

Unveiling the potential of wine yeasts as lipid supplements

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Introduction

- Lipids: natural fats and fat-like substances that are insoluble in water, but soluble in organic solvents
- The lipids essential to yeasts are:
 - Fatty acids (saturated and unsaturated)
 - Sterols (ergosterol, phytosterols)
- Fatty acids can be incorporated into components of the plasma membrane
 - Phospholipids
 - Sphingolipids
- Fatty acids and sterols can also be stored (TAG, steryl esters)

Smith *et al.* (2000); Mbuyane *et al.* (2021)

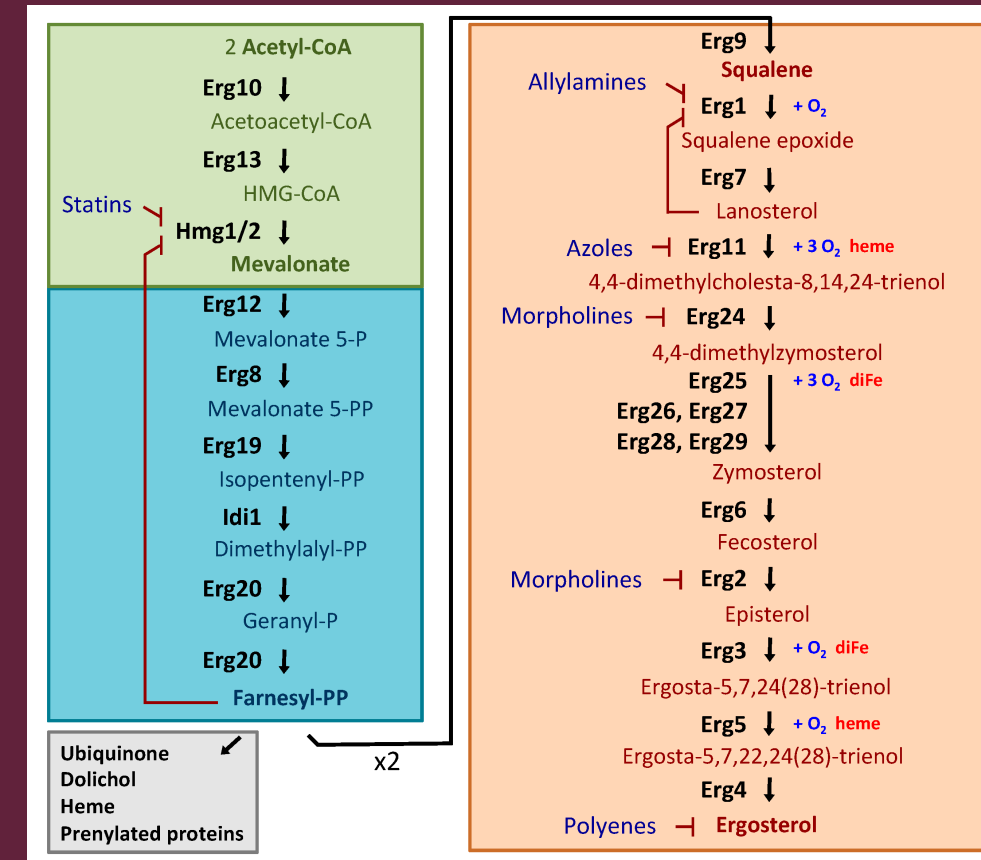
Role of lipids in yeast

- Important for cell survival throughout fermentation
 - Sterols required for initiation of yeast cell growth
- Role in nitrogen assimilation
- Adaptations to stress
 - Anaerobiosis, ethanol toxicity, nutrient stress, temperature variation, weak acid stress
 - Brought about by varying membrane lipid composition
- Important for aroma
 - Excess lipids and fatty acids used to produce aroma compounds
 - Maintaining a lipid balance - too much MCFA = toxic

Luparia *et al.* (2004); Mbuyane *et al.* (2021), Girardi-Piva *et al.* (2022)

Lipid availability during alcoholic fermentation

- Under anaerobiosis, yeasts are unable to synthesize ergosterol and unsaturated fatty acids
 - Depend on alternative sources
- Onset of climate change brings about challenges
 - Faster ripening of berries
 - Imbalances in nutrients and nitrogen
 - pH fluctuations
- Winemaking practices changed to accommodate for these challenges
 - Lower temperature fermentations
 - Excessive clarification of grape must



Jorda & Puig (2020)

Singleton *et al.* (1975); Yunoki *et al.* (2004); Nicolini *et al.* (2011)

Impact of over-clarification

- Over-clarification of white grape juice may result in lipid deficiency
- Winemakers retain a certain level of grape solids to ensure complete AF
- Grape lipid composition is highly variable
 - Cultivar and region-dependent
- Exploring yeast-derived lipids may be warranted

Lipid diversity in yeasts

- Yeasts exhibit species-specific lipid profiles
 - Large variations also exist between strains
- Explained by variations in:
 - Natural habitat
 - Growth conditions
 - Strain-specific genotypic and/or phenotypic adaptations
- Yeasts that accumulate >20% (w/w) total lipids - oleaginous yeasts
 - Lipid composition is largely UFA and PUFA
 - Lipid accumulation impacted by medium and culture conditions

Smith *et al.* (2000); Mbuyane *et al.* (2021)

Harnessing oleaginous yeasts as lipid supplements

- Oleaginous yeast species occur in the broader wine environment
 - Must be explored in greater depth
- Inactivated yeast supplements are commercially available
 - None of these produced from non-*Saccharomyces* for lipid supplementation
 - Following recent resolution by OIV (Resolution OIV-OENO 740-2024), non-*Saccharomyces* allowed
 - Opportunity to utilize yeasts richer in lipid composition

Aims and Objectives

- The project aimed to assess the viability of using wine yeasts as lipid supplements in fermentation, while simultaneously investigating the lipid profile of *Saccharomyces cerevisiae* and selected non-*Saccharomyces* wine yeasts.

Objective 1: Screening *S. cerevisiae* and non-*Saccharomyces* wine yeasts by quantifying their composition of fatty acids and sterols, total lipids and intracellular lipids.

Objective 2: Producing and testing a small selection of inactivated yeasts screened in Objective 1, and assessing their impact on fermentation kinetics and metabolite production in fermentations inoculated with a commercial *S. cerevisiae* strain.

Yeast selection

Yeast isolate	Previously described as oleaginous
<i>Candida oleophila</i>	✓
<i>Candida railensis</i>	✓
<i>Metschnikowia pulcherrima</i>	✓
<i>Rhynchogastrema visegradensis</i>	✓
<i>Rhodotorula glutinis</i>	✓
<i>Rhodotorula mucilaginosa</i>	✓
<i>Wickerhamomyces anomalus</i>	✓
<i>Yarrowia lipolytica</i>	✓
<i>Hanseniaspora guilliermondii</i>	
<i>Hanseniaspora uvarum</i>	
<i>Saccharomyces cerevisiae</i>	
<i>Starmerella bacillaris</i>	
<i>Torulasporea delbrueckii</i>	

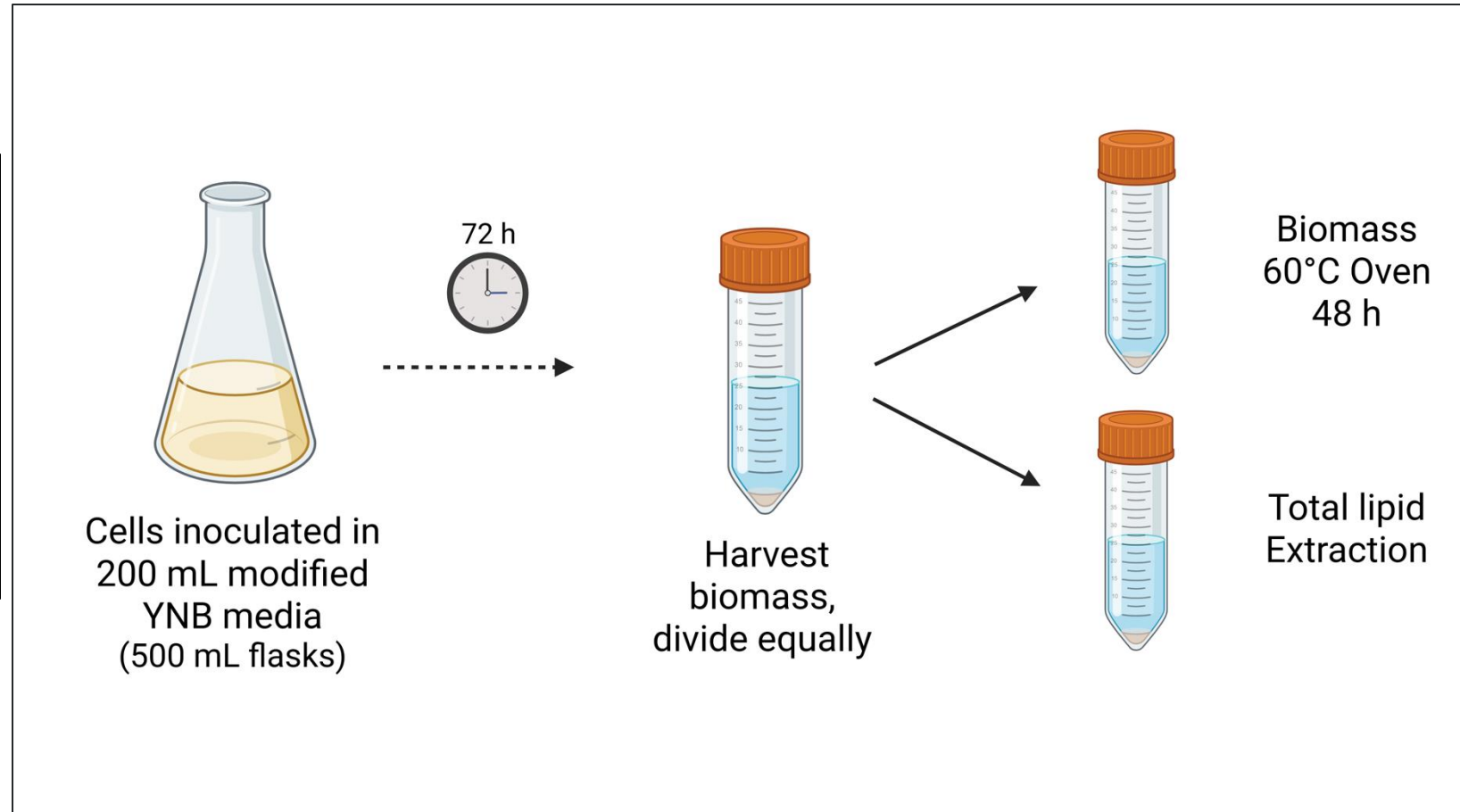
Thorpe & Ratledge. (1972); Rattray *et al.* (1975);
 Piña *et al.* (2003); Beopoulos *et al.* (2012);
 Kolouchová *et al.* (2016); Kot *et al.* (2016);
 Arous *et al.* (2017); Tatay-Núñez *et al.* (2024)

