



SCIENCE AND
EDUCATION **FOR**
SUSTAINABLE
LIFE

TEMPEH

Fermentation

for Healthier Foods

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Providing more food
choices can support
the transition to

PLANT-RICH DIETS

Reducing meat and dairy
consumption can lower
the environmental
impact of food systems.

but nutritional quality and
health effects remain concerns.

Plant-based Fermentation

Solid-state fermentation is a bioprocess that uses fungi or bacteria to transform solid plant materials

- **To preserve raw materials**
- **To create flavour, develop texture**
- **Food more practical to eat**



Sufu



Actinomucor elegans

Tofuyo



Aspergillus oryzae

Image source: Drakfrukt Sverige AB Wangzhihe

China

Japan

Tempeh



Rhizopus oligosporus

Oncom



Neurospora intermedia

Indonesia

Soybean and
Rhizopus
starter

Very adapted
to other
legumes

Firm, cake-like
beans and
fungal
mycelium

Reduction of
Anti-nutrients

High protein
and DIETARY
FIBER

Bioactive
compounds



TEMPEH

Faba beans



Oats

Nordic crops



Fermentation



Tempeh



1

Nutrient
composition

Anti-nutrient
degradation

2

Mineral
uptake
(*in vitro*
model)

3

Structural
and Sensory
properties

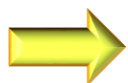
4

Novel
co-fermentation

Tempeh Lab-scale Production

Flow chart

Raw material



Soaking

Heating (boiling)



Started culture inoculation



Fermentation

Fernandez et al., (2024)

Faba bean tempeh production

A) Protocol for producing 1 kg of tempeh

Raw material

A1. Raw materials

Faba bean



Inspect whole dried beans

A2. Dehulling



Remove the seed coats/hulls mechanically or manually.
500 g of dehulled beans

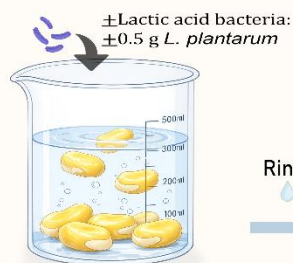
A3. Washing



Rinse with tap water

Pre-treatment

A4. Soaking



Water: ~1.5 L
Sodium bicarbonate: 5 g
Time: 12 h
Temperature: ~25 °C

Rinse

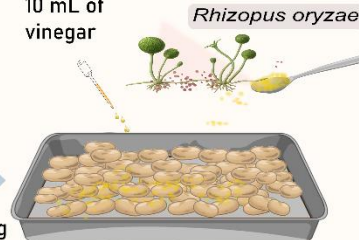
A5. Heat treatment (boiling)



New water: ~5 L
Time: 8 min

Rinse

A6. Acidification



R. oryzae powder ~6 g

A7. Inoculation

Rhizopus oryzae

Fermentation

A8. Packaging



Perforated LDPE zip-lock bag
100 × 150 × 0.05 mm
or wrap in plant leaves
150 g inoculated beans per bag

A9. Incubation



Fermentation
Time: ~40 h

A10. Fresh tempeh



~1 kg faba bean tempeh
Cooking
*Fresh tempeh: 4 °C, ≤3 days
*Frozen tempeh: -18 °C, ≤3 months

RESULTS

Nutrient composition

Nutritional profile

In vitro digestion Protein and
Starch degradation

How does fermentation change the nutritional value legumes/cereals?

