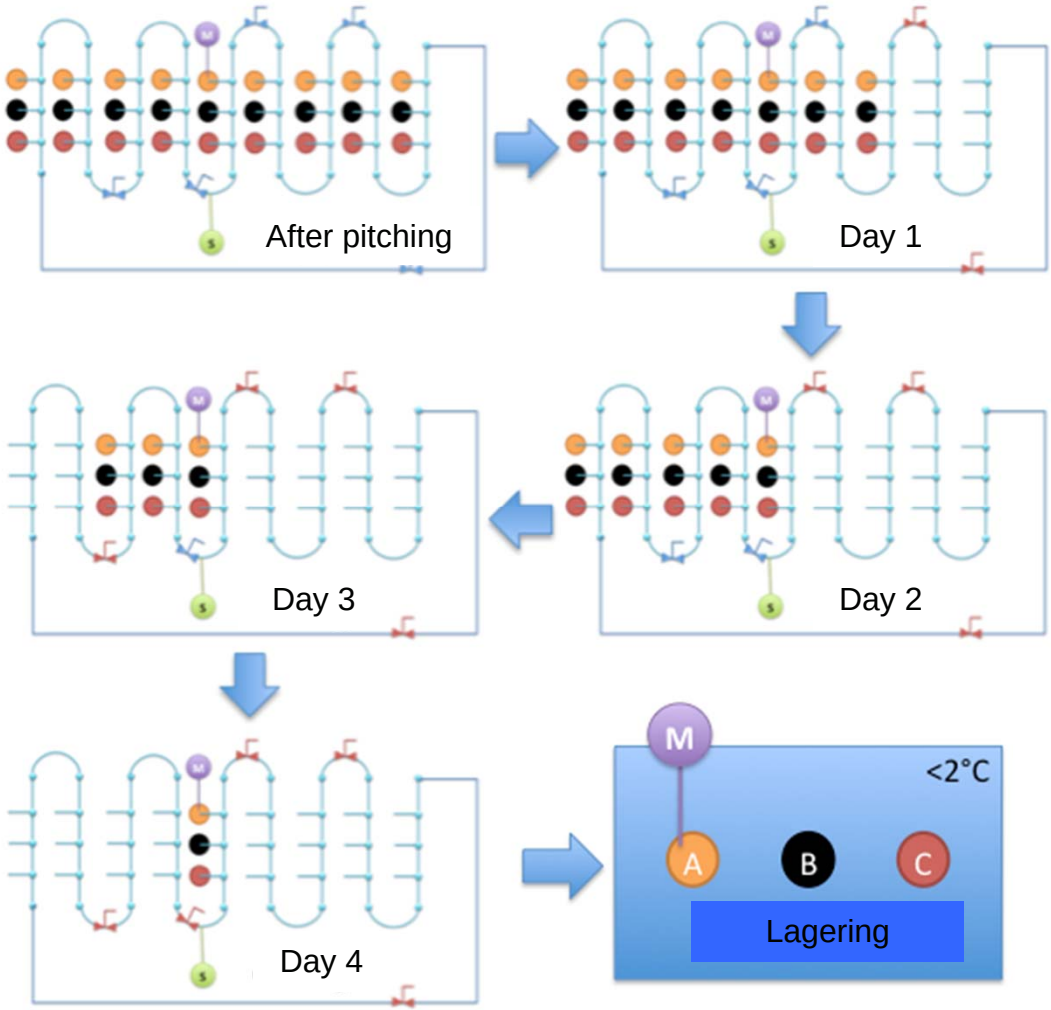
Scientific Approach…

Most important parameters for brewers

- Fermentation performance and extract utilization
- Carbohydrate and nitrogen metabolism
- Decline in pH
- Yeast reproduction
- Formation of flavor compounds
- Development of volatile aroma compounds
- Duration of lag phase and fermentation
- Concentration of yeasts in suspension
- Sedimentation characteristics
- Influence on foam characteristics
- Influence on the sensorial impact of the product

