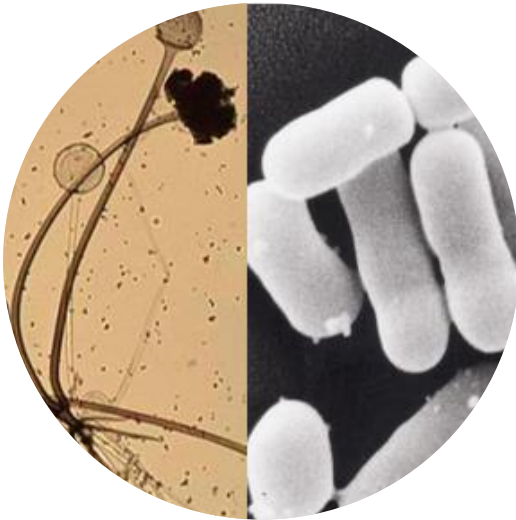


Vitamin B₁₂ fortification of lupin tempeh

Judith Wolkers-Rooijackers & Eddy J. Smid

Symposium, 22 April 2021



SUSTAINABILITY and HEALTH

- ✓ Exploit use of different substrates
- ✓ Innovate by exploiting global microbial diversity

New concepts:

"Cross-over-Foods"¹ and ***"Tinder-for-Microbes"***²

¹ Eddy J. Smid; Seminar at DSM (Biotechnology center Delft, 7 June 2018)

² Eddy J. Smid; Rotzooi met Katz festival (Mediamatic, Amsterdam, 15th June 2017)

Approach

Tempeh: Indonesian fermented product:

- Raw material: Soy beans
- Fermented with: *Rhizopus* spp.
- High protein content



Desire: nutritious meat replacer should contain vitamin B₁₂

Fact: no vitamin B₁₂ in lupine beans

Fact: fungi do not produce vitamin B₁₂

Fact: vitamin B₁₂ is a bacterial metabolite

Develop lupine tempeh by fermentation with a mixed culture:
Rhizopus sp. + B₁₂ producing bacterium

Propionibacterium freudenreichii: food grade multi-vitamin producer!



Generally Recognized As Safe

At start of project

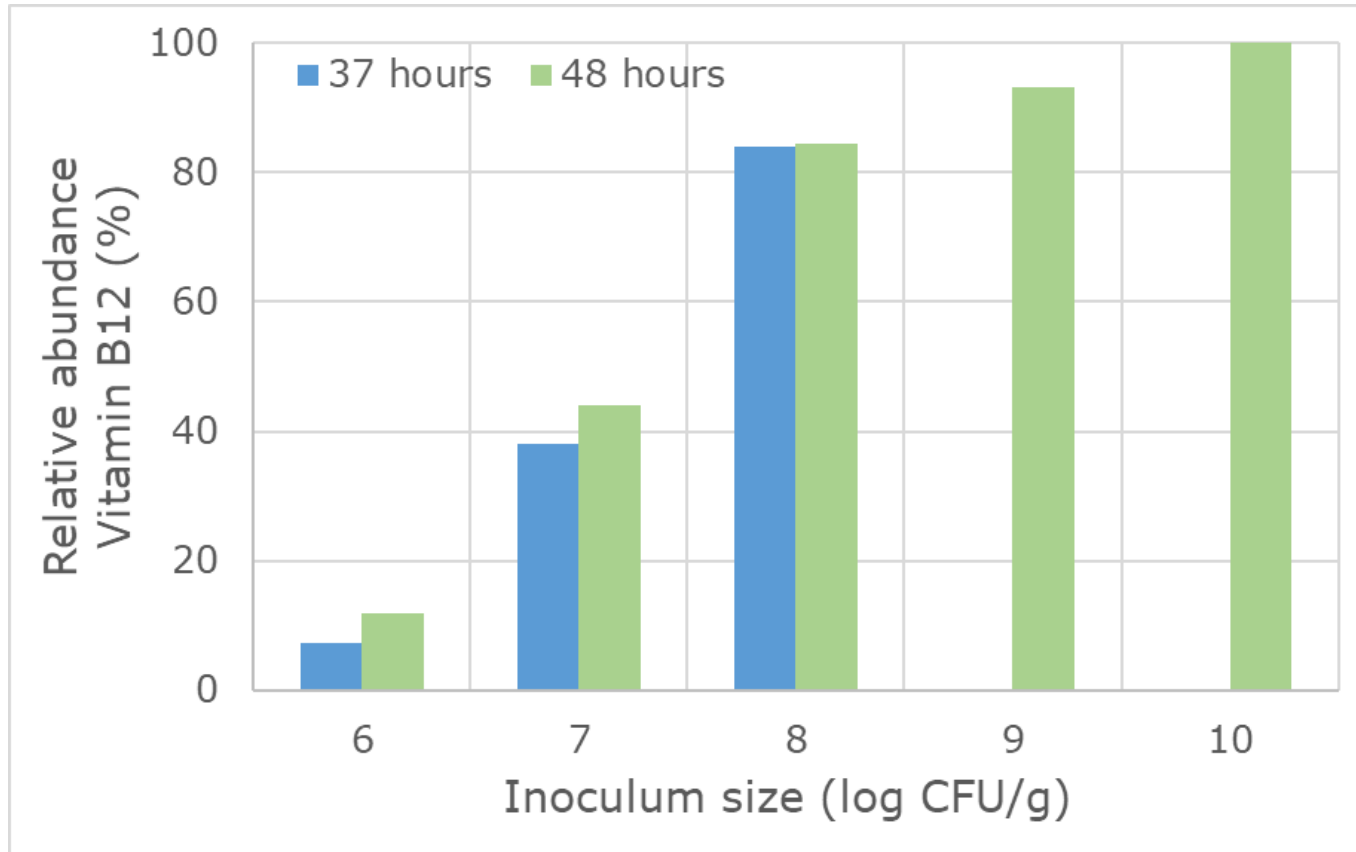
Optimizing co-fermentation and B₁₂ production