Methodology: Objective 1

Total lipid extraction

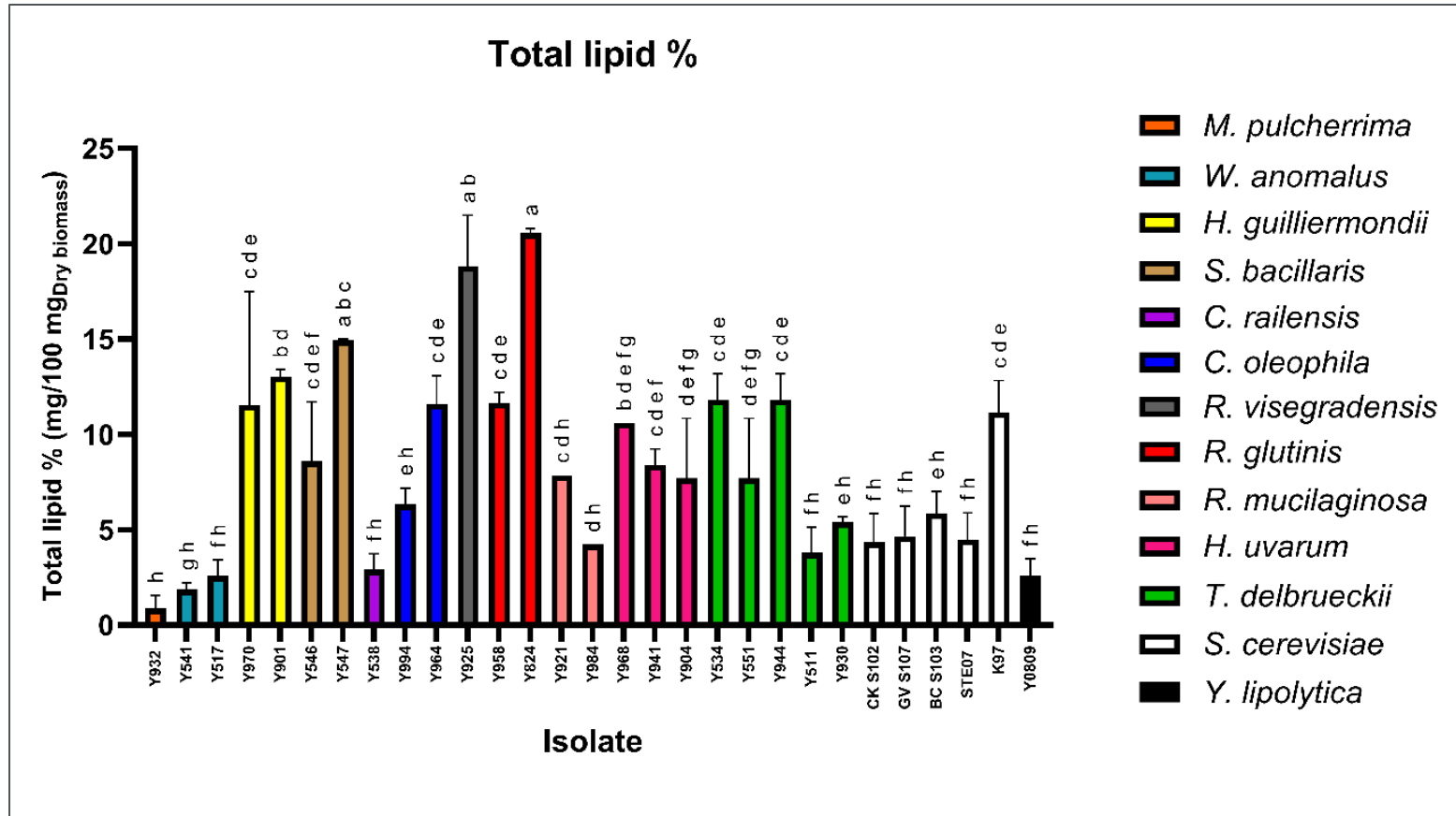
MODIFIED YNB:

- YNB without amino acids and ammonium sulphate
- 50 g/L glucose
- 1 g/L ammonium sulfate



Total lipid extraction Results and Discussion

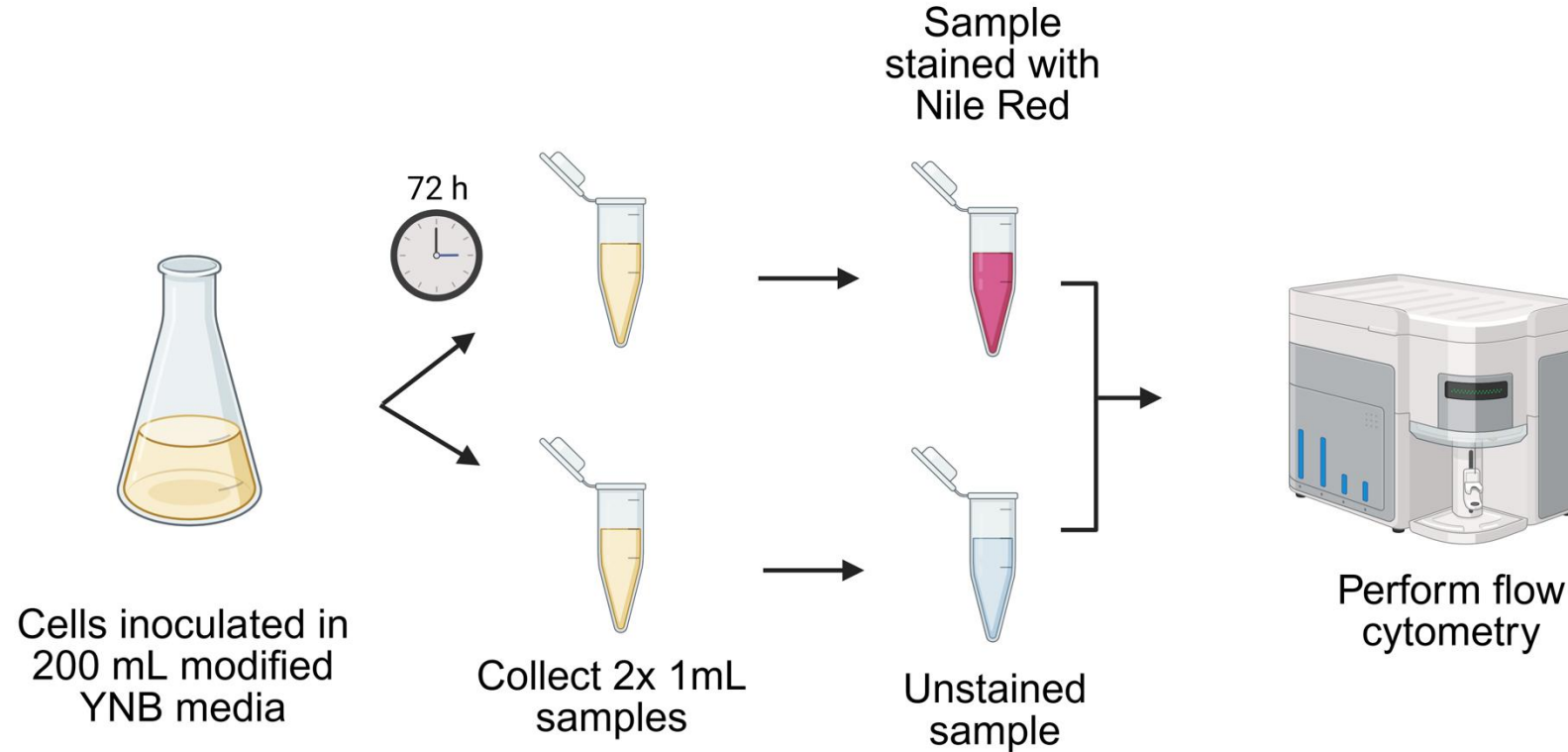
- Large differences in lipid diversity observed
 - Strain-specific variability
- Little variation observed for strains of *S. cerevisiae*
 - *S. cerevisiae* SafAle™ K97
 - Likely due to evolution
- Performance of some yeasts was interesting
 - *T. delbrueckii*, *Y. lipolytica*, *R. mucilaginosa*, *Candida spp*