Processing


N2
25kg-H
Scale
N2
25kg
Scale
N2
Scale
9X 2kg
Scale

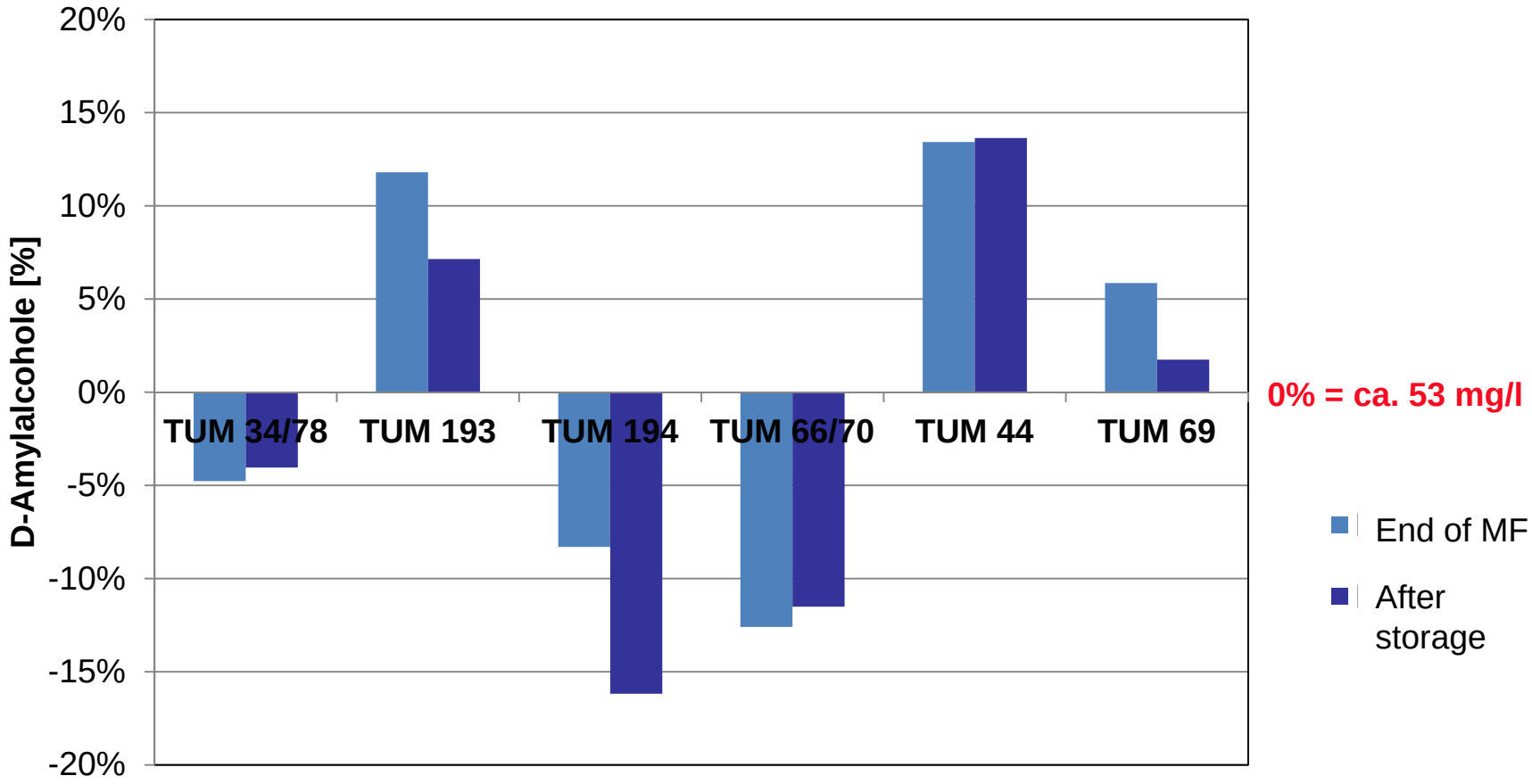

After pitching
Day 1
Day 2
Day 3
Day 4
M
<2°C
A
B
C
Lagering

Plan for Sampling & Analysis

Parameter	Pitching yeast	Inoculated wort	1st day of fermentation	2nd day of fermentation	3rd day of fermentation	4th day of fermentation	Storage
CO_2 production			X	X	X	X	X
Extract		X	X	X	X	X	X
FAN		X	X	X	X	X	X
pH		X	X	X	X	X	X
Color		X				X	X
Sugar spectrum		X				X	X
Final attenuation		X	X	X	X	X	X
Total yeast concentration	X	X	X	X	X	X	X
Yeast in suspension			X	X	X	X	X
Surface charge	X		X	X	X	X	X
Turbidity			X	X	X	X	X
VDK (total)			X	X	X	X	X
VDK (free)							X
By-products						X	X
SO_2						X	X
Foam							X
Sedimentation			X	X	X	X	X
Degustation							X

Visualization of the Test Results

Example of a graph showing test results



Results: Fermentation Properties

Fermentation properties	TUM 34/78	TUM 193	TUM 194	TUM 66/70	TUM 44	TUM 69
Fermentation performance						
Extract reduction	↓	--	--	↓	--	↓
Sugar uptake	↓	--	↓↓	↑	↓↓	↓
CO_2 production	↓	↑	↑	--	--	↓
CO_2 concentration	↓	↓	↓	↑	↓	↓
Duration of Fermentation	↓	--	--	↓	--	↓
Maltotriose uptake	↓	↓↓	↓↓↓	↑	↓↓	↓
pH reduction						
pH reduction	--	↓	↑	--	↑↑	↑
Acid formation	↑	--	↑	--	↑↑	↑

- Yeast TUM: 44, 34/78 and 69 ferment the wort more slowly.
- Maltotriose metabolism is strain-specific, and the uptake is significantly slower for the TUM strains 193, 194 and 44.
- TUM yeast strains 44, 194 and 69 show a more rapid decline in pH and result in lower final values for pH **compared to 34/70!**