- Inoculum dose
- Size bioreactor - heat transfer
- Company parameters
- Oxygen supply
- Monitor fungal biomass



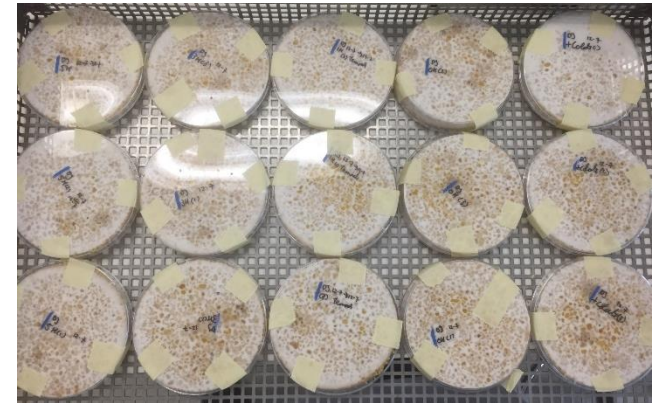
Martha Endika

Results optimization inoculum size



Jasper Zwinkels

Results optimization size bioreactor

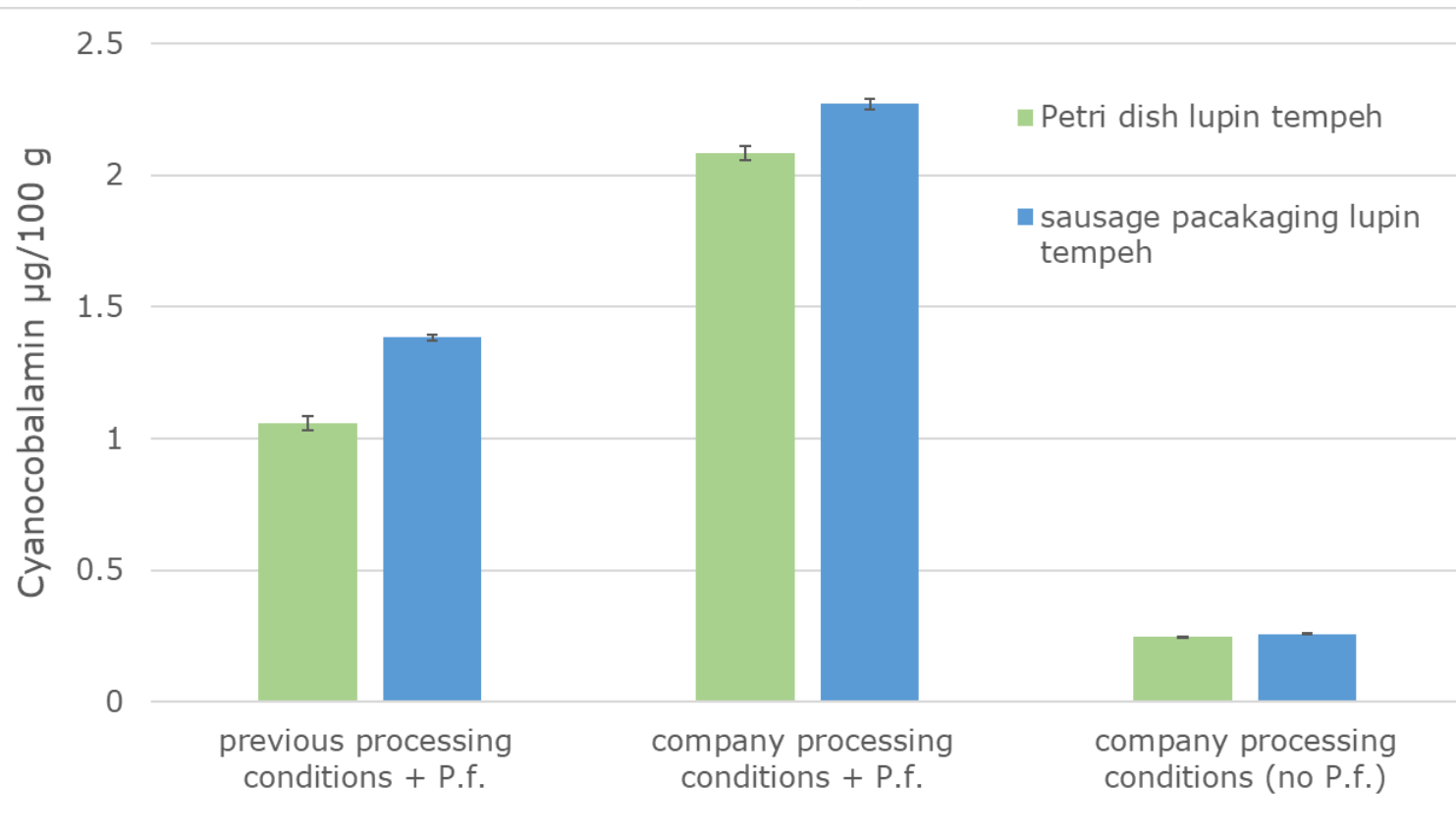


David Janssen

1.4 $\mu\text{g}/100\text{ g}$ tempeh (wet weight)

Results company parameters