(Rupcic & Juresic., 2010; Bonatto., 2022)

Methodology: Objective 1

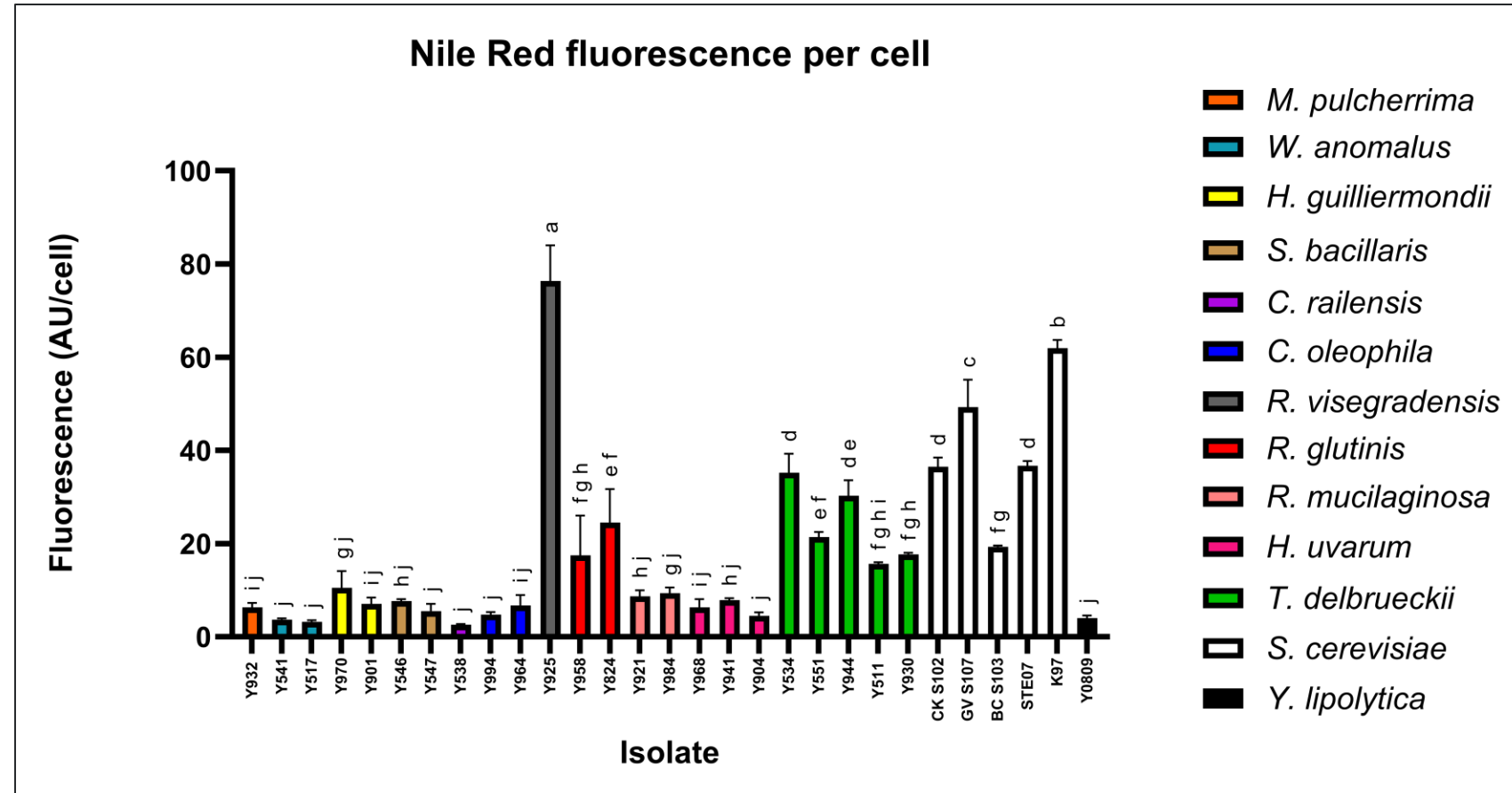
Intracellular lipid quantification



Intracellular lipid quantification

Results and discussion

- *R. visegradensis*: highest intracellular lipid concentration
- Similar trends as total lipid quantification
 - *S. cerevisiae*, *T. delbrueckii*, *R. visegradensis*
 - Suggests lipid composition mostly consists of intracellular/stored lipids
- Oleaginous yeasts performed poorly
 - Culture medium not optimal
 - Selected as best compromise



AU: Arbitrary Units - Ratio of relative change in fluorescence between the sample and a reference sample (calibration standard)

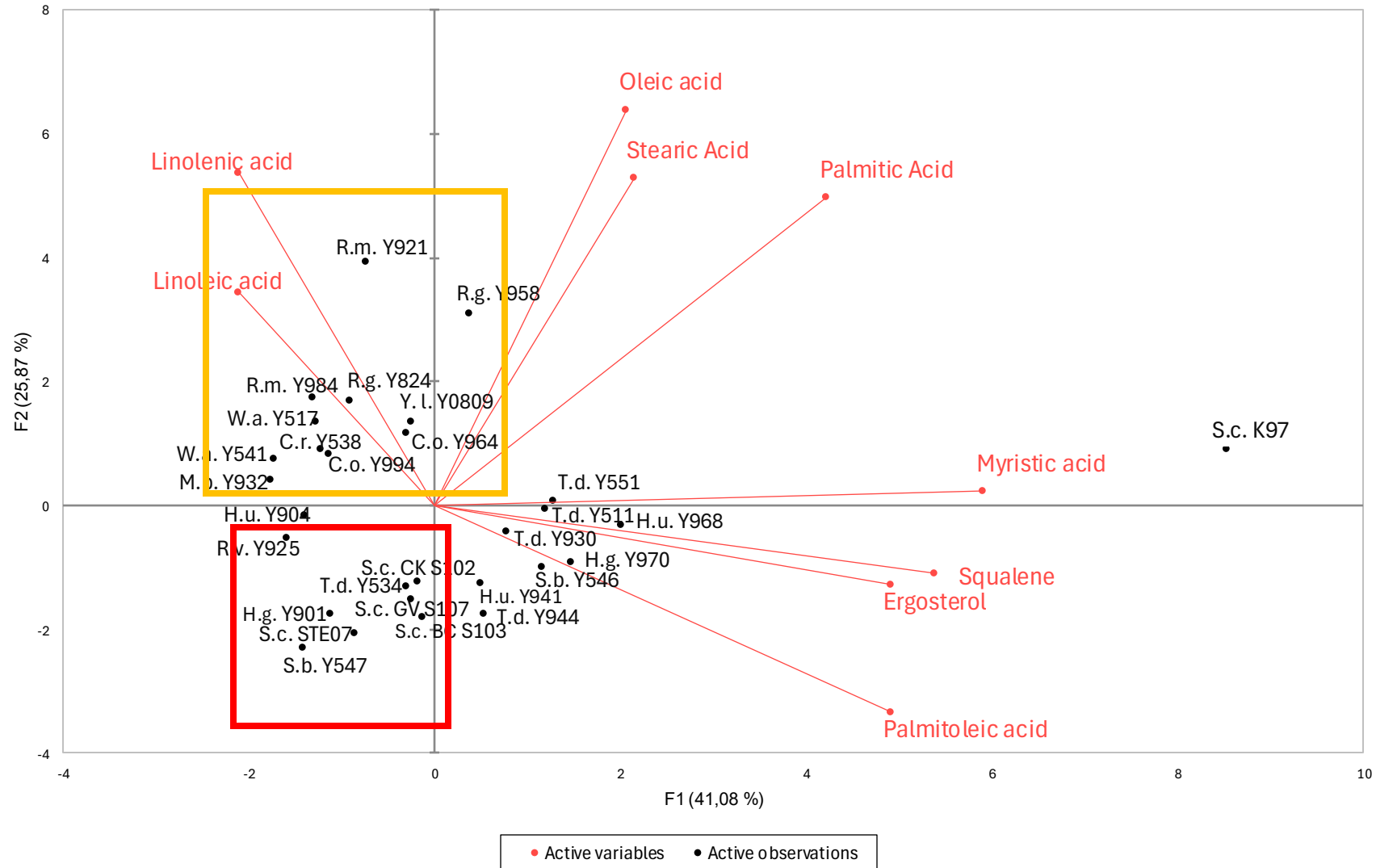
Methodology: Objective 1

Fatty acid and Sterol Quantification

- Cells were incubated in 200 mL modified YNB for 72h
- Extracted according to Williams *et al.* (2021), briefly:
 1. Non saponifiable lipids removed with solvent wash
 2. Biomass freeze-dried
 3. Lipid saponification (Tumanov *et al.*, 2016)
 4. Acid hydrolysis of lipids
 5. Centrifugation and organic layer collected
 6. Derivatization to volatilize lipids
 7. GCMS analysis

