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SAGWRI South African Grape and Wine Research Institute

Evaluating Lactic Acid Bacteria for Volatile Phenol Release in Smoke Tainted Pinotage Must.

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Recap…

- Increased frequency of wildfires as a consequence of climate change has elevated the risk of producing smoke tainted wines.

- Smoke gets absorbed by the berries and vines
  - VPs glycosylated for storage - odourless

- Smoke (VPs) can negatively alter the aroma and flavour profiles of wine.
  - Smoky
  - Ashy
  - Burnt

(Ristic *et al*. 2011, Parker *et al*. 2013)

Expanding on prior studies

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Daniel Zieff

- Screened 30 non-*Saccharomyces* yeast strains for volatile phenol release in smoke exposed Pinotage grape juice.
- 3 yeast strains selected for further investigation:
  - *Lachancea thermotolerans* Y940
  - *Stamerella bacillaris* Y550
  - *Wickerhanomyces anomalus* Y541
- Quantified 6 target volatile phenols using GC-MS.
- Measured glycosidase enzyme activity of 3 selected strains with artificial substrates.

Phuti Lekokotla

- Screened 42 **LAB isolates** using an arbutin plate assay and experiments with smoke-exposed Pinotage grape juice – to assess their **ß-glycosidase activity.**
- Quantified the release of free volatile phenols using GC-MS.
- Evaluated LAB performance in small scale vinification scenarios.
- Identified LAB strains that displayed high release of VPs
  - *Latilactobacillus sakei* 115
  - *Lactiplantibacillus pentosus* 65.1
  - *Lentilactobacillus hilgardii* 87.2
  - *Oenococcus oeni* 192
  - *Lactiplantibacillus plantarum* 56

Aim

To assess the use of lactic acid bacteria in winemaking to maximize the enzymatic release of volatile phenols from their glycosylated precursors, thereby facilitating their downstream removal and reducing smoke taint in wine.

Experimental Design
Grape must & Fermentation trials

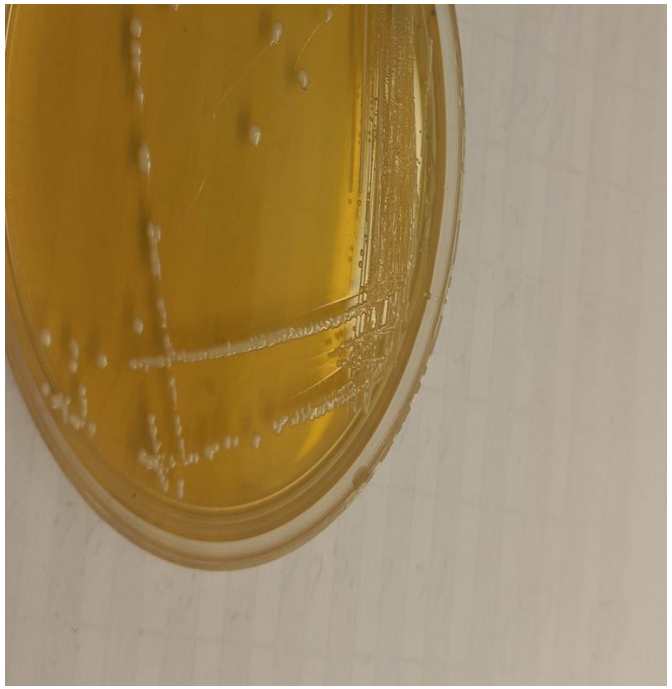
- Grape must preparation
  - Controlled smoking treatment of harvested Pinotage grapes.
  - Processing of smoked bunches.
    - Crushing
    - Cold maceration (48 hours)
    - Pressing
  - Clarification

- Fermentation scenarios:
  1. LAB inoculated 48 hours before *S. cerevisiae*
  2. Co-inoculation (LAB inoculated 24 hours after *S. cerevisiae*)
  3. Sequential inoculation (LAB inoculated after alcoholic fermentation)

Experimental Design
Microbial strains

- LAB Strains:
  - *Latilactobacillus sakei* 115
  - *Lactiplantibacillus pentosus* 65.1
  - *Lactiplantibacillus plantarum* 56
  - *Lentilactobacillus hilgardii* 87.2
  - *Oenococcus oeni* 192