Together with “De Hobbit” company parameters were transferred to a lab-scale product



Zikrina Istighfarah

Results oxygen supply



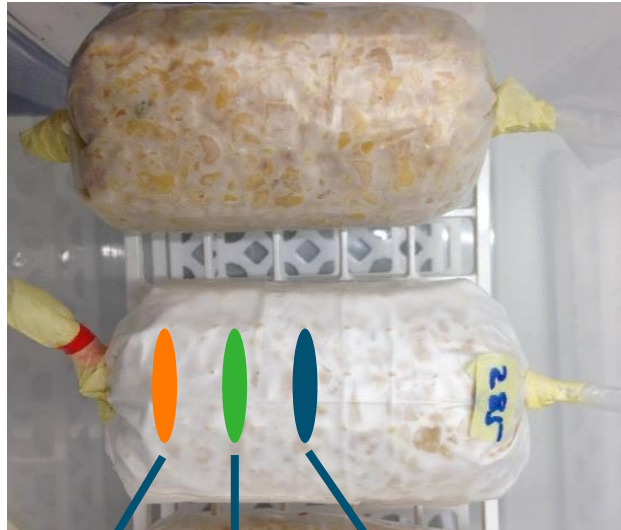
Need of oxygen... a balancing act

- Fungus **needs** oxygen to grow
- *Propionibacterium freudenreichii* grows best with **limited access** to oxygen *BUT*
- **Needs** sufficient oxygen for final vitamin B₁₂ production step

Results oxygen supply

Rhizopus sp.