Fatty acid and sterol quantification Results

Biplot (axes F1 and F2: 66,95 %)



Yeast selection for Objective 2

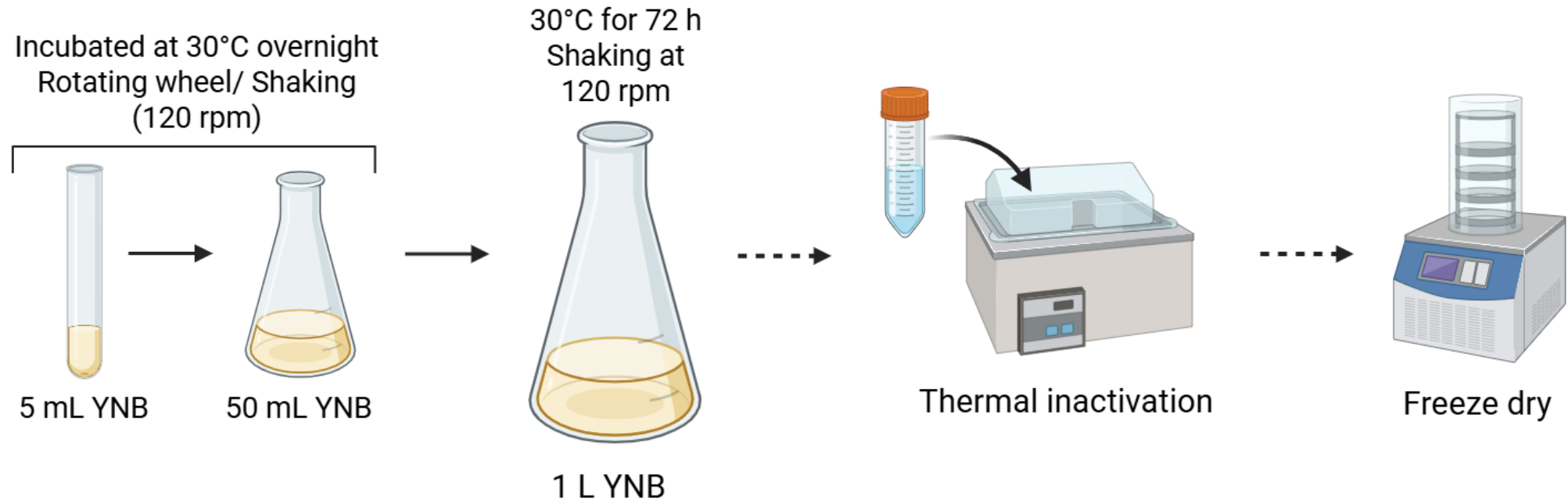
- Following screening experiments, six yeast isolates were selected for use in objective 2.
- Selected based on:
 - Total lipid accumulation
 - Intracellular lipid content
 - Fatty acid and sterol composition

Selected isolates

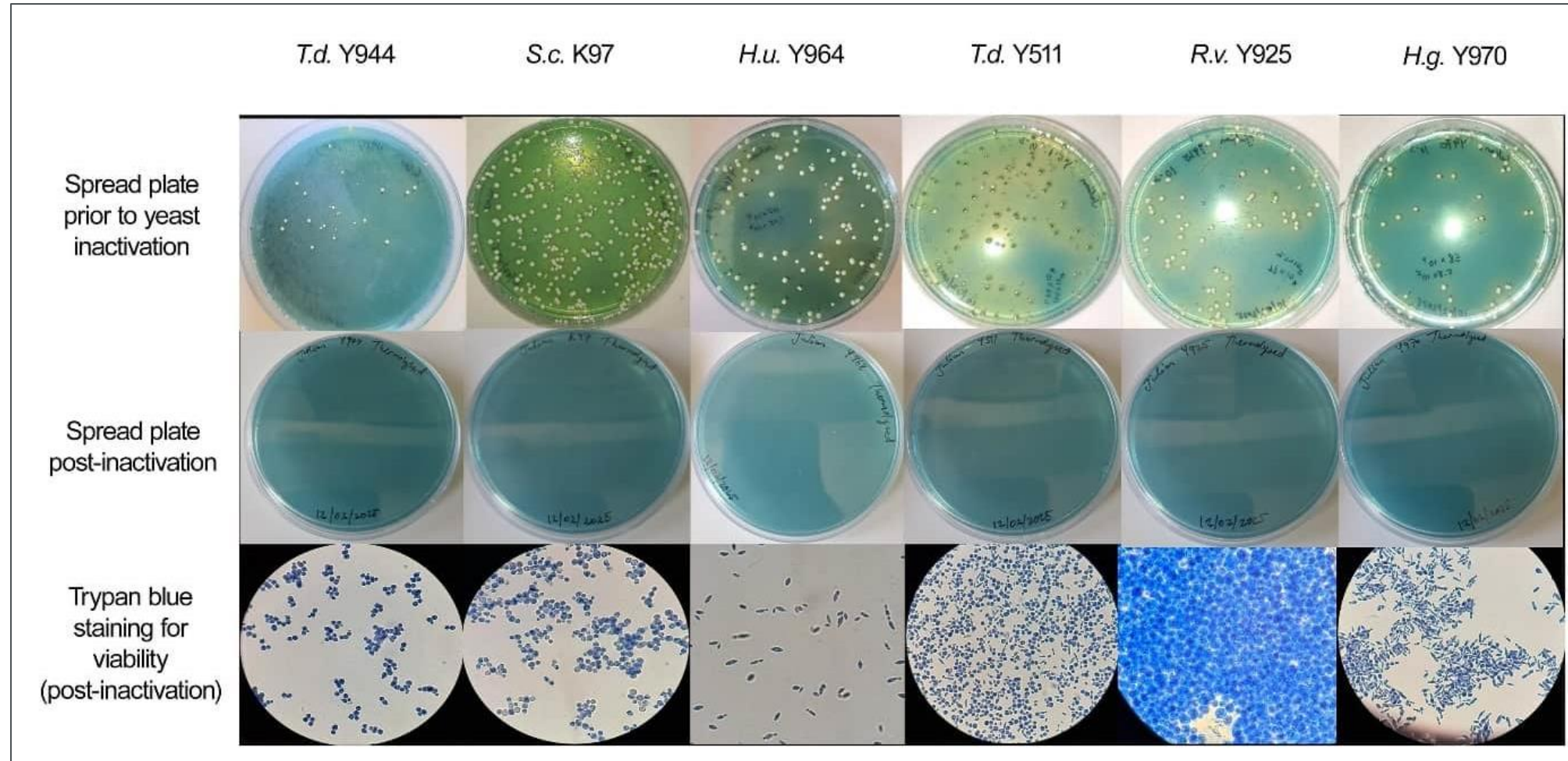
Yeast strain	High total lipid %	High intracellular lipid content	High fatty acid and sterol concentrations
<i>S. cerevisiae</i> SafAle™ K97	✓	✓	✓
<i>T. delbrueckii</i> Y511			✓
<i>H. uvarum</i> Y968			✓
<i>T. delbrueckii</i> Y944	✓	✓	
<i>R. visegradensis</i> Y925	✓	✓	
<i>H. guilliermondii</i> Y970	✓		✓