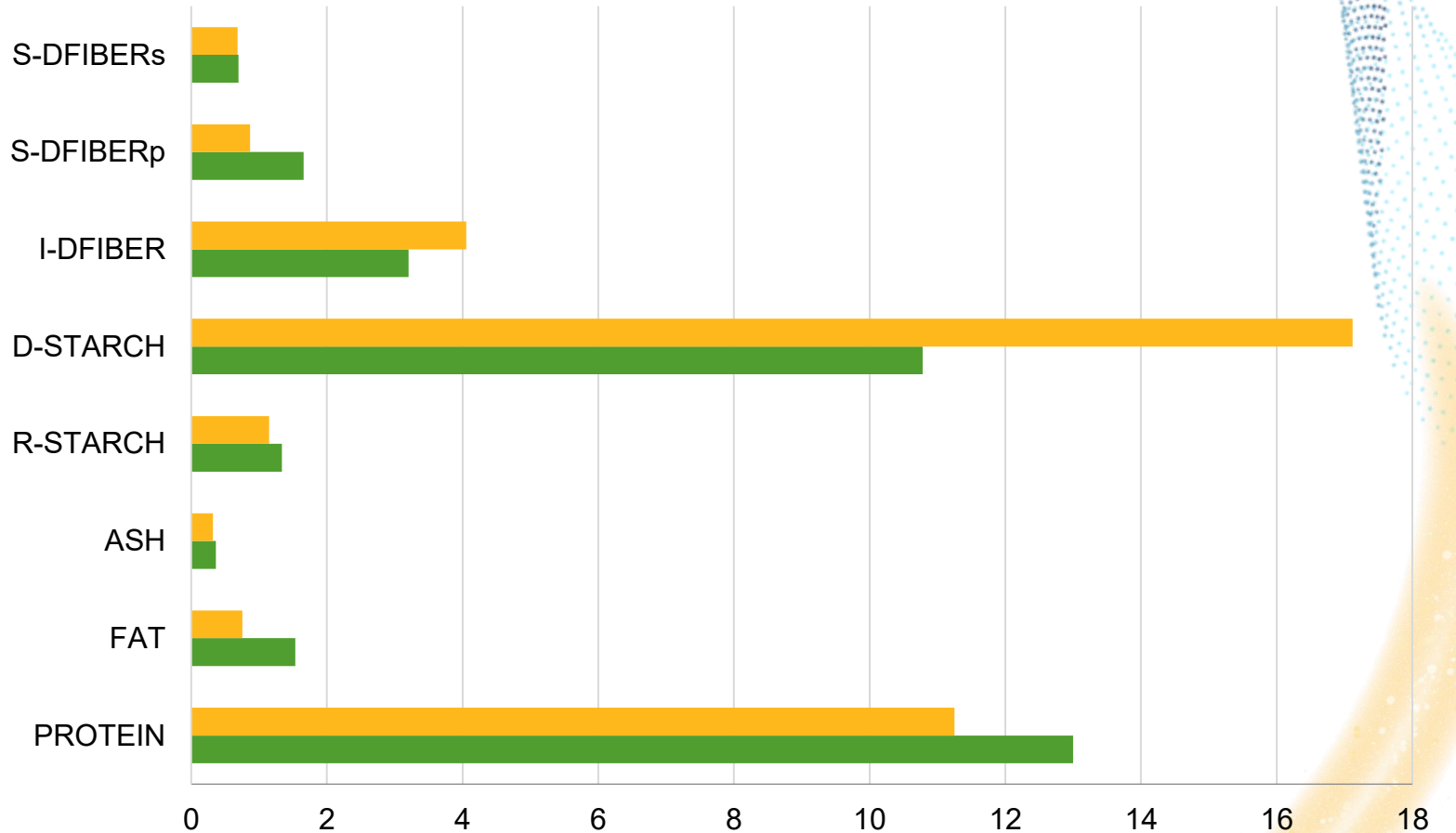
LEGUME/
CEREAL



TEMPEH

Faba-Oat Tempeh

Nutritional Composition (g/100 g Wet Weight)



Fernandez et al., (2026a)

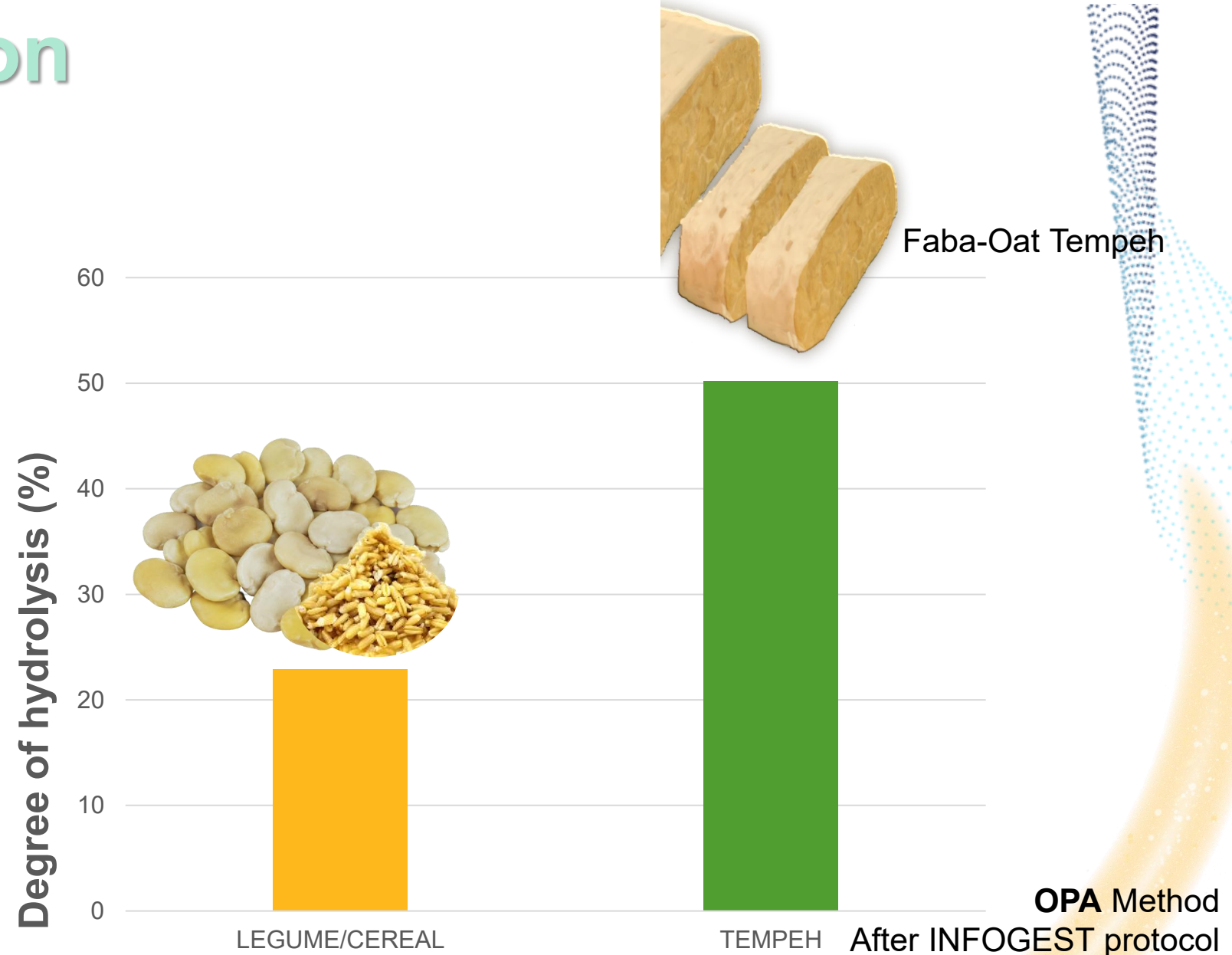
Nutrients:

In vitro digestion

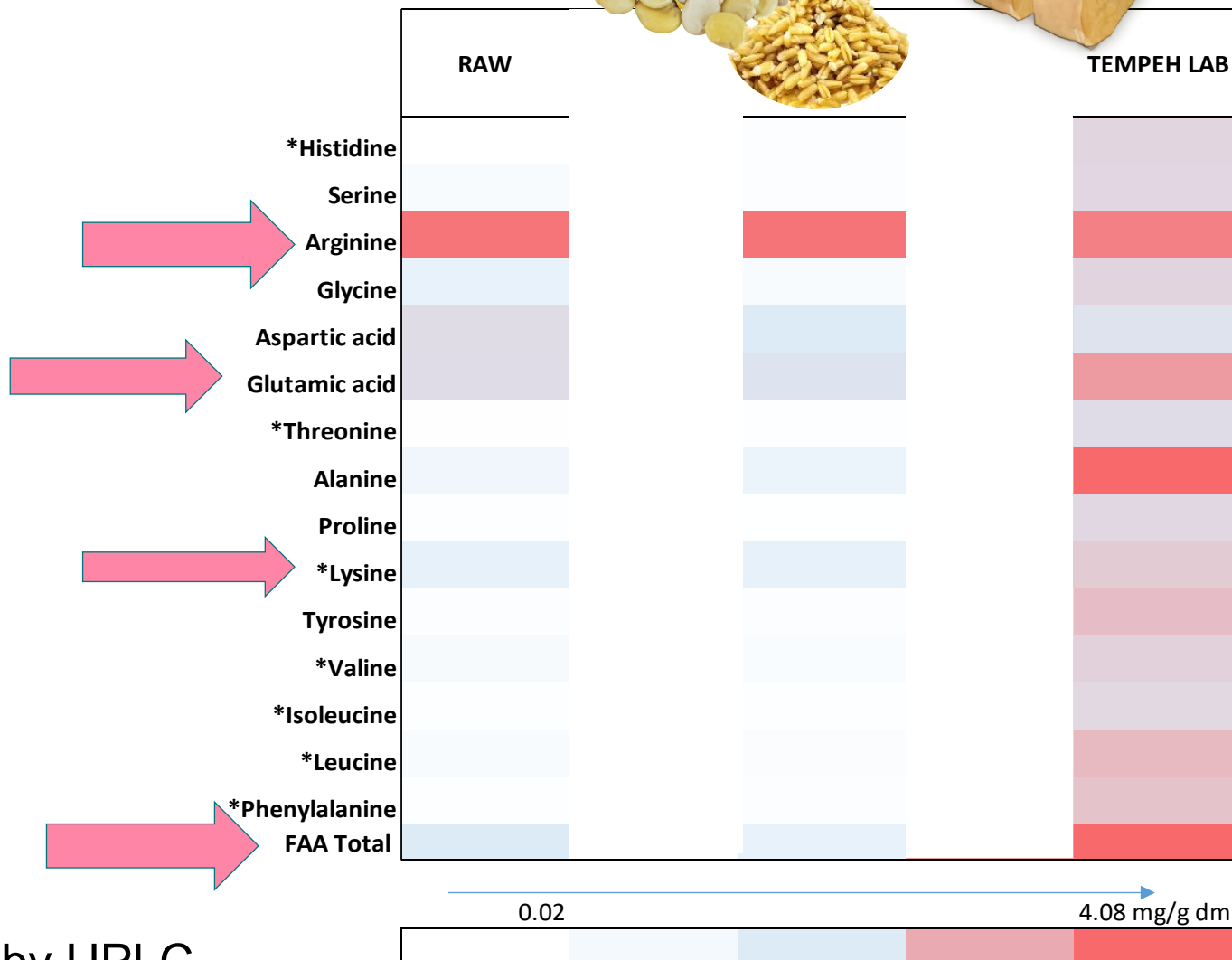
Degree of Hydrolysis (DH)

Nutritional Quality:

A higher DH generally indicates **better** protein digestibility and a higher release of bioaccessible amino acids ready for intestinal absorption.