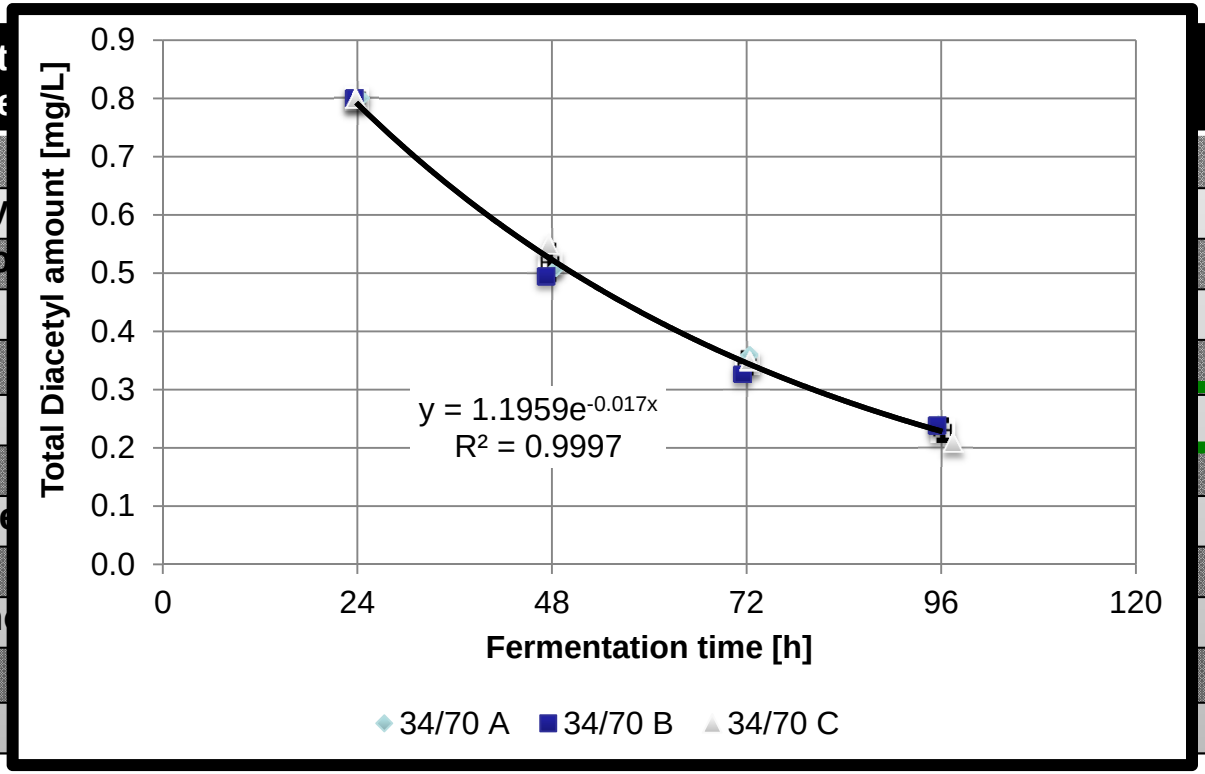
Results: Sedimentation Properties

Fermentation properties	TUM 34/78	TUM 193	TUM 194	TUM 66/70	TUM 44	TUM 69
Physical properties of the yeast cells						
Suspended yeast	↑	--	↓	↑↑	--	↑
Sedimentation	--	--	--	--	↑	--
Turbidity	↑↑	--	↑↑↑	--	--	↑
Fermentation By-Products						
Diacetyl	↓	--	--	↓↓	↓	↓
SO_2	↓↓	↑↑↑	↓↓	↑	--	--
Acetaldehyde	↓	↑	↓↓	--	--	↑
Esters	↓	↑	↓	↓	--	↓
Higher alcohols	↓	↑	↓	↓	↑	--
Other quality characteristics						
Foam	↑↑	--	↓↓	--	↓↓	↓

- Yeast TUM 66/70 remains longer in suspension, followed by 34/78 and 69.
- By contrast, yeast TUM 44 sediments vey rapidly.
- The turbidity did not correlate with the other parameters of sedimentation, possibly due to movement prior to the measurement.

Results: Diacetyl Reduction

Fermentat properti	TUM 69
Suspended y	↑
Sedimentatio	--
Turbidity	↑
Diacetyl	↓
SO_2	--
Acetaldehyd	↑
Esters	↓
Higher alcoh	--
Foam	↓



- Yeast TUM 66/70 reduces diacetyl very slowly by comparison.
- Strains TUM 34/78, 44 and 69 also reduce the diacetyl slightly more slowly than yeast strain TUM 34/70, used here as control.

Results: SO_2 Production

Fermentation properties	TUM 34/78	TUM 193	TUM 194	TUM 66/70	TUM 44	TUM 69
Physical properties of the yeast cells						
Suspended yeast	↑	--	↓	↑↑	--	↑
Sedimentation	--	--	--	--	↑	--
Turbidity	↑↑	--	↑↑↑	--	--	↑
Fermentation By-Products						
Diacetyl	↓	--	--	↓↓	↓	↓
SO_2	↓↓	↑↑↑	↓↓	↑	--	--
Acetaldehyd	↓	↑	↓↓	--	--	↑
Esters	↓	↑	↓	↓	--	↓
Higher alcohols	↓	↑	↓	↓	↑	--
Other quality characteristics						
Foam	↑↑	--	↓↓	--	↓↓	↓

- Yeast strains TUM 34/78 and 194 produce less SO_2.
- By contrast, TUM 193 and 66/70 produce more SO_2 than the control strain TUM 34/70.