Methodology: Objective 2

Preparing inactivated yeasts



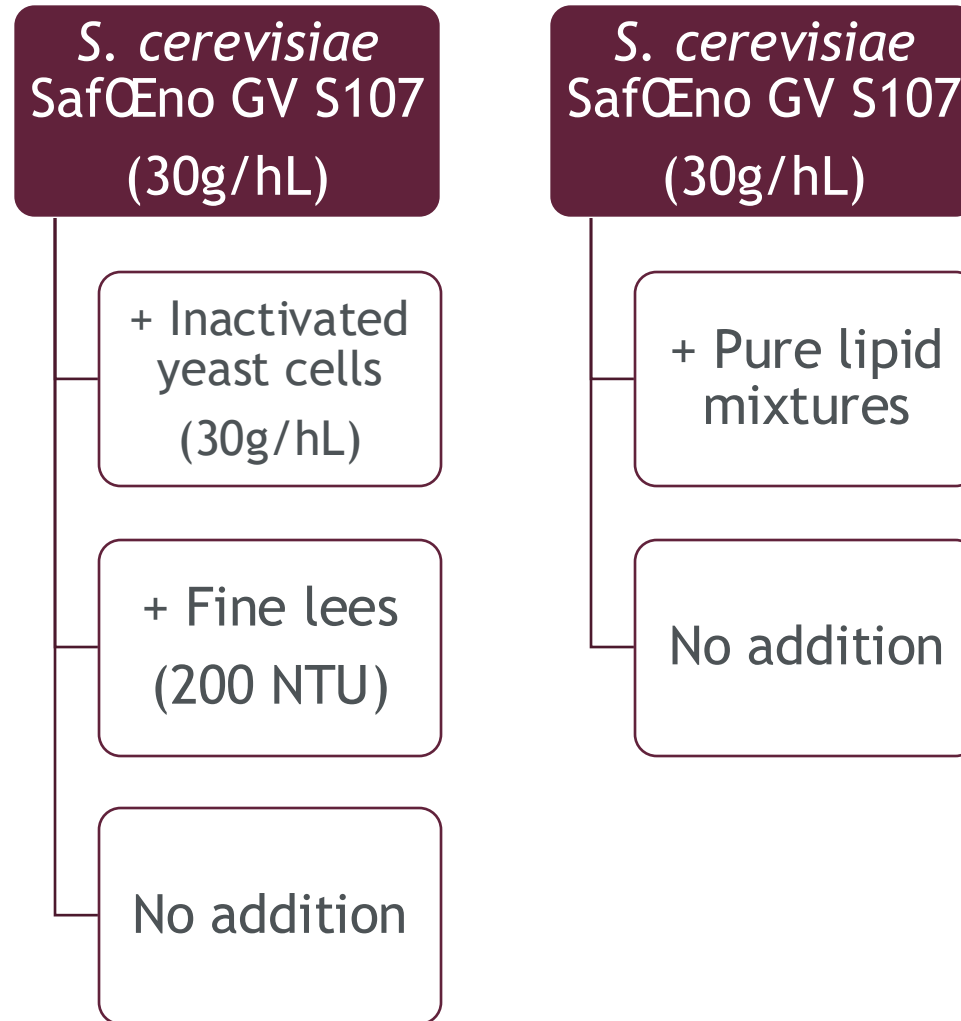
Yeast inactivation Confirmation



Grape juice parameters

- Chenin Blanc
- 25° Brix
- 17.7 NTU
- YAN adjusted to 225 mg/L
- pH: 3.49

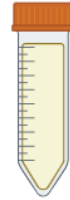
Fermentations Experimental Design



Fermentation monitoring and chemical analyses



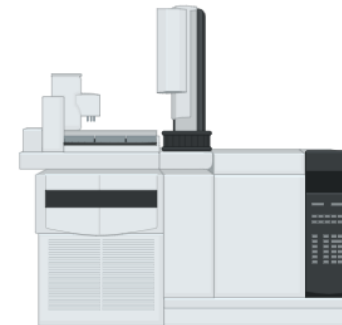
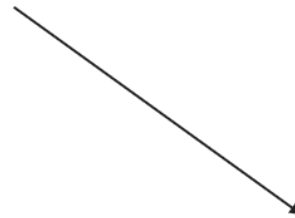
Measure
Fermentation
kinetics
(cumulative
weight loss -
CO₂ release)



Wine Sample



HPLC: Sugars and
Organic acids

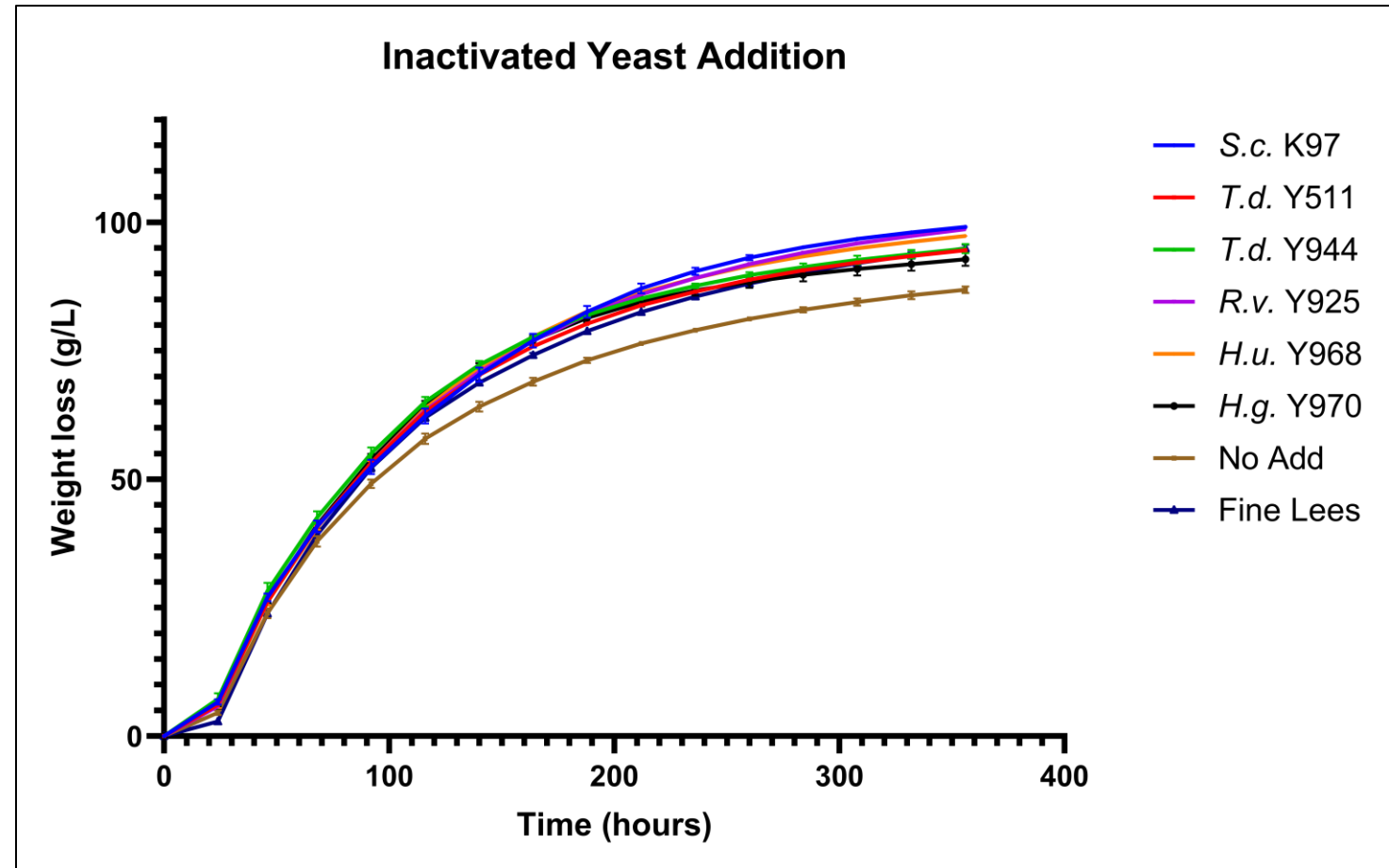


GCMS: Volatile
Organic Compounds

Objective 2: Results and Discussion

Fermentation Kinetics

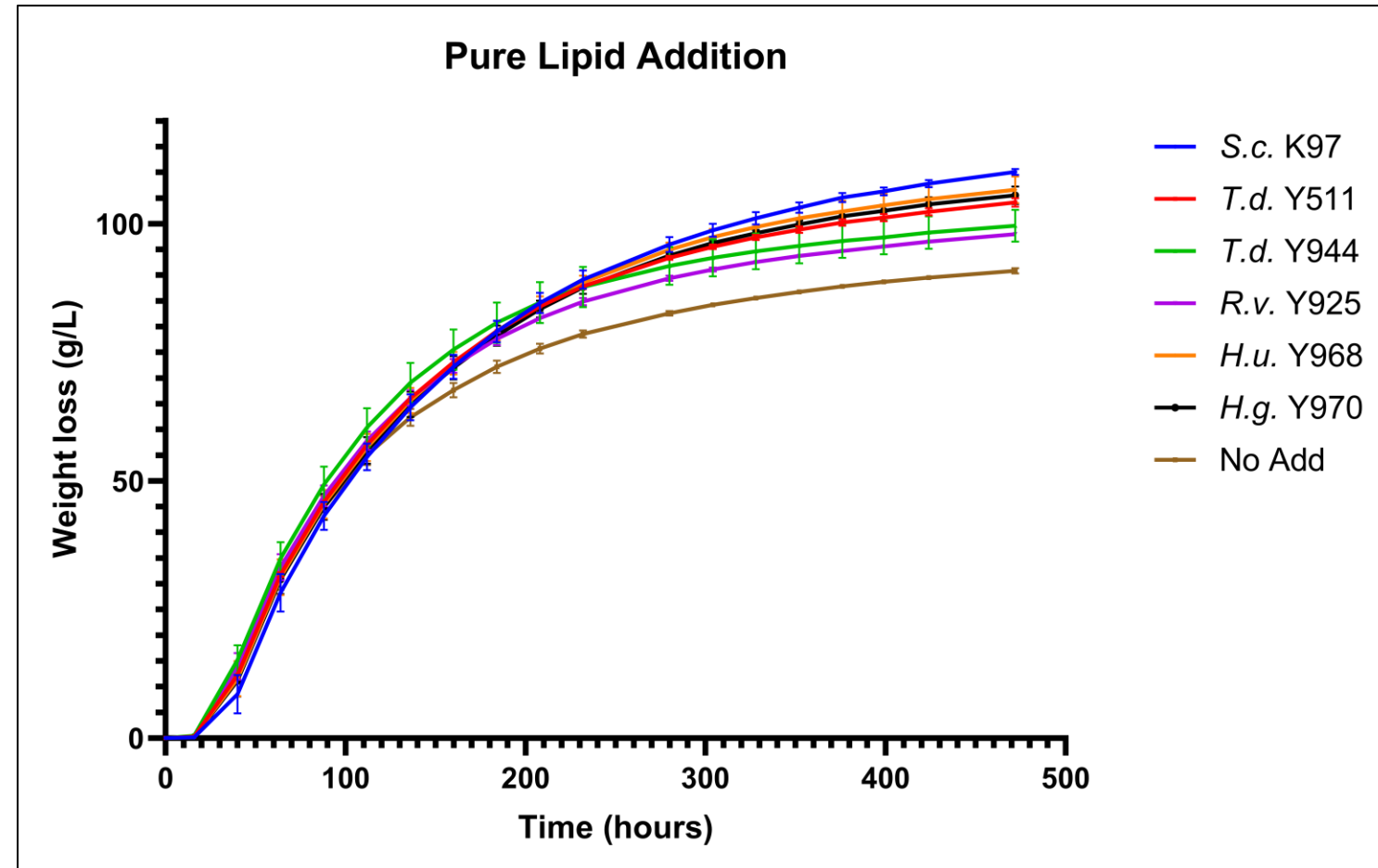
- No addition performed the worst
- Addition of grape solids shown to improve fermentation kinetics
- Addition of inactivated *S. cerevisiae* SafAle™ K97: Best performing
- All fermentations ceased prematurely
- Supplementing with inactivated yeasts more effective
- Assimilation of phytosterols not as efficient as that of ergosterol and fatty acids