Free amino acids



Faba-Oat Tempeh

* EAA: Essential amino acids

FAA by UPLC



Vicine and Convicine (faba bean)

Natural compounds in plant chemical defence

↓ *During digestion*

Divicine and isouramil

Cause oxidative stress

↓ *In people with G6PD deficiency*

Red blood cell destruction (favism)

G6PD deficiency affects around 400 million people worldwide.



Phytic acid

Beans and cereals contain phytic acid
The main phosphorus-storage compound in seeds

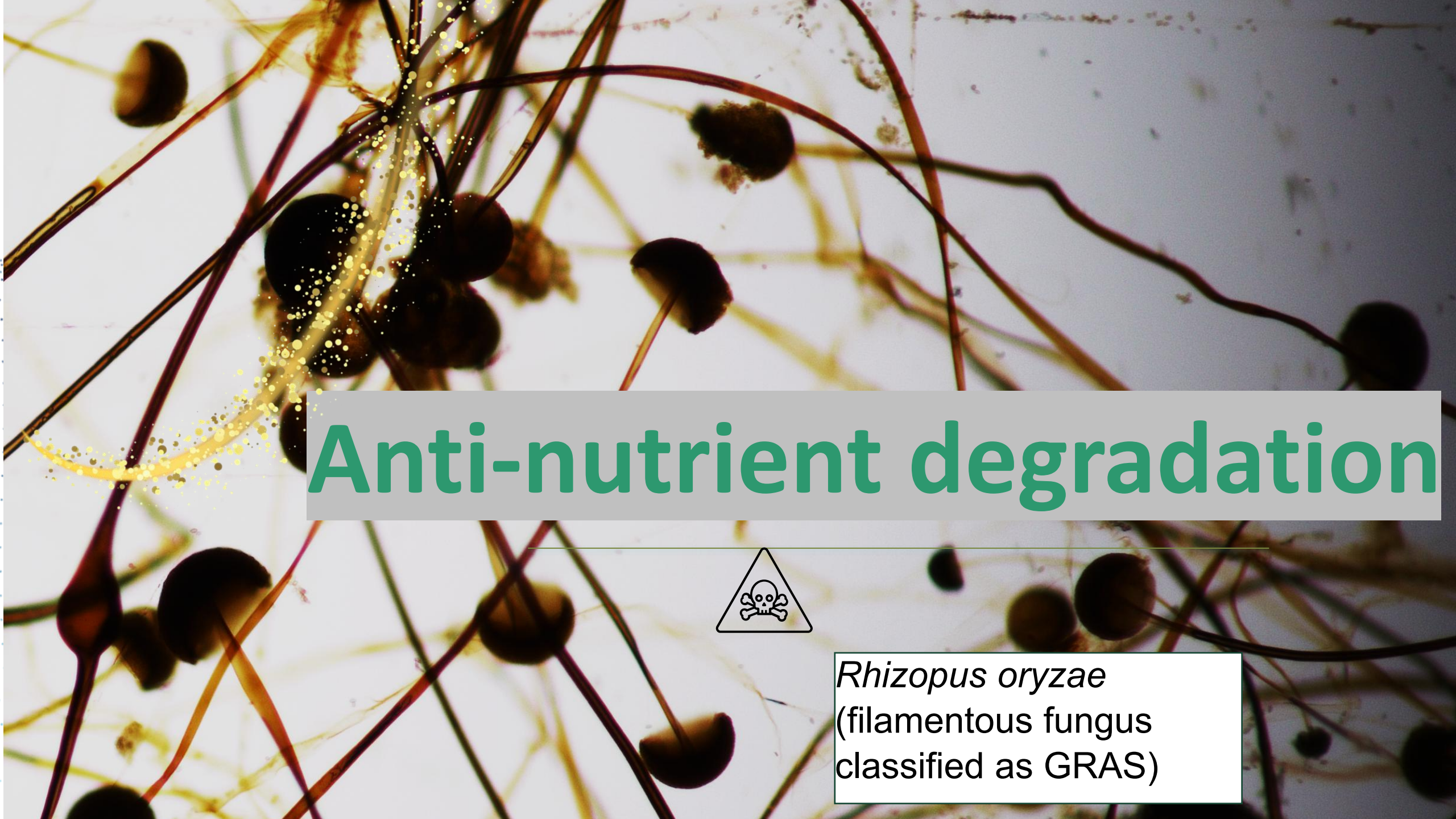
↓ *During digestion*

Phytic acid binds minerals

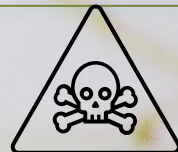
Iron, zinc and calcium

↓ **Lower mineral absorption**

Populations with **poorly diversified diets** and limited access to fortified foods.