Results: Acetaldehyde Production

Fermentation properties	TUM 34/78	TUM 193	TUM 194	TUM 66/70	TUM 44	TUM 69
Physical properties of the yeast cells						
Suspended yeast	↑	--	↓	↑↑	--	↑
Sedimentation	--	--	--	--	↑	--
Turbidity	↑↑	--	↑↑↑	--	--	↑
Fermentation By-Products						
Diacetyl	↓	--	--	↓↓	↓	↓
SO_2	↓↓	↑↑↑	↓↓	↑	--	--
Acetaldehyde	↓	↑	↓↓	--	--	↑
Esters	↓	↑	↓	↓	--	↓
Higher alcohols	↓	↑	↓	↓	↑	--
Other quality characteristics						
Foam	↑↑	--	↓↓	--	↓↓	↓

- Yeast TUM 34/78 and 194 produced less acetaldehyde.
- Whereas, TUM strains 193 and 69 produced more acetaldehyde than yeast strain 34/70.

Results: Ester Production

Fermentation properties	TUM 34/78	TUM 193	TUM 194	TUM 66/70	TUM 44	TUM 69
Physical properties of the yeast cells						
Suspended yeast	↑	--	↓	↑↑	--	↑
Sedimentation	--	--	--	--	↑	--
Turbidity	↑↑	--	↑↑↑	--	--	↑
Fermentation By-Products						
Diacetyl	↓	--	--	↓↓	↓	↓
SO_2	↓↓	↑↑↑	↓↓	↑	--	--
Acetaldehyde	↓	↑	↓↓	--	--	↑
Esters	↓	↑	↓	↓	--	↓
Higher alcohols	↓	↑	↓	↓	↑	--
Other quality characteristics						
Foam	↑↑	--	↓↓	--	↓↓	↓

- Yeast strains TUM 34/78, 194, 66/70 and 69 produce less ethyl acetate.
- Strain TUM 193 in contrast produces more ethyl acetate than the control strain TUM 34/70.

Results: Production of Higher Alcohols

Fermentation properties	TUM 34/78	TUM 193	TUM 194	TUM 66/70	TUM 44	TUM 69
Physical properties of the yeast cells						
Suspended yeast	↑	--	↓	↑↑	--	↑
Sedimentation	--	--	--	--	↑	--
Turbidity	↑↑	--	↑↑↑	--	--	↑
Fermentation By-Products						
Diacetyl	↓	--	--	↓↓	↓	↓
SO_2	↓↓	↑↑↑	↓↓	↑	--	--
Acetaldehyde	↓	↑	↓↓	--	--	↑
Esters	↓	↑	↓	↓	--	↓
Higher alcohols	↓	↑	↓	↓	↑	--
Other quality characteristics						
Foam	↑↑	--	↓↓	--	↓↓	↓

- Yeast TUM 34/78, 194, 66/70 produced less amyl alcohols.
- Strains TUM 193 and 44 produce more amyl alcohols than the control strain TUM 34/70.

Results: Foam Quality

Fermentation properties	TUM 34/78	TUM 193	TUM 194	TUM 66/70	TUM 44	TUM 69
Physical properties of the yeast cells						
Suspended yeast	↑	--	↓	↑↑	--	↑
Sedimentation	--	--	--	--	↑	--
Turbidity	↑↑	--	↑↑↑	--	--	↑
Fermentation By-Products						
Diacetyl	↓	--	--	↓↓	↓	↓
SO_2	↓↓	↑↑↑	↓↓	↑	--	--
Acetaldehyde	↓	↑	↓↓	--	--	↑
Esters	↓	↑	↓	↓	--	↓
Higher alcohols	↓	↑	↓	↓	↑	--
Further quality characteristics						
Foam	↑↑	--	↓↓	--	↓↓	↓