(Nicolini *et al.*, 2011; Guittin *et al.*, 2021; Girardi-Pivá *et al.*, 2022)

Objective 2: Results Fermentation Kinetics

- When supplementing with pure lipid mixtures, broader trends conserved
- *T. delbrueckii* Y511, Y944
 - Y511 produced more UFA than Y944
 - UFA may play greater role than sterols
- Likely due to bioavailability of pure lipid mixtures
- Targeted GCMS analysis did not account for all lipids and sterol intermediates



Discussion

Fermentations with inactivated yeasts and pure lipids

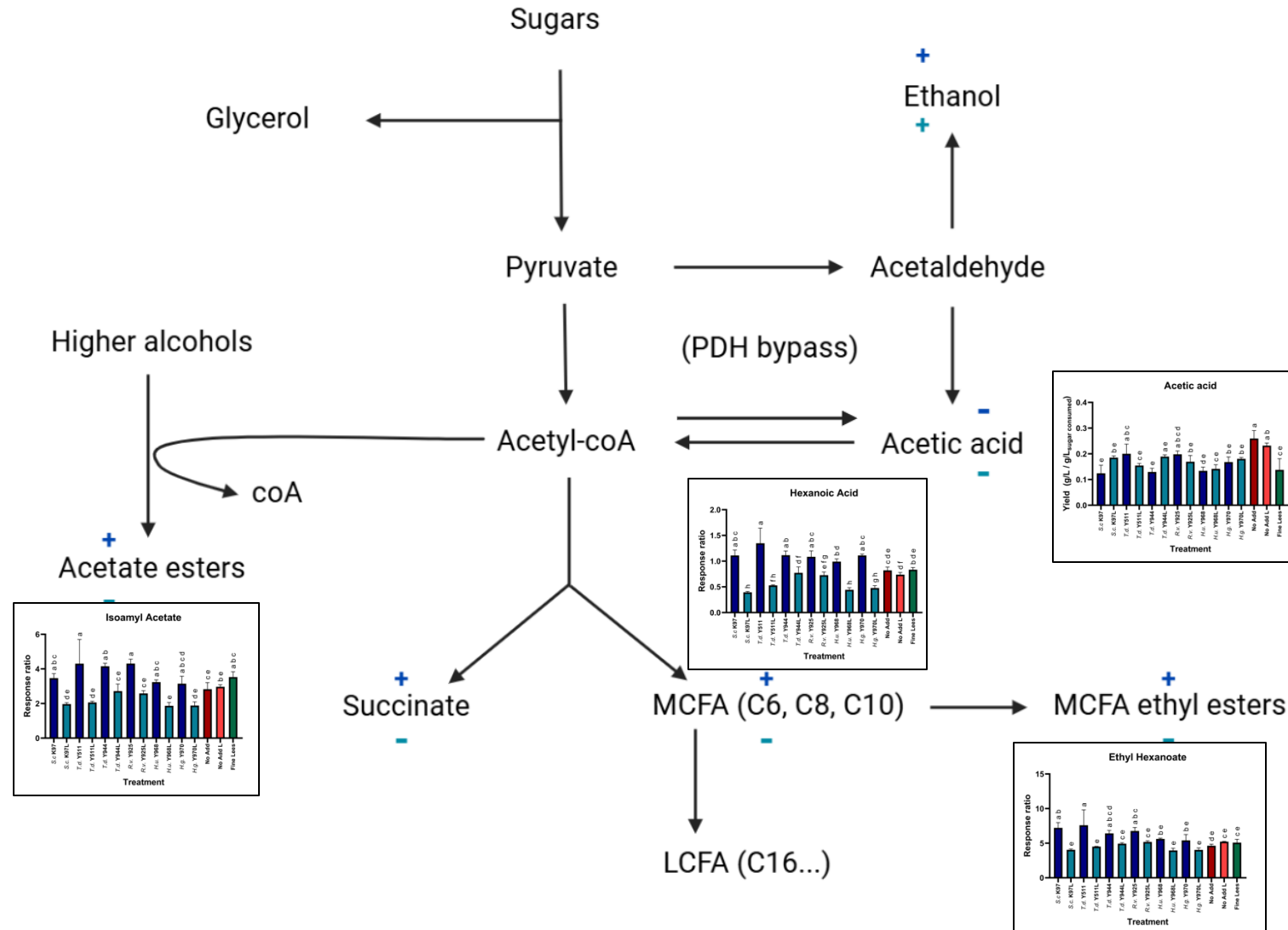
- Improvement of fermentation kinetics confirms findings of previous studies
(Belviso *et al.*, 2016; Luparia *et al.*, 2004; Ochando *et al.*, 2017; Fairbairn *et al.*, 2019)
- Fermentations ceased before dryness
- Likely due to high sugar concentration, overclarification and yeast strain used
 - Likely sensitive to the nitrogen:lipid imbalance (Tesnière *et al.*, 2013; Rollero *et al.*, 2016)
- Yeasts with high ergosterol and UFA had best fermentation performance