A microscopic image of the filamentous fungus Rhizopus oryzae. The image shows a dense network of long, thin, reddish-brown hyphae. Interspersed among these hyphae are numerous dark, spherical spores. A decorative yellow and orange particle trail is visible on the left side of the image, curving around the central text area.

Anti-nutrient degradation



Rhizopus oryzae
(filamentous fungus
classified as GRAS)

Vicine

Convicine

Phytic acid

Raw material

Soaking

Heating (boiling)



-13%

-9%

-75%



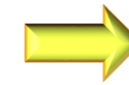
Mineral Uptake Assessed by Caco-2/HT29 Cell Model



Tofu



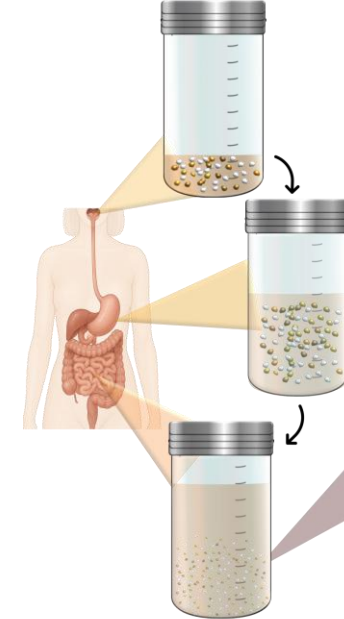
Tempeh



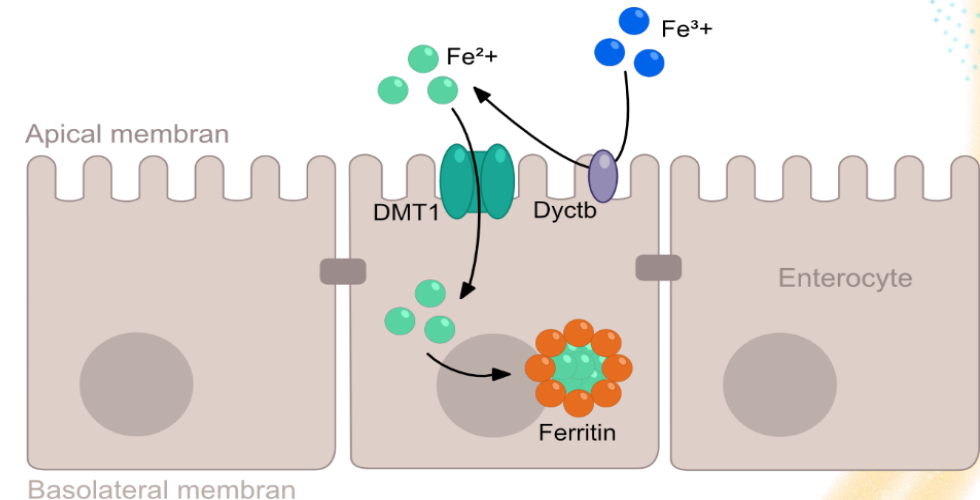
Digested food samples applied to intestinal cell monolayers