R. oryzae

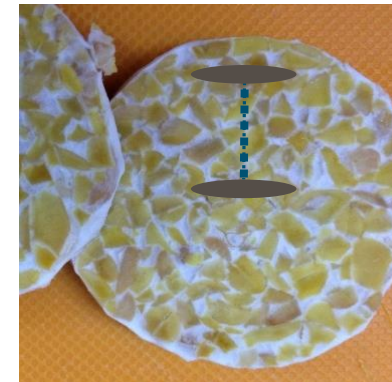


EDGE **MID** **CENTER**



0.5 cm

2.5 cm



0.5 cm

2.5 cm



Maja Starovic



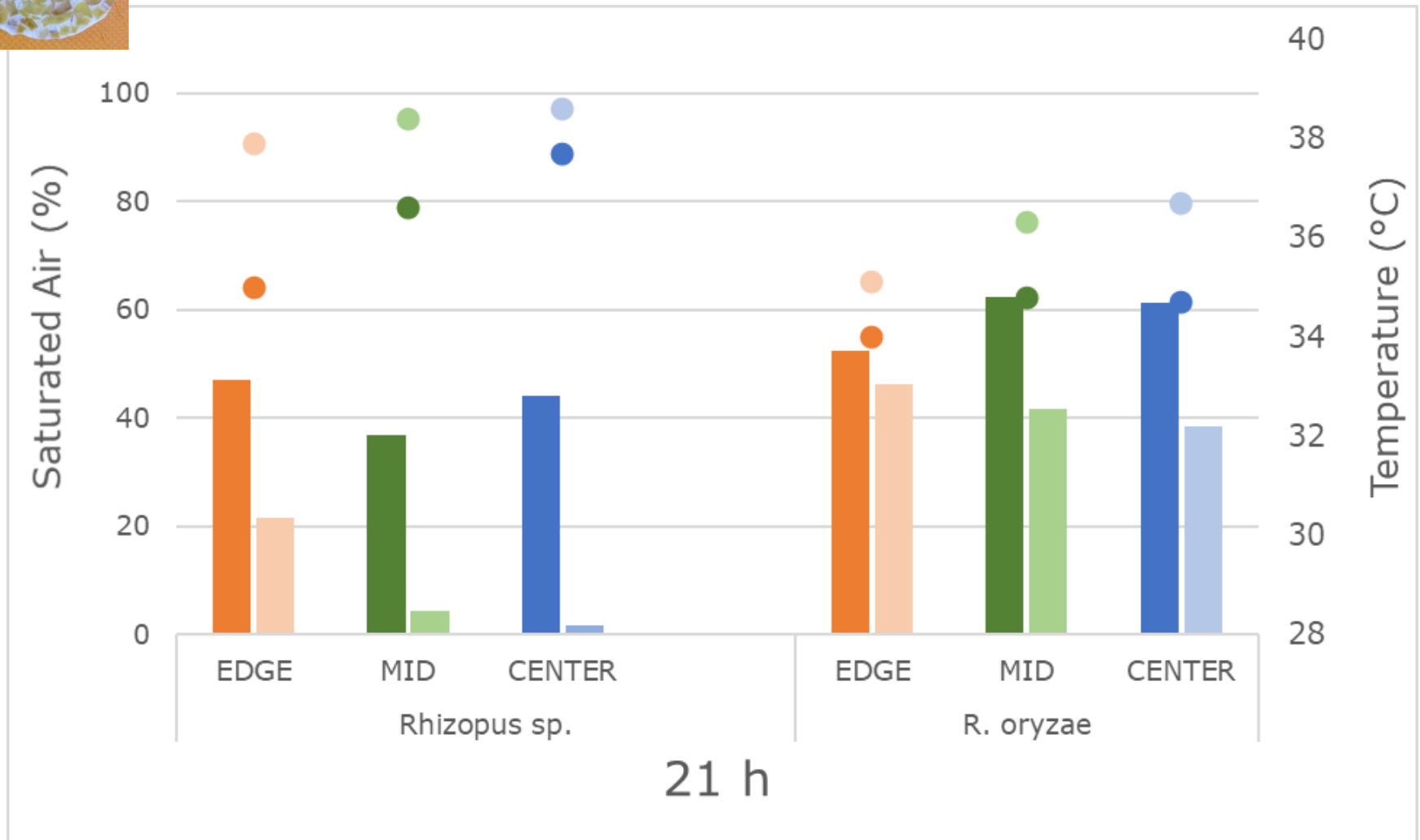
..... 0.5 cm
— 2.5 cm



0.5 cm