Results and Discussion

Chemical analyses

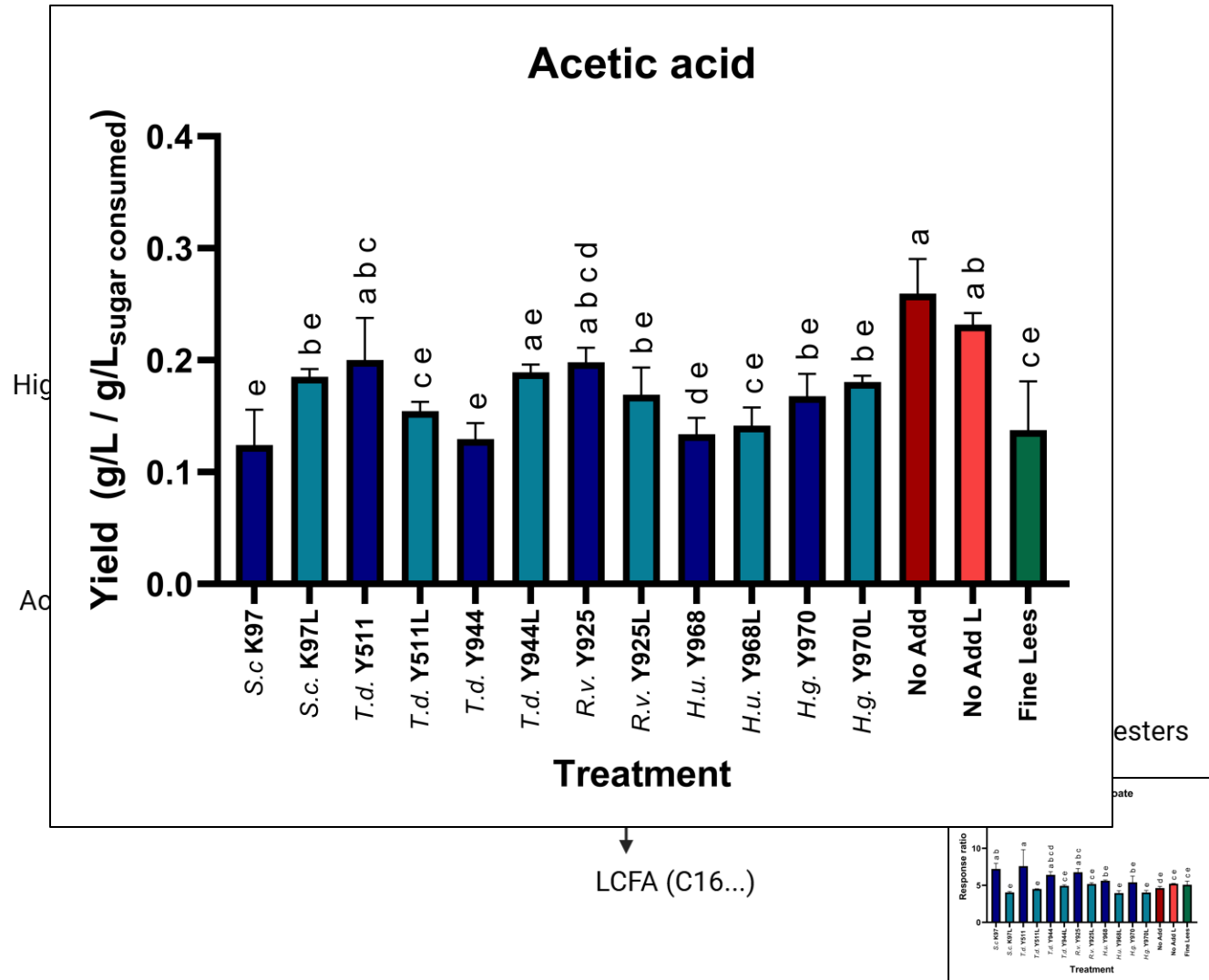
Objective 2: Results

Impact of lipids and inactivated yeasts on yeast metabolism: Overview



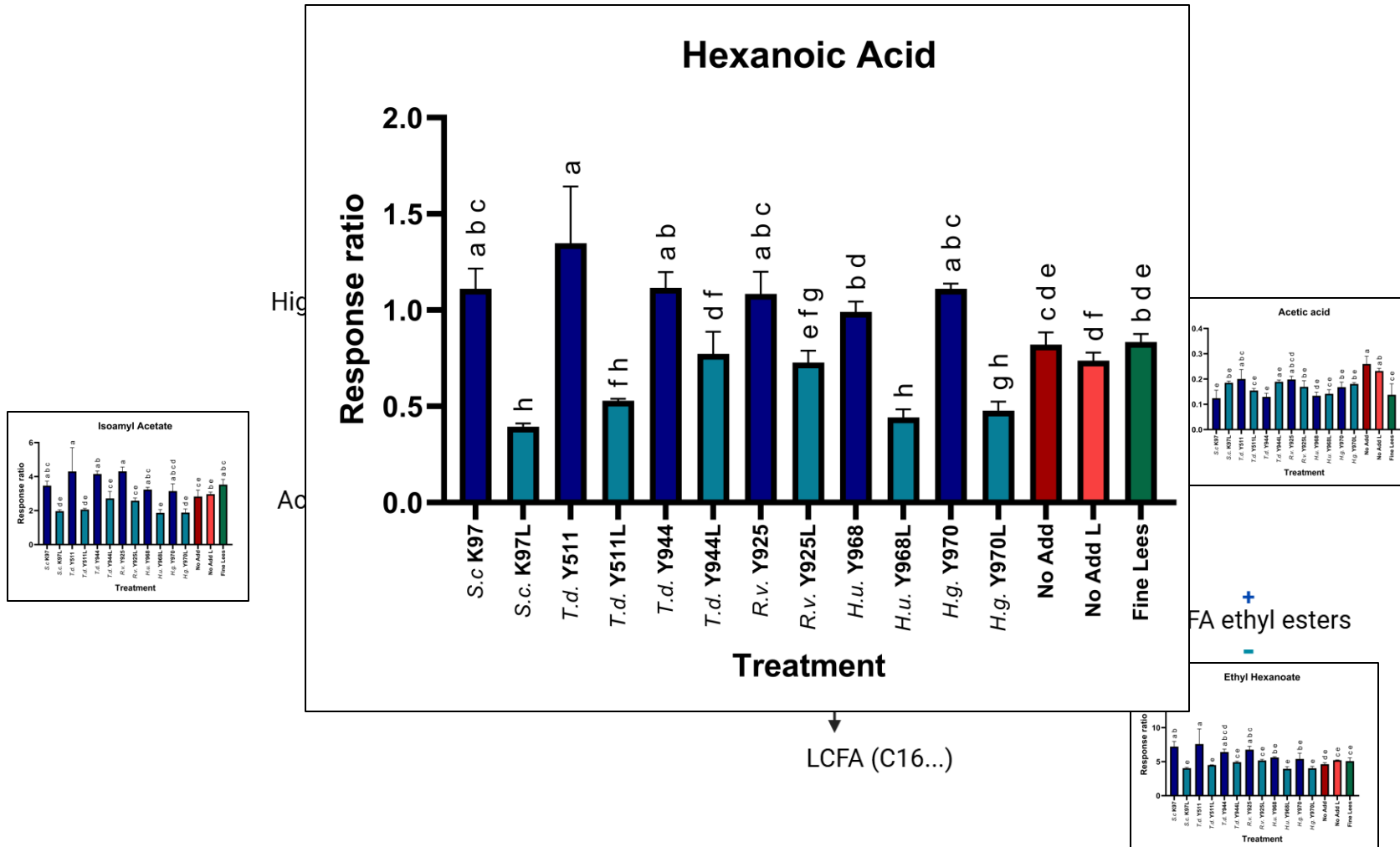
Objective 2: Results

Impact of lipids and inactivated yeasts on medium-chain fatty acids



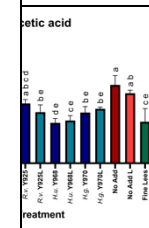
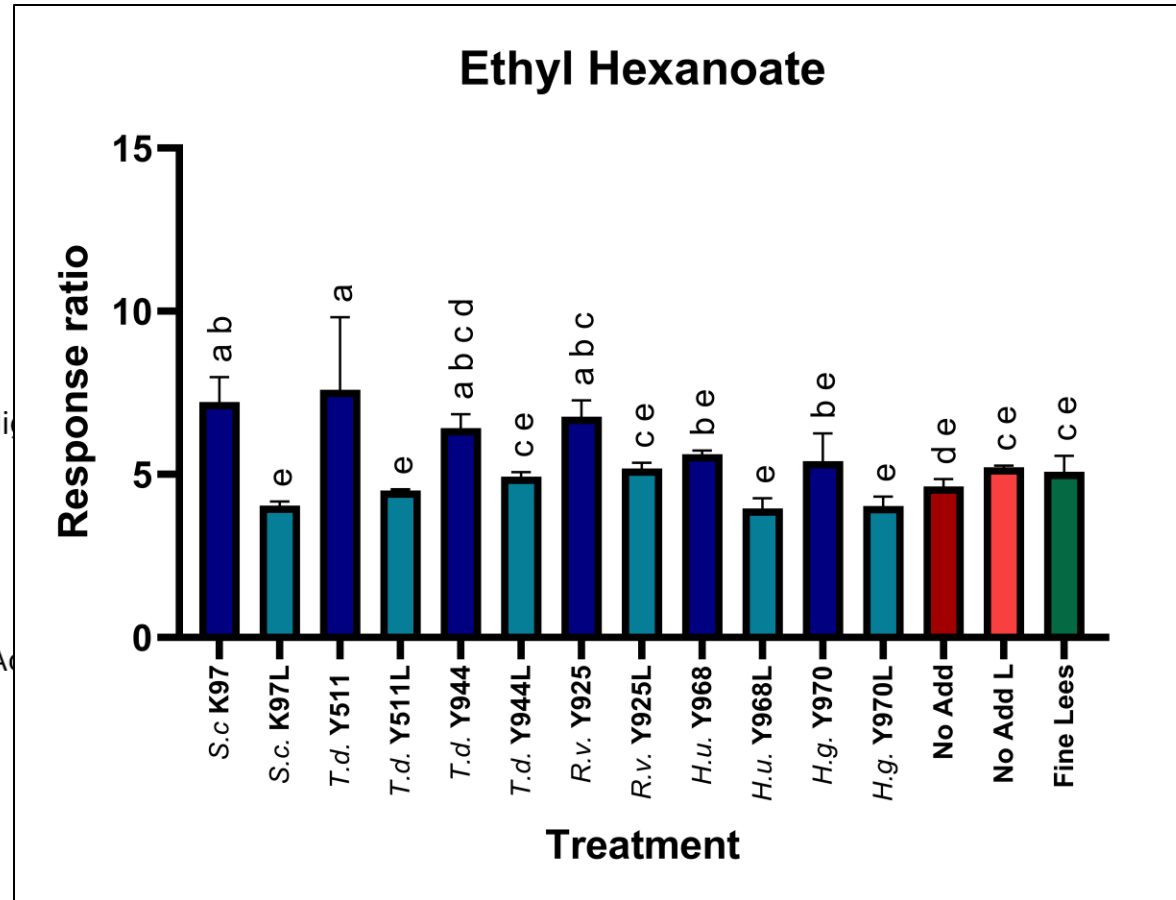
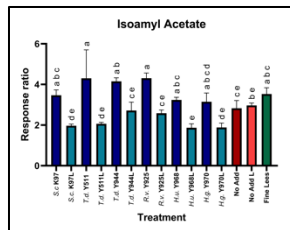
Objective 2: Results