Ferritin formation measured after 24 h as a proxy for iron uptake

INFOGEST 2.0



In vitro Digestion



Mineral Uptake Assessed by Caco-2/HT29 Cell Model



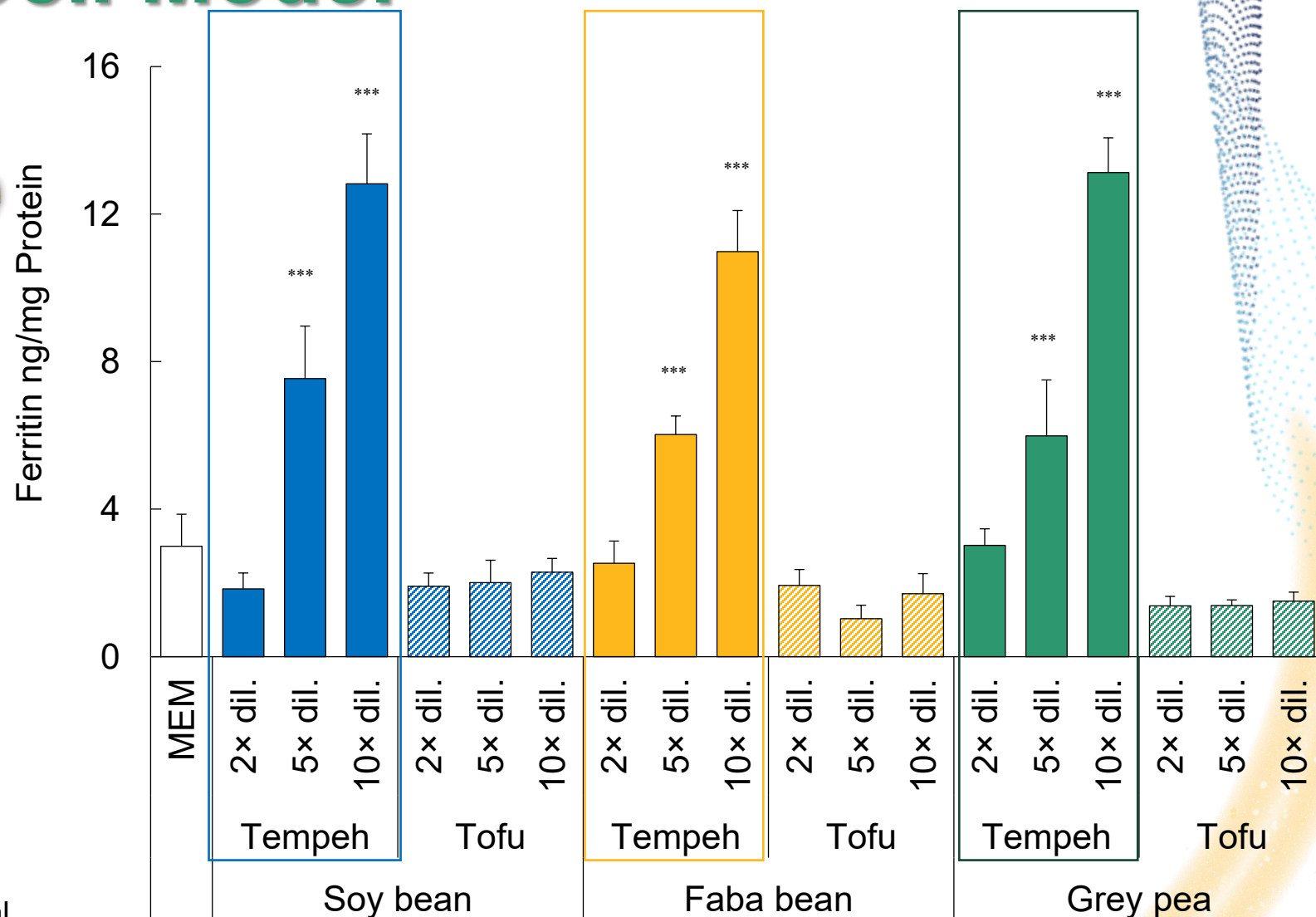
Tofu

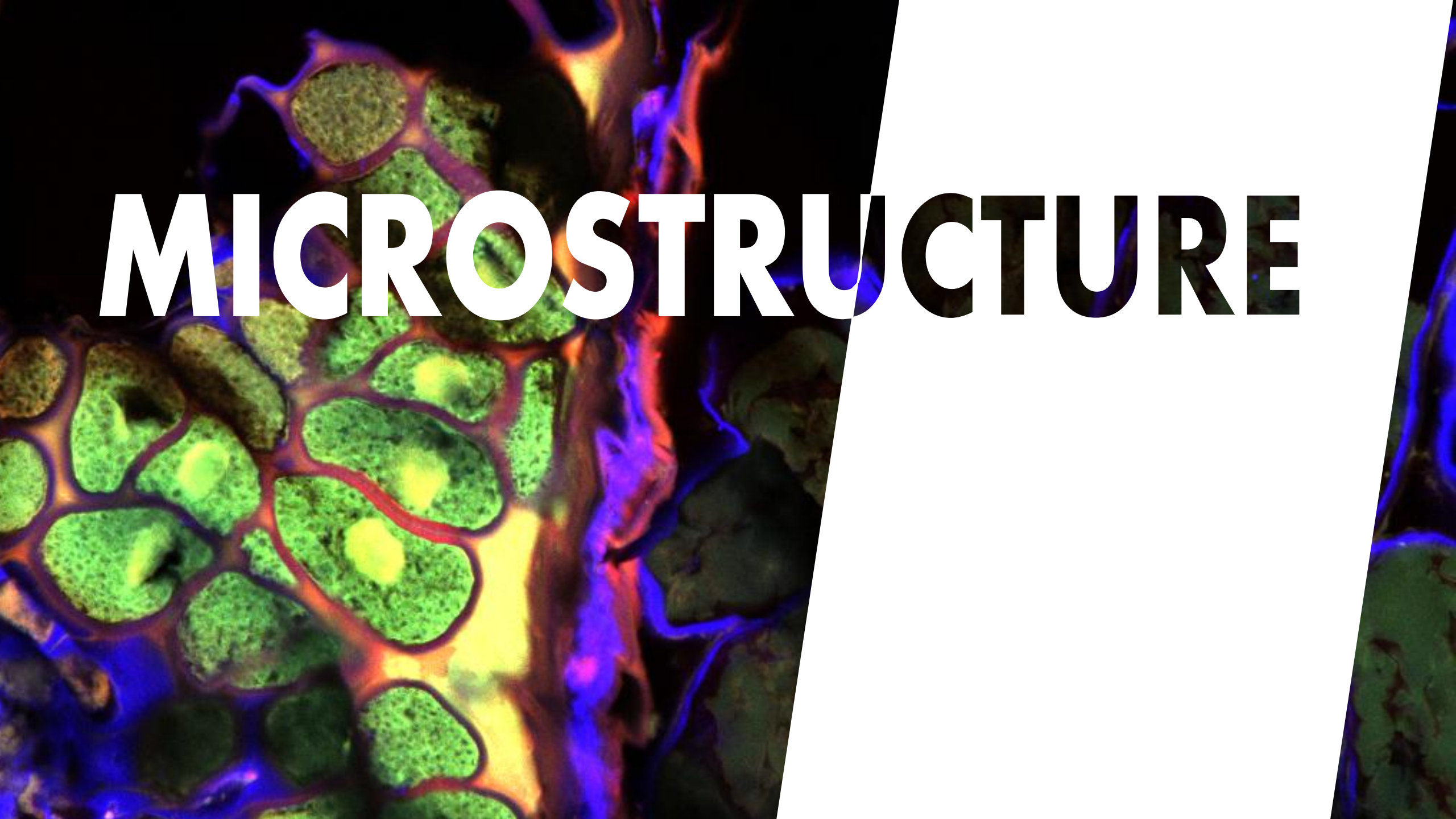


Tempeh

Fermented (tempeh) samples induced significantly higher ferritin levels than tofu