- Yeast strain for alcoholic fermentation:
  - *Saccharomyces cerevisiae* NT 112



Preliminary Fermentations
Alcoholic Fermentation Kinetics

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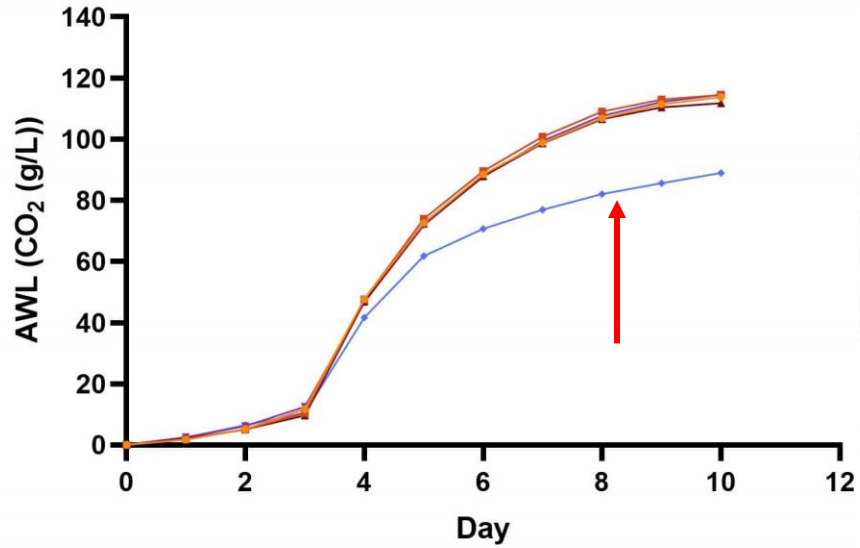
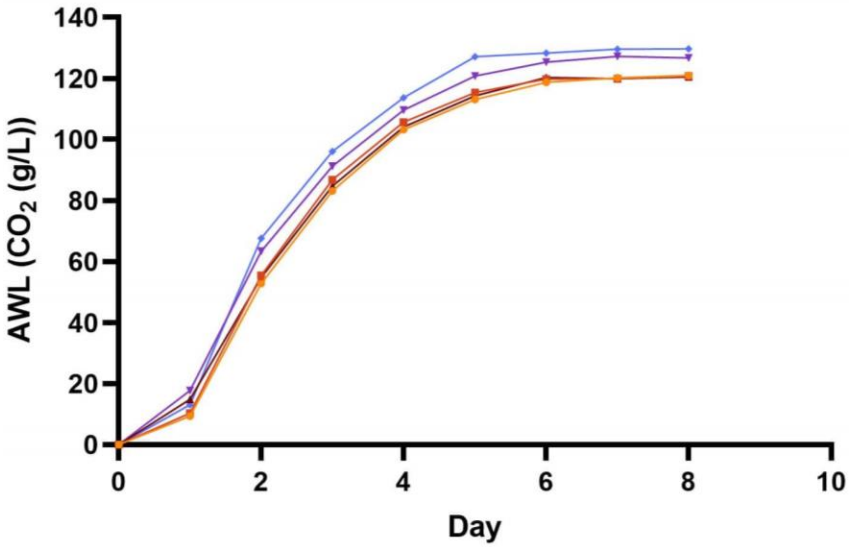
- Lat. sakei 115
- Lac. pentosus 65,1
- Lac. plantarum 56
- Len. hilgardii 87,2
- O. oeni 192

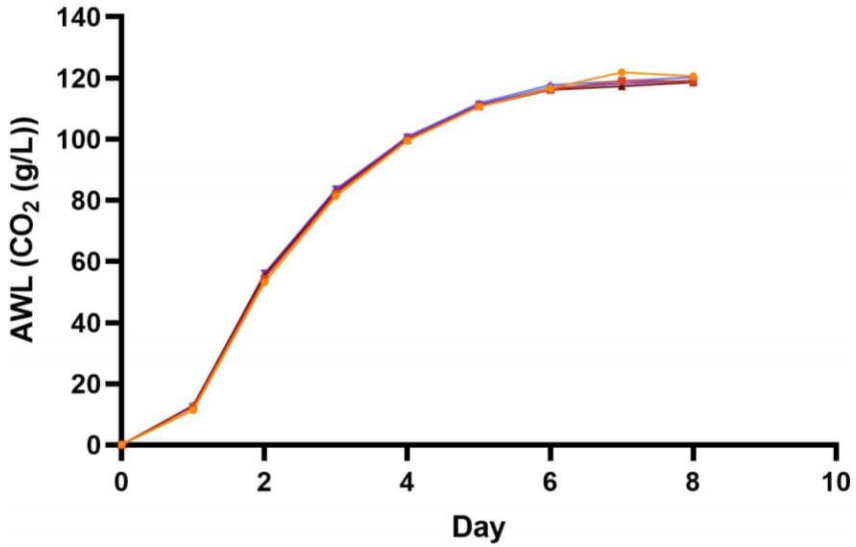
- Sugar: 240.80g/L
- pH: 3.39
- TA: 8.80 g/L
- YAN: 348.92 mg/L