Impact of lipids and inactivated yeasts on medium-chain fatty acids



Objective 2: Results

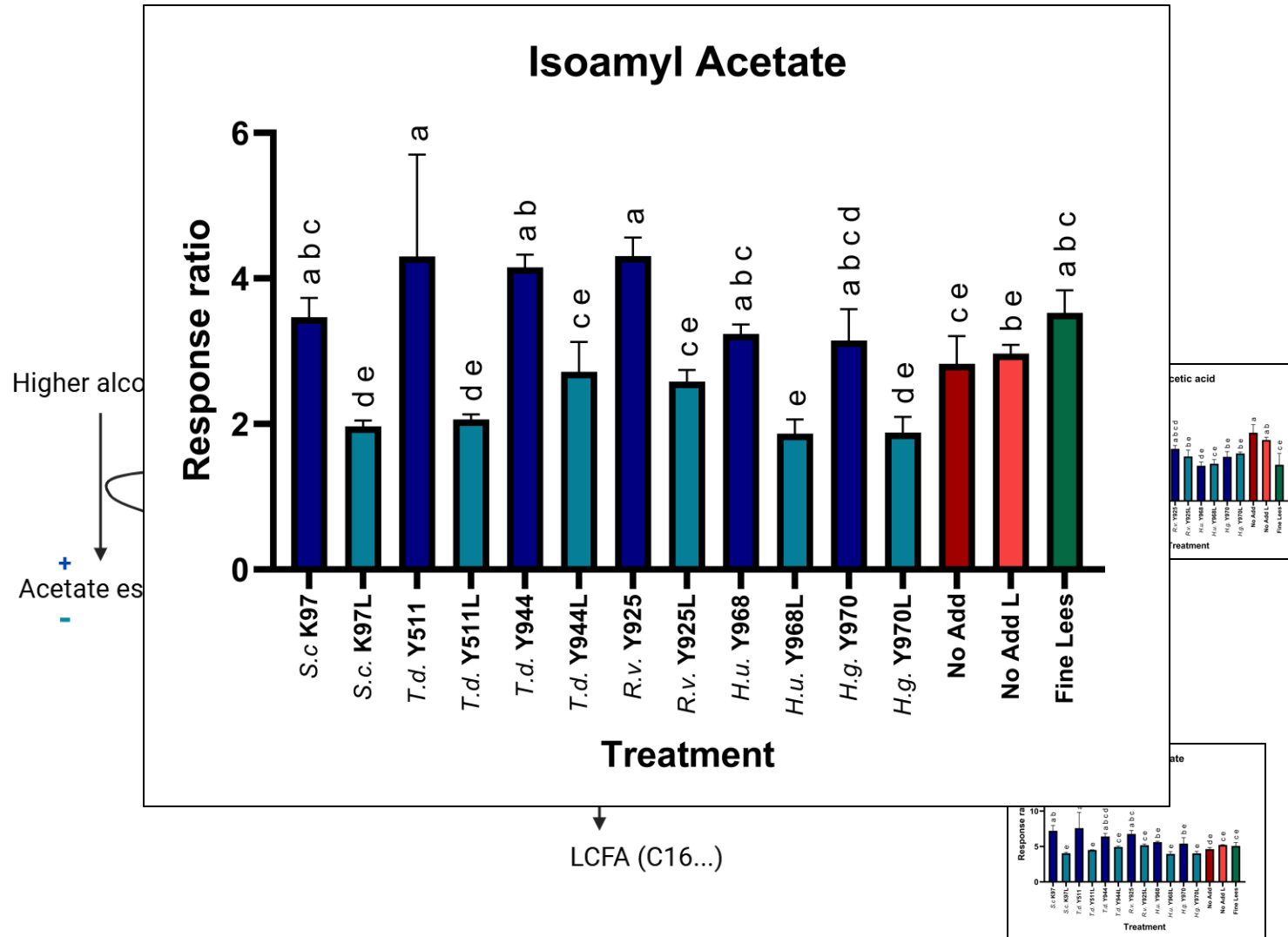
Impact of lipids and inactivated yeasts on ethyl esters



LCFA (C16...)

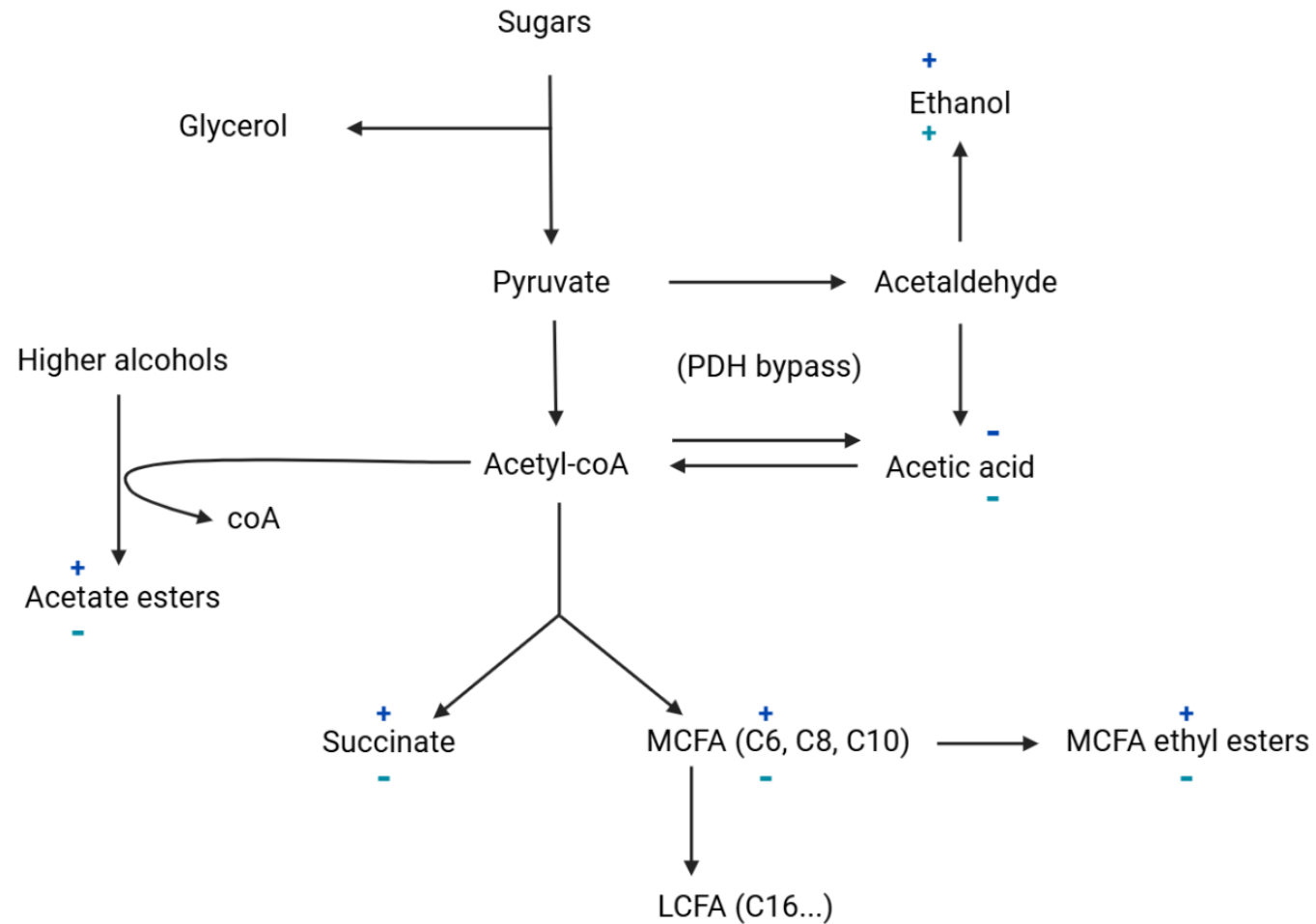
Objective 2: Results

Impact of lipids and inactivated yeasts on acetate esters



Objective 2: Results

Impact of lipids and inactivated yeasts on yeast metabolism: Overview



Conclusions

- In this study, a diversity of lipid profiles in wine yeasts were observed
 - Strain-specific variations in lipid composition
- Novel insights into lipid diversity of wine yeasts were provided
- Supplementation of inactivated yeasts more effective
 - Improved fermentation kinetics and aroma compound production over reintroducing grape solids
- Effect of lipid bioavailability was highlighted in this study
 - Requires further investigation

Future outlooks

- Improved culture medium and/or culture method
- Screening a larger number of strains
 - Contribute to the knowledge of wine yeast lipid diversity
- Quantifying lipid composition of grape juice
 - Confirm hypotheses regarding lipid bioavailability
- Optimizing the type of yeast derivative used as a nutritional supplement
- Conducting larger scale fermentations - observe larger differences

ACKNOWLEDGEMENTS



- Dr Hans Eyeghe-Bickong, Dr Anke Berry, Mr Michael Weightman (Chemical analyses)
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Thank you
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Dankie
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