2.5 cm





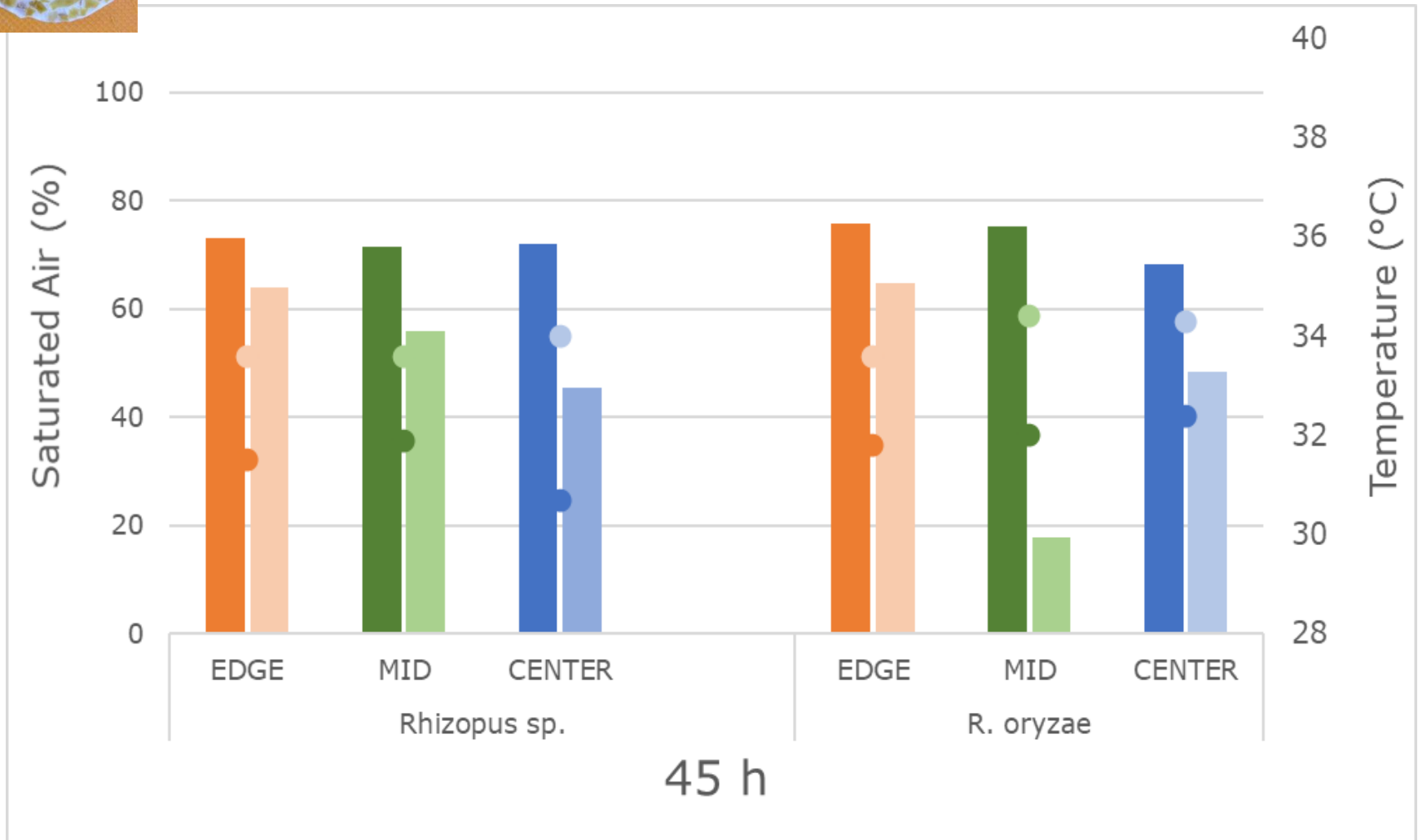
..... 0.5 cm
— 2.5 cm



0.5 cm



2.5 cm





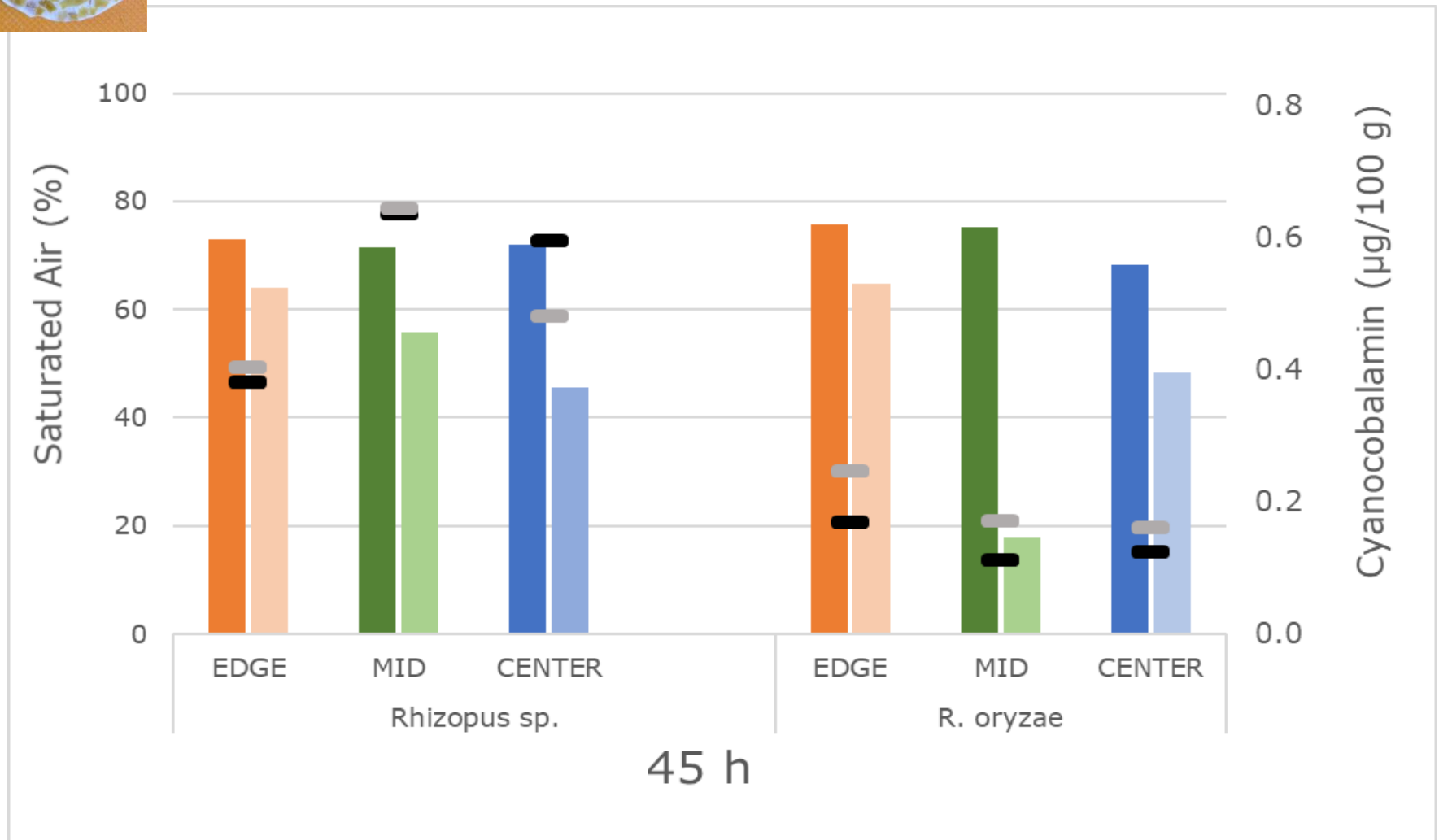
..... 0.5 cm
— 2.5 cm



0.5 cm



2.5 cm



Results fungal biomass

Rhizopus spp.
Aerobic



Oxygen Consumption



Heat Production



P. freudenreichii
**Anaerobic/
Microaerophilic**

No mixing or forced aeration



Gradients across matrix



How is microbial growth affected?