LAB inoculated 48 hours before *S. cerevisiae*.

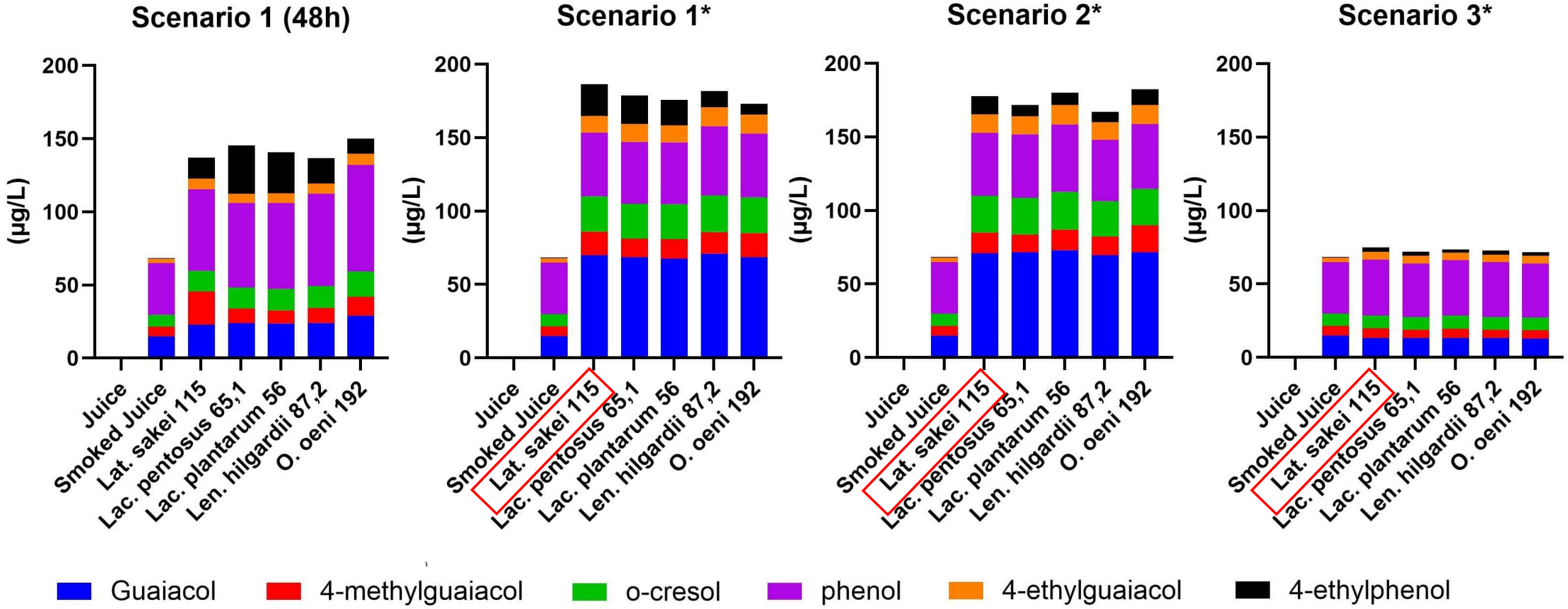


Co-inoculation (LAB inoculated 24 hours after *S. cerevisiae*).



Sequential inoculation (LAB inoculated after alcoholic fermentation).