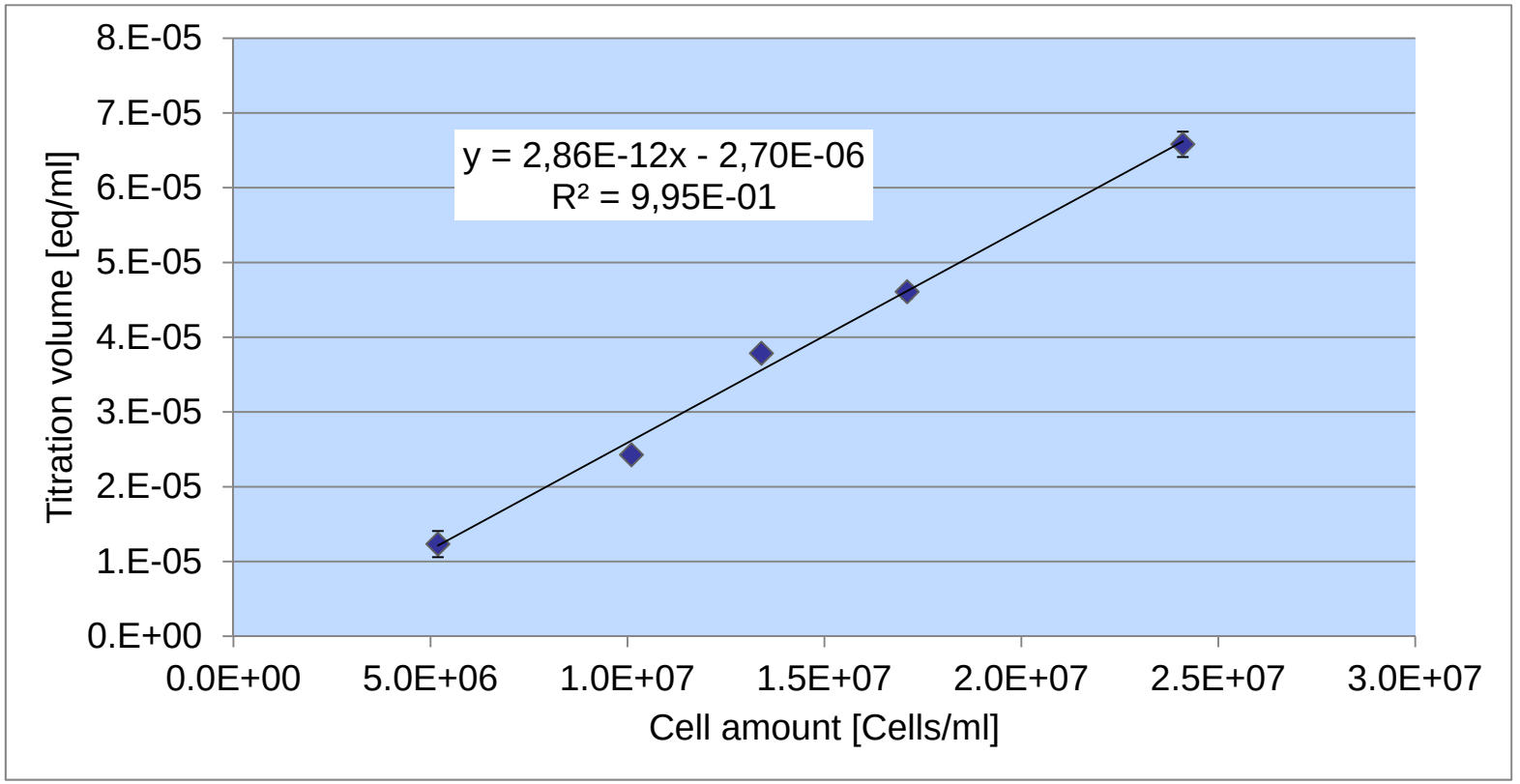
- Yeast strains TUM 194, 44 and 69 produced beers with lower foam stability.
  - By contrast, TUM 34/78 produced better foam than TUM 34/70.