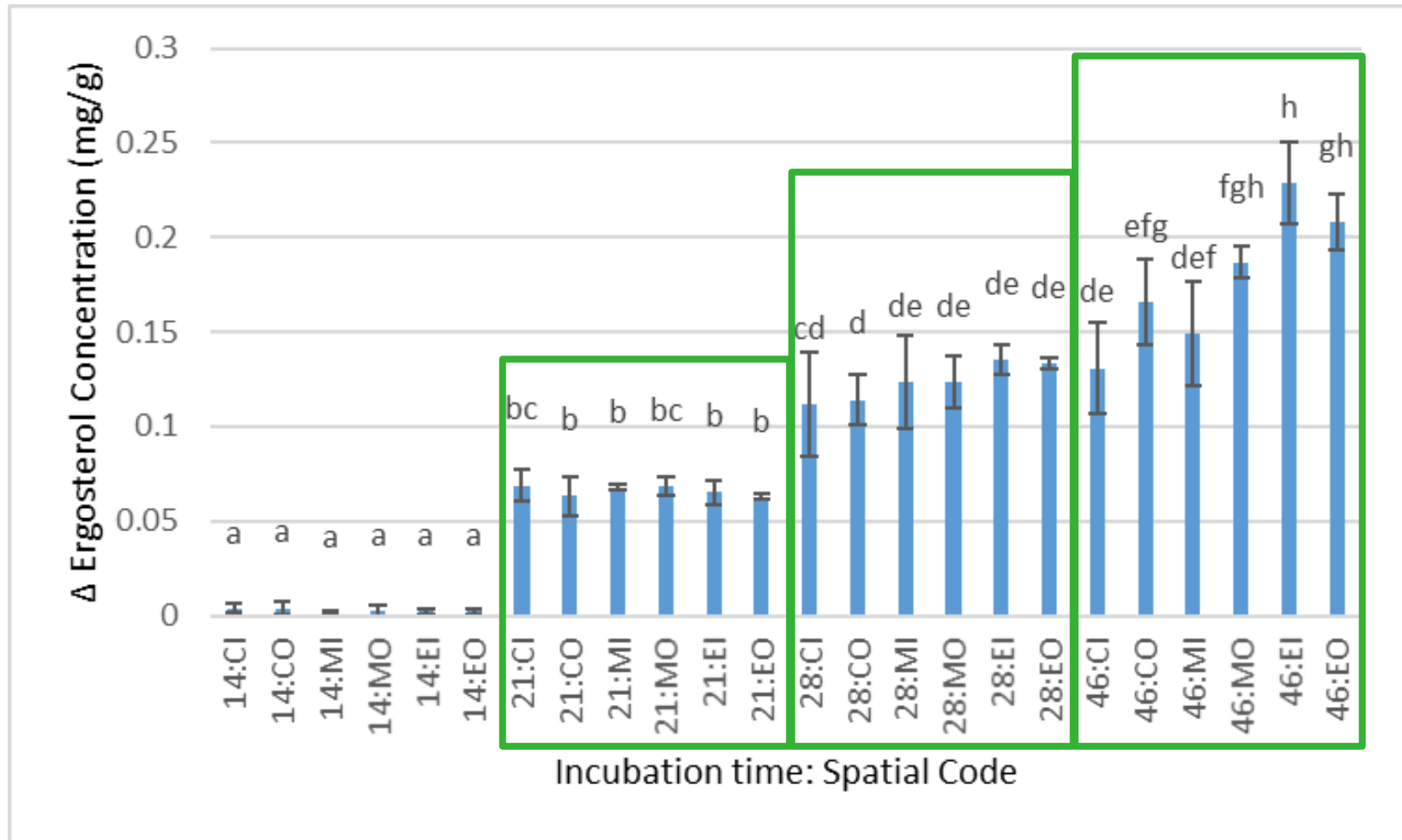


Ergosterol: membrane lipid in fungi

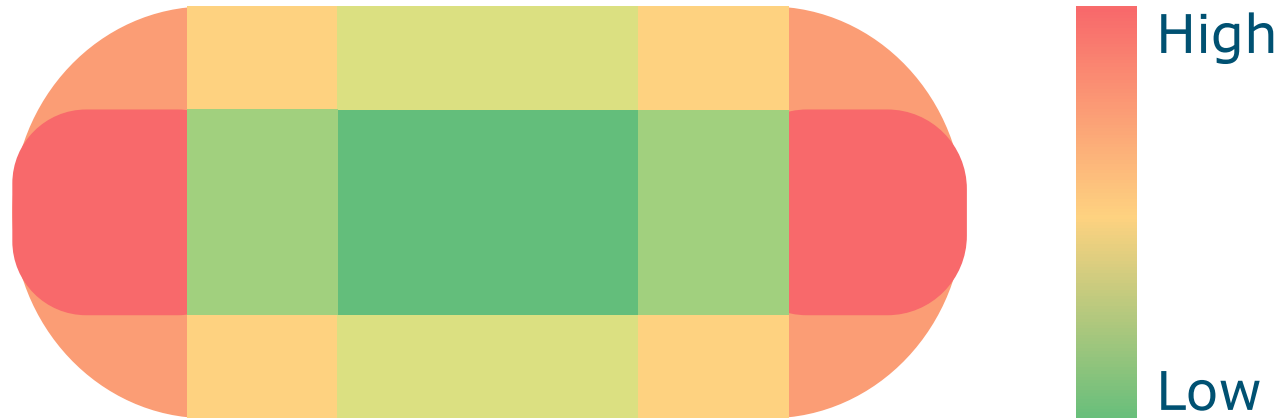


Owen Turner

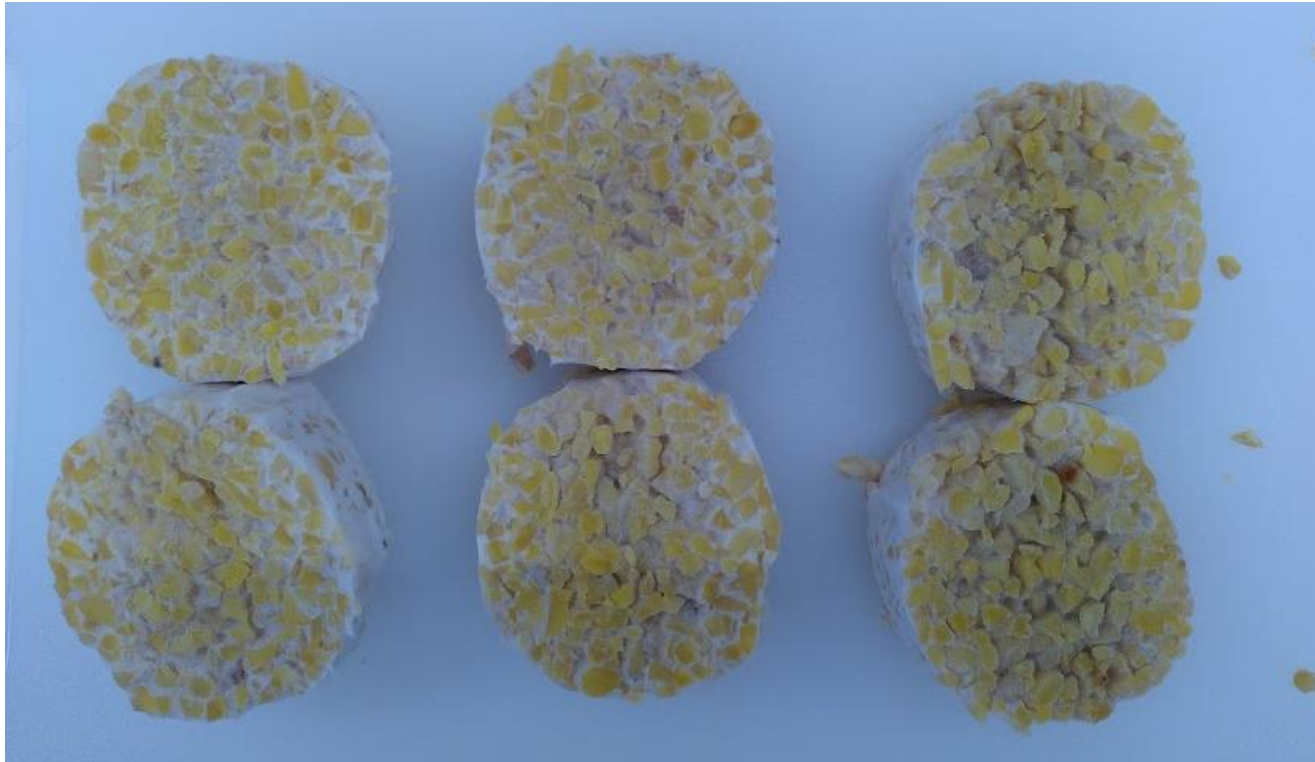
Results ergosterol quantification



Results: Ergosterol quantification



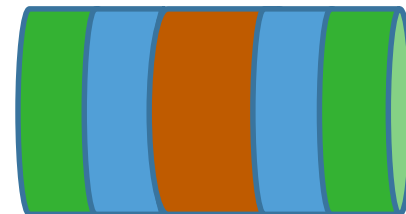
Results Ergosterol quantification



Edge

Middle

Centre



Take home messages

- ✓ Co-fermentation of *P. freudenreichii* with *Rhizopus* sp. consistent in vitamin B₁₂ production in lupin tempeh
- ✓ *Rhizopus* sp. consumes more oxygen and faster than *R. oryzae*
 - ✓ Leads to more vitamin B₁₂ production
 - Interaction fungus/bacterium plays a role
- ✓ Fast oxygen consumption seems key!
- ✓ Ergosterol good way to monitor fungal growth

Thank you for your attention!

Questions?



<http://www.hobbit.be/recipes/burger-met-lupine-pepp-en-henneburgers-paprika>