Preliminary Fermentations
Volatile Phenols (GC-MS)



*End of AF & MLF

Preliminary Fermentations
Summary

- Co-inoculation resulted in the highest release of VPs.
- *Lat. sakei* 115 released the most VPs amongst the unconventional strains.
- MLF progressed to varying extents across strains.
- *O. oeni* 192 resulted in high acetic acid, causing sluggish or stuck alcoholic and malolactic fermentations – confirming previous findings by Lekokotla (2024).
- Moving forward: *Lat. sakei* 115 and *S. bacillaris* Y550 will be used in a co-inoculation fermentation scenario.
  - To validate the preliminary fermentation results.
  - Assess if the combination of these strains resulted in a higher VP release.

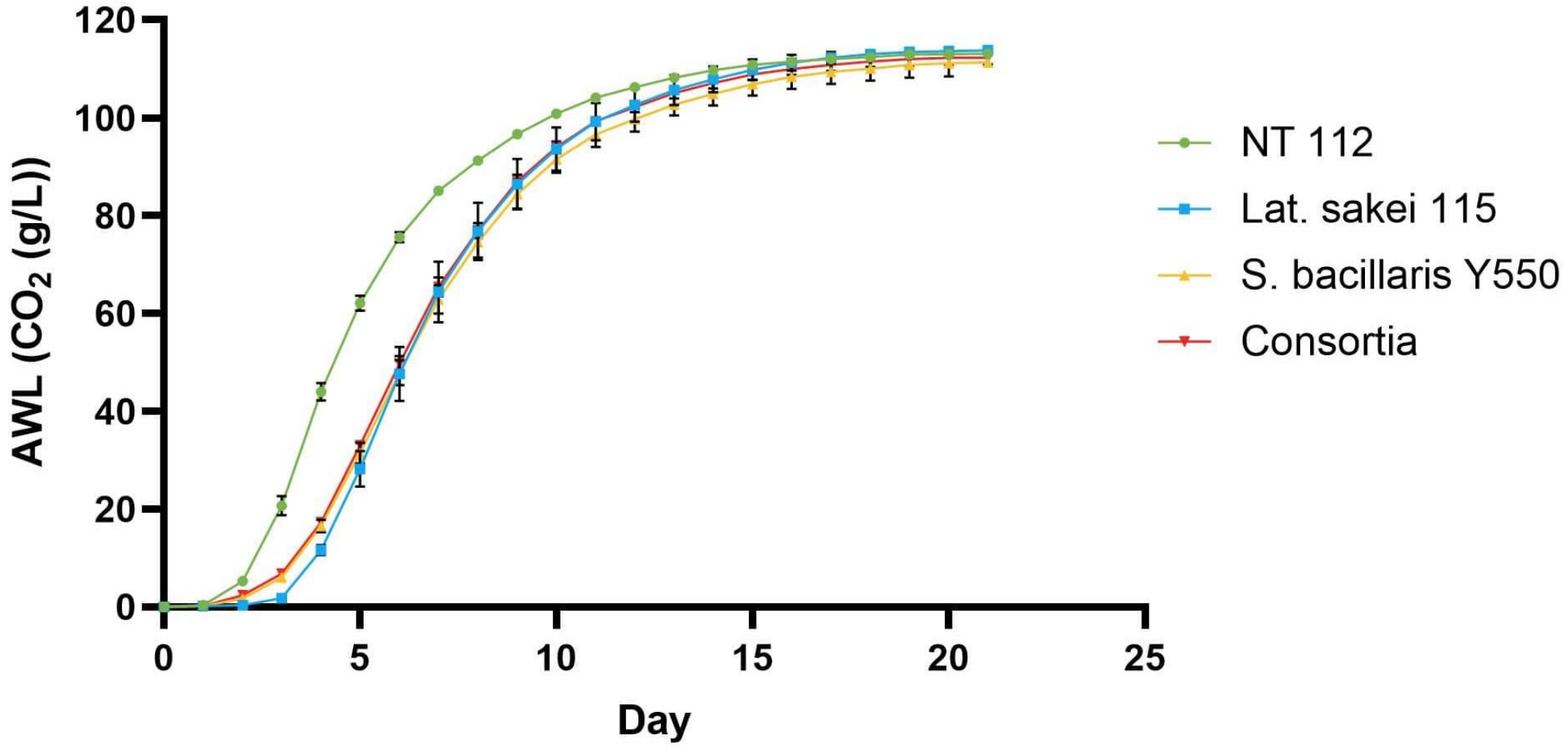
Follow-up Fermentations
Juice Analysis & Fermentation Scenarios