➔Note: The foam results were measured in unfiltered samples…←

➔ They may not be representative of filtered/packaged beer!←

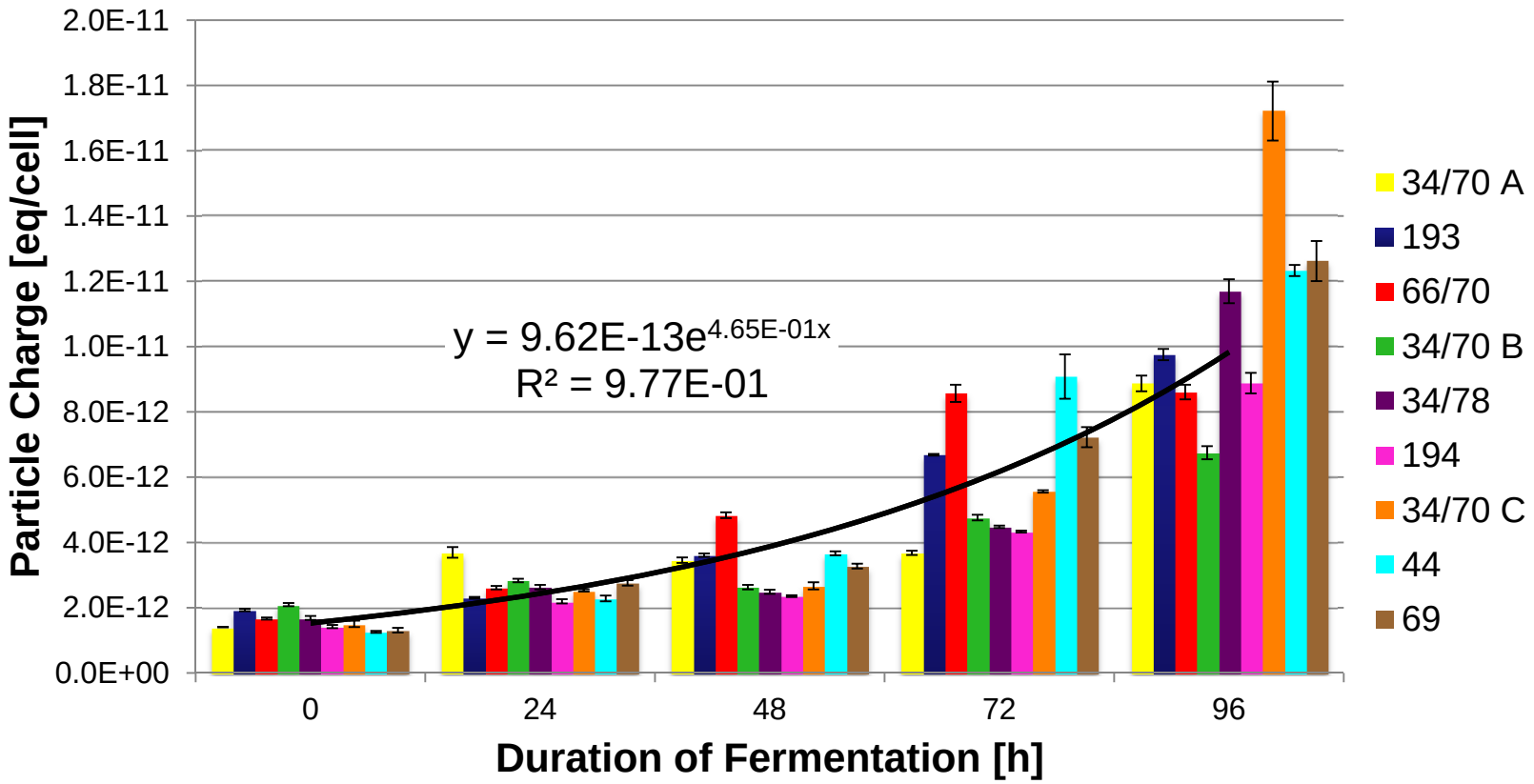
Results: Particle Charge

Particle Charge // Surface Charge



- Titration volume and cell amount correlate linear

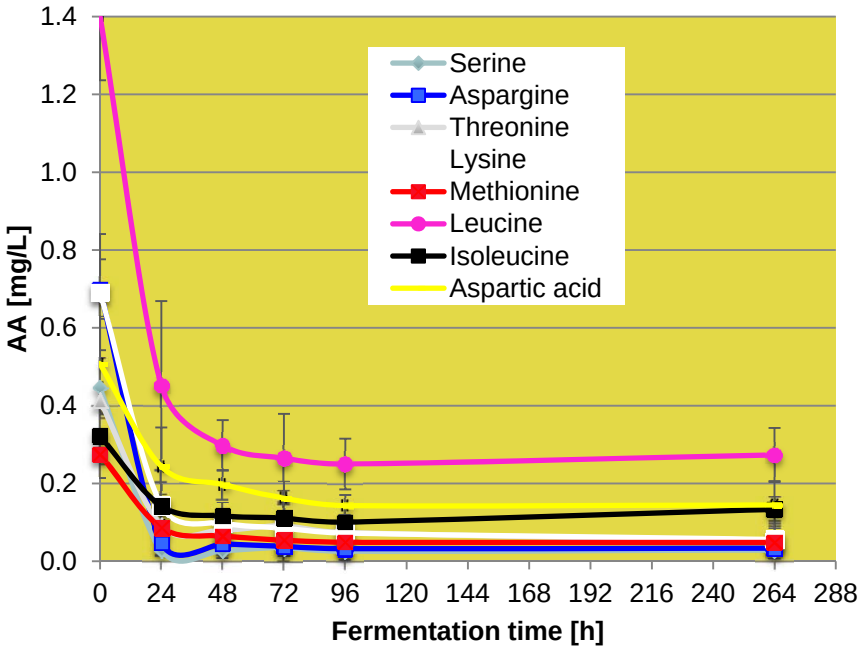
Results: Surface Charge of Cells in Suspension

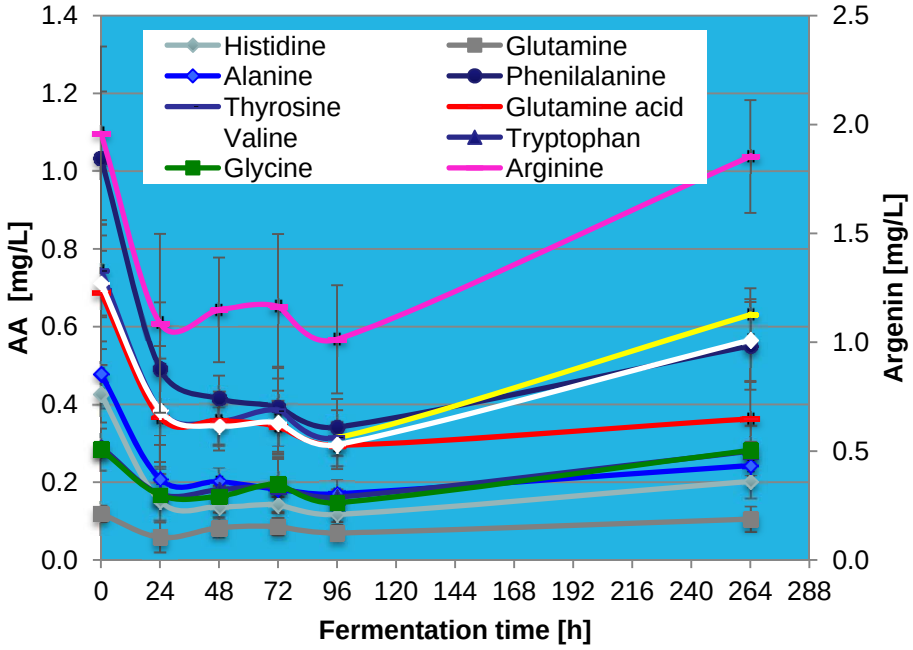


- The surface charge of the cells/particles in suspension increases independently of the yeast strain used for fermentation
- In the sedimented yeast cells/particles, no such an effect could be observed