- Sugar: 240.80g/L
- pH: 3.39
- TA: 8.80 g/L
- YAN: 348.92 mg/L

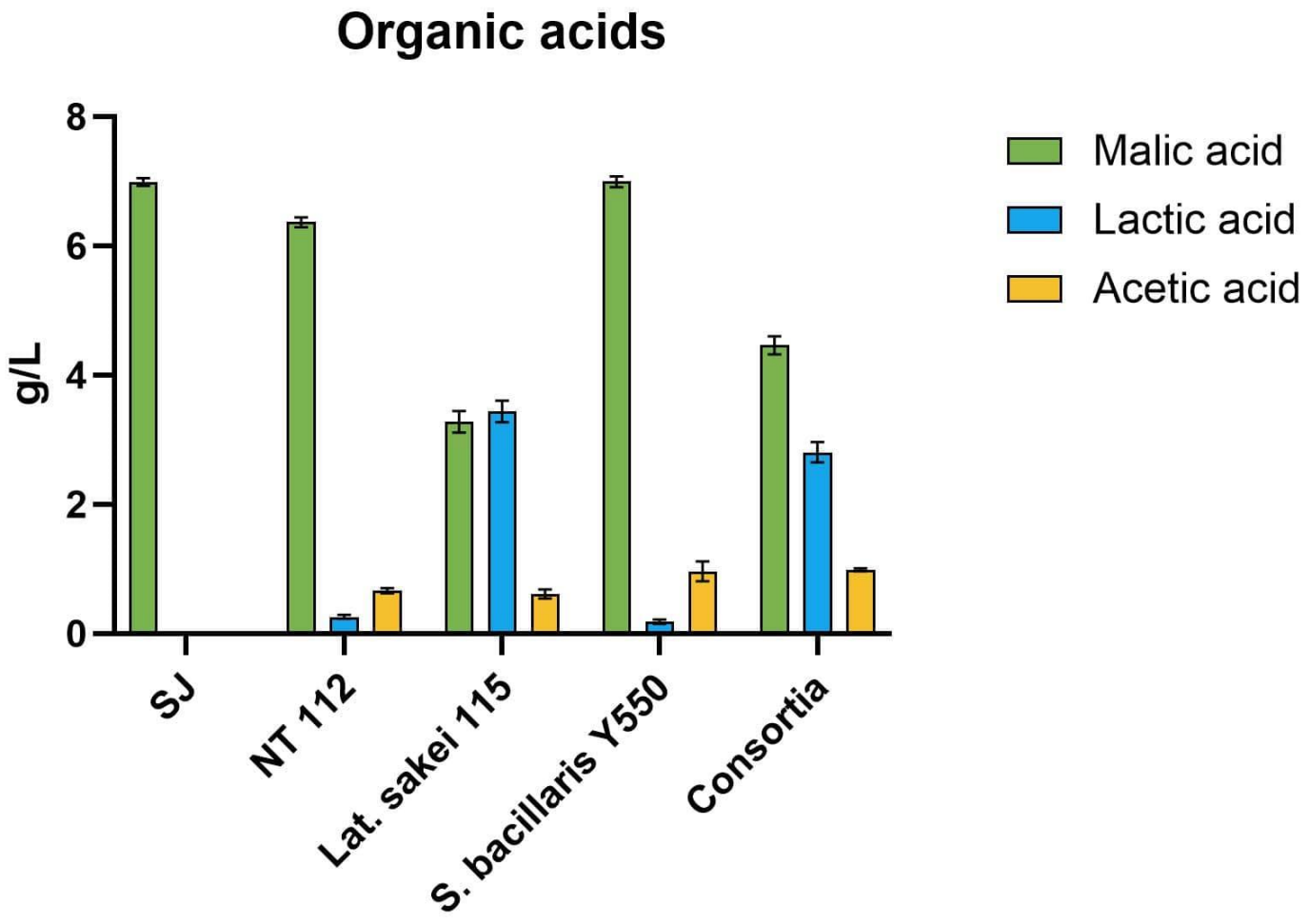
1. *S. cerevisiae* NT 112
2. *Lat. sakei* 115*
3. *S. bacillaris* Y550*
4. Consortia*:
   - *Lat. sakei* 115
   - *S. bacillaris* Y550
   - *S. cerevisiae* NT 112

*NT 112 was added after 48h

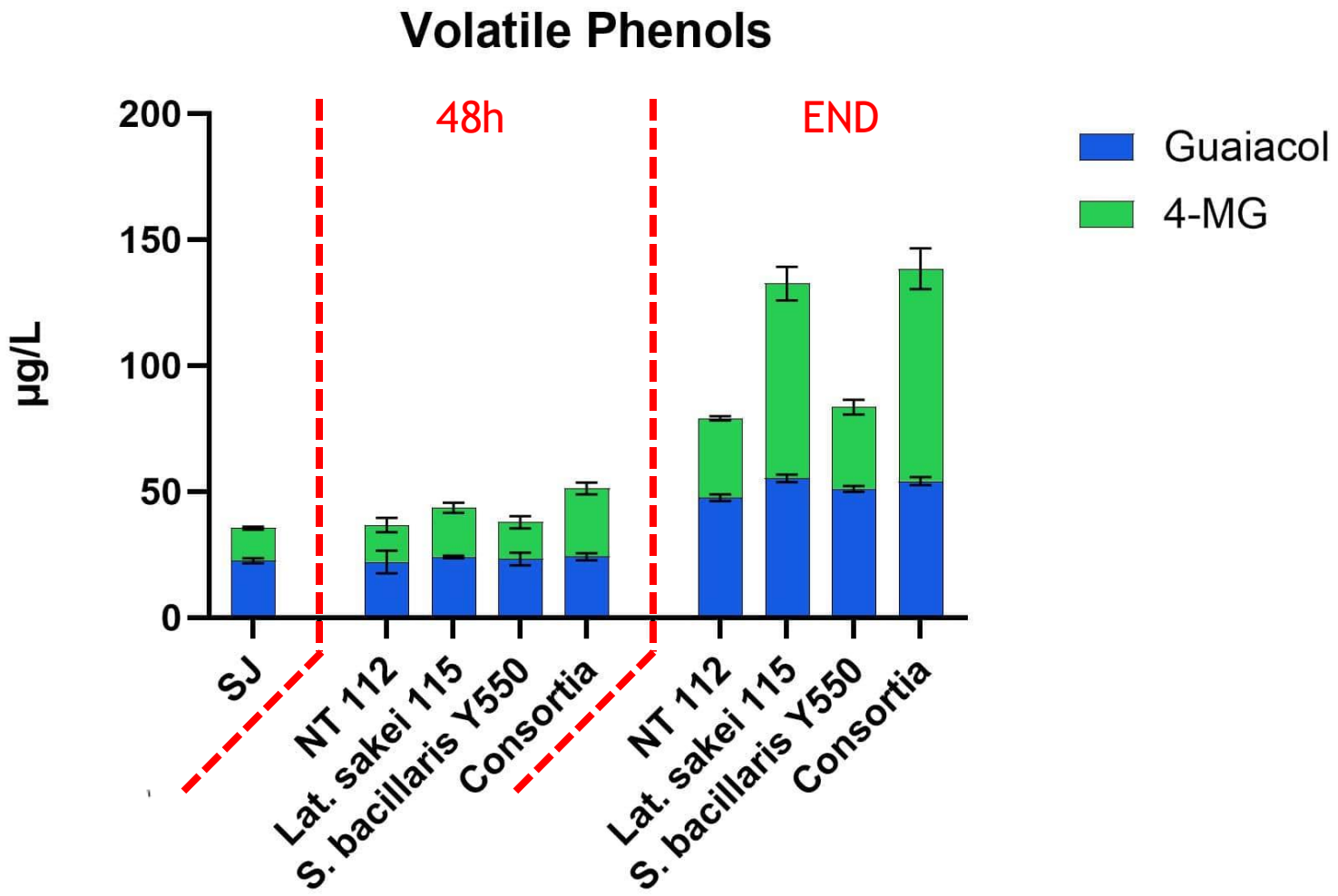
Follow-up Fermentations
Alcoholic Fermentation Kinetics