Results: Amino Acid Uptake

Amino Acid Consumption during Fermentation



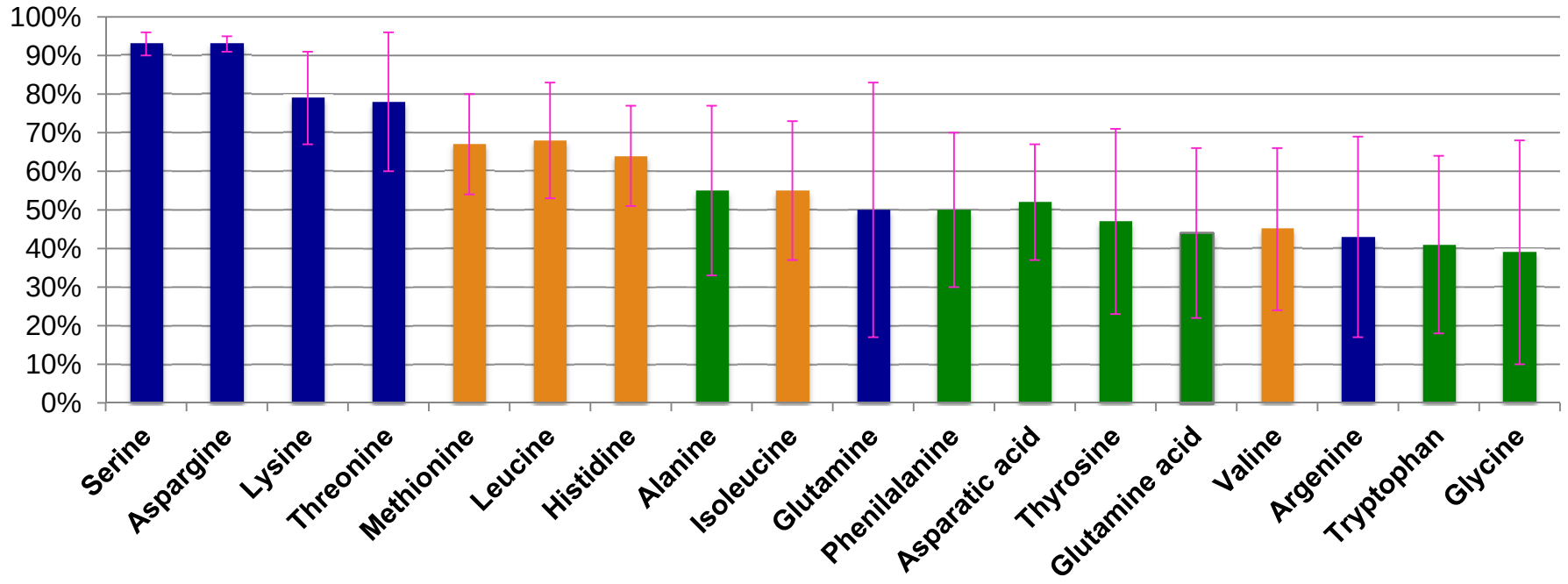


- **Group I** : The AA are consumed to a certain extent during primary fermentation

- **Group II**: After primary fermentation, the AA are again released into the beer

Results: Amino Acid Uptake

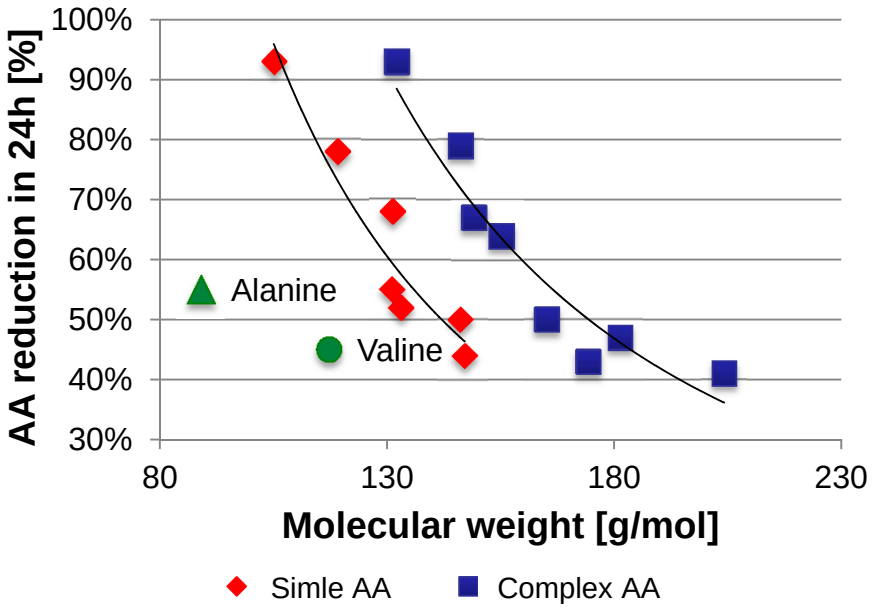
Amino Acid Consumption in 24 h Fermentation