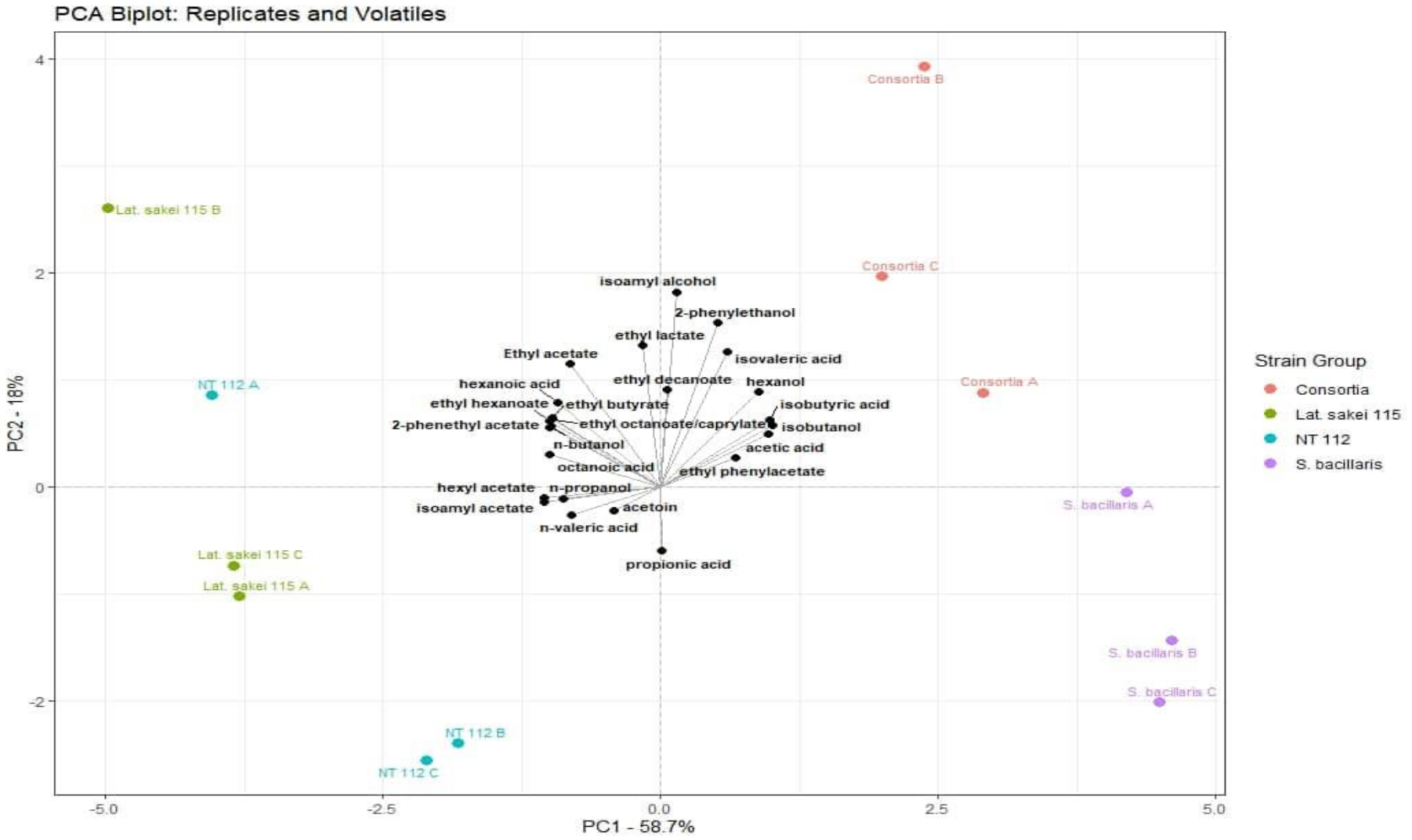
Follow-up Fermentations
Organic Acids (HPLC)



Follow-up Fermentations
Volatile Phenols (GC-MS)



Follow-up Fermentations
Major Volatiles (GC-FID)



Follow-up Fermentations
Summary

- All the fermentations were completed to dryness.
- *Lat. sakei* 115 released the most volatile phenols and was able to drive MLF (albeit partially).
- *S. bacillaris* Y550 drove acetic acid production.
- Major volatiles:
  - NT 112 & *Lat. sakei* 115 – esters and medium chain fatty acids
  - *S. bacillaris* Y550 & Consortia – higher alcohols and fusel acids

Take Home Message

- LAB can significantly enhance the release of VPs associated with smoke taint, but performance is strain-dependent.
- Co-inoculation resulted in the highest VP release.
- *Lat. sakei* 115 shows strong potential as part of a microbial strategy to mitigate smoke taint.
- Utilising a microbial strategy provides a non-chemical, safer approach for managing smoke-affected wines.

Acknowledgements

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Thank you
Dankie