- The AA serine and asparagine were consumed most quickly and independently of the applied yeast stem.
- The uptake rates of the described groups published in the literature (here indicated in different colors) could only partially be confirmed…

Results: Amino Acid Consumption within 24h

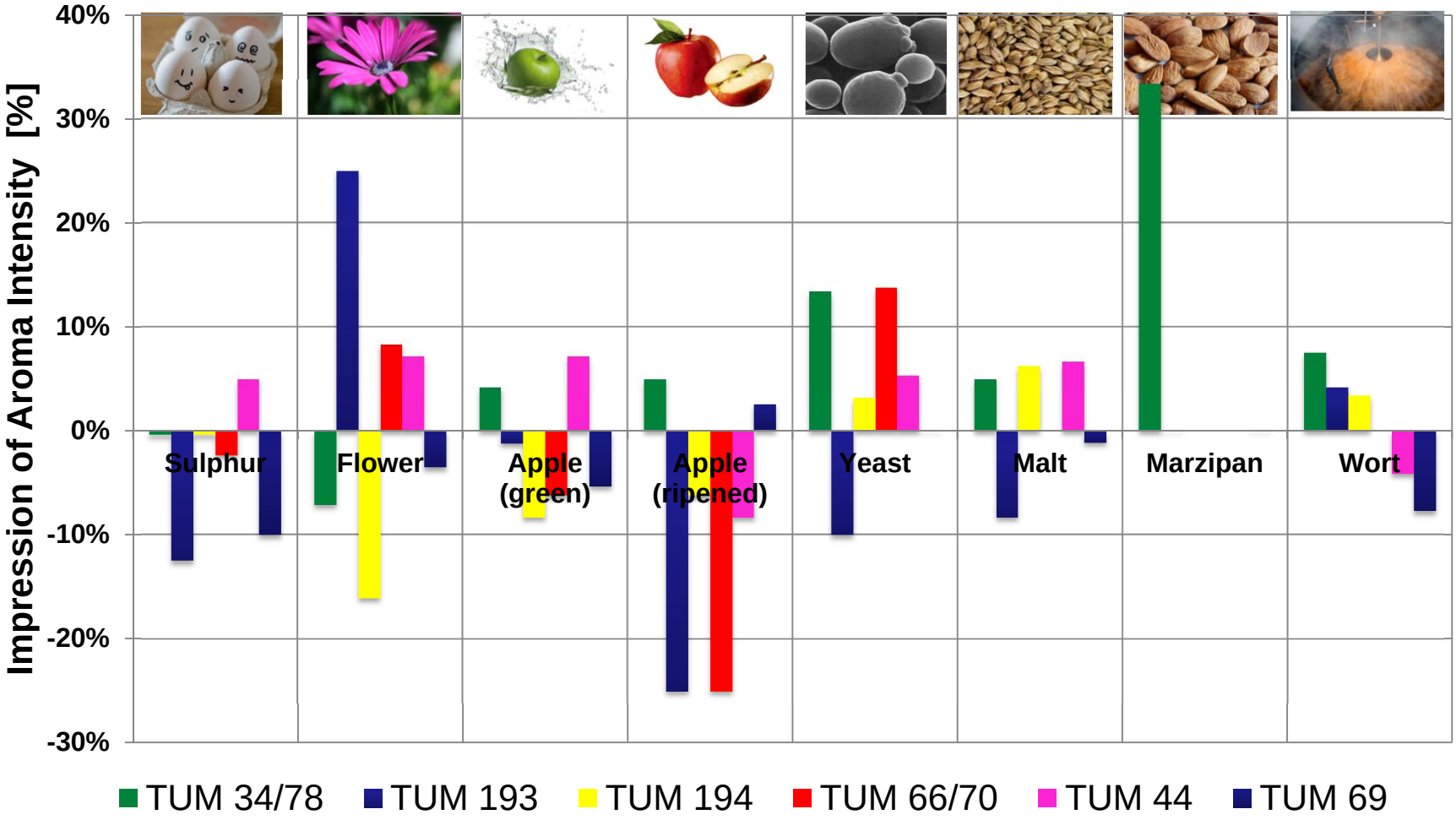
Simple AA	Complex AA
Serine	Aspargine
Threonine	Lysine
Leucine	Methionine
Isoleucine	Histidine
Aspartic acid	Phenilalanine
Glutamine	Argenine
Glutamine acid	Thyrosine
Alanine	Tryptophan
Valine	



- The smaller the molecular weight, the quicker the uptake in 24h
- Alanine und Valine exhibit different behavior…

- The more complex the AA (at similar molecular weight), the more quickly they are consumed within 24h (eg.: Argenine (mw:132) 2 x fast as Leucine (mw: 131))

Flavor and Aroma Profiles



Summary

- In this trials, six commercially available yeast strains were compared with one another under conditions simulating industrial beer production, in order to determine the differences in their characteristics.
- In doing so, 18 different properties were described under the application of uniform fermentation parameters.
- The yeast strains varied analytically and sensorially in numerous respects.
- New insights into the fermentation process, such as changes to the surface charge during fermentation or differences in amino acid reduction rates, were gained and need to be evaluated through further research.
- More yeast strains (also those exclusively used and owned by breweries) may be tested in order to compare their characteristics.

Thank you very much for your attention!

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