→ Low phytate levels enhance iron uptake

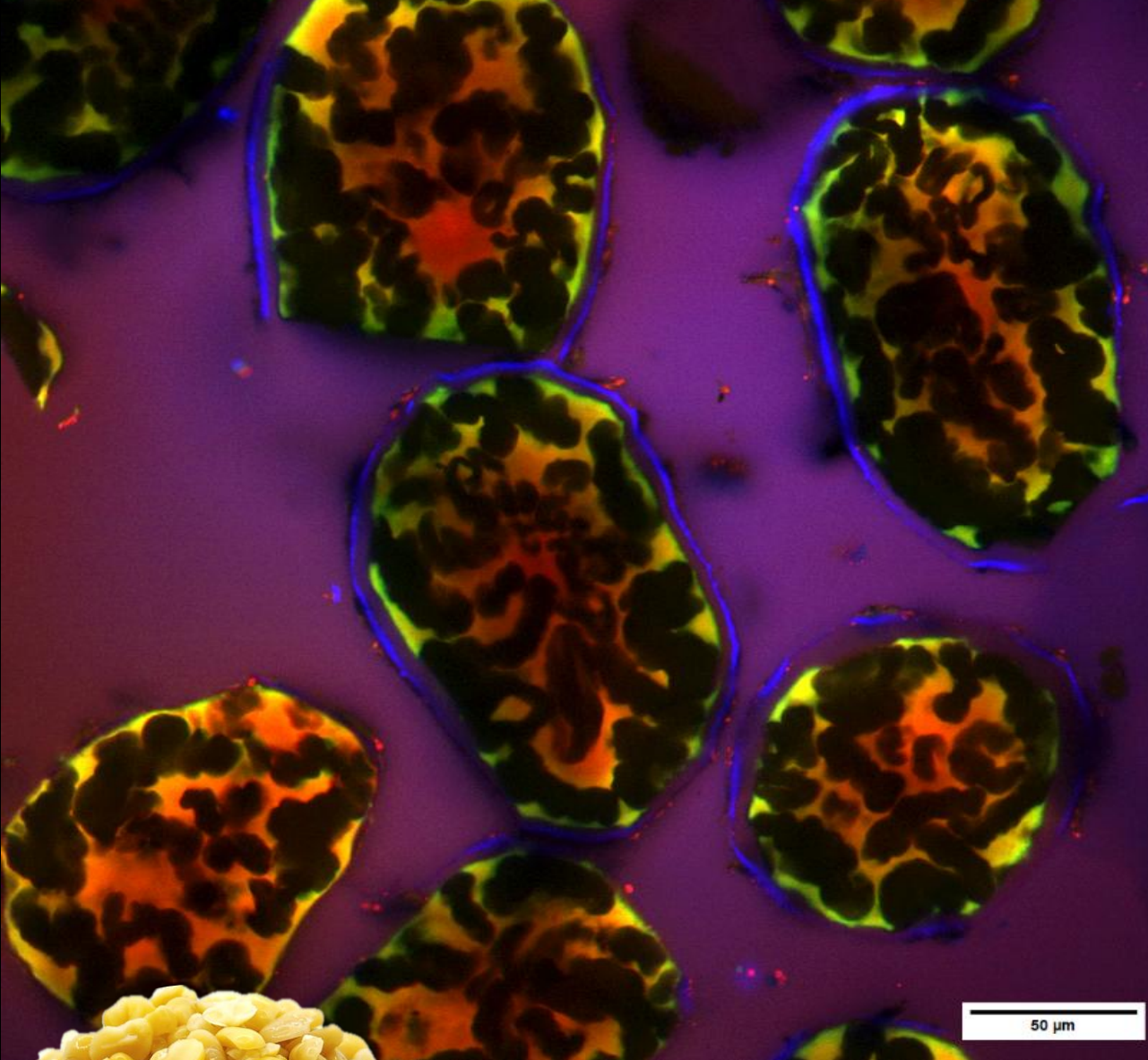
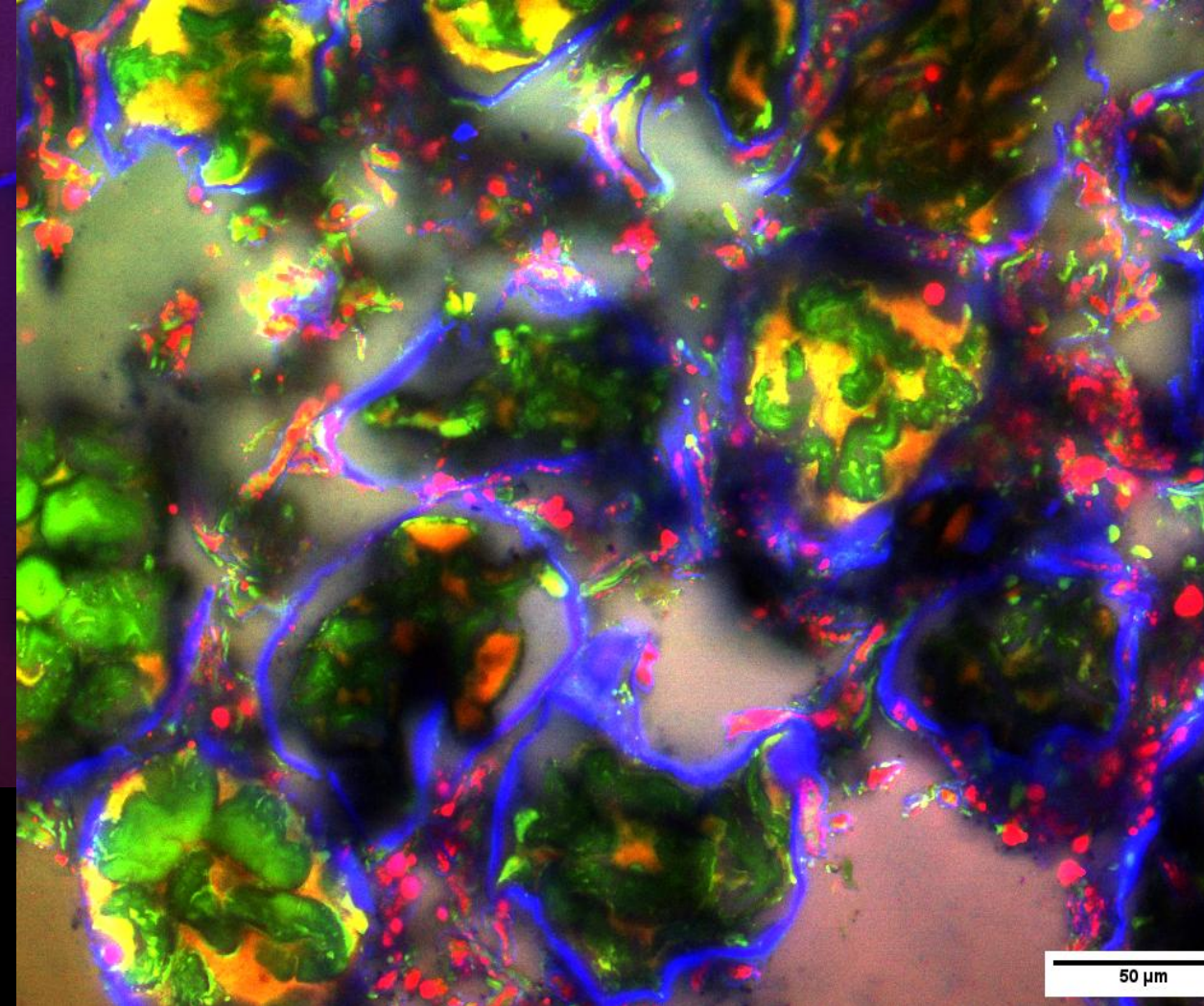




MICROSTRUCTURE



Tempeh



Faba bean

Confocal light microscopy 40X

Calcofluor White → Cell walls
 Rhodamine → Proteins
 FITC → Starch

Sensory analysis

Volatiles organic compounds

Descriptive (8 pannelist)

Liking (107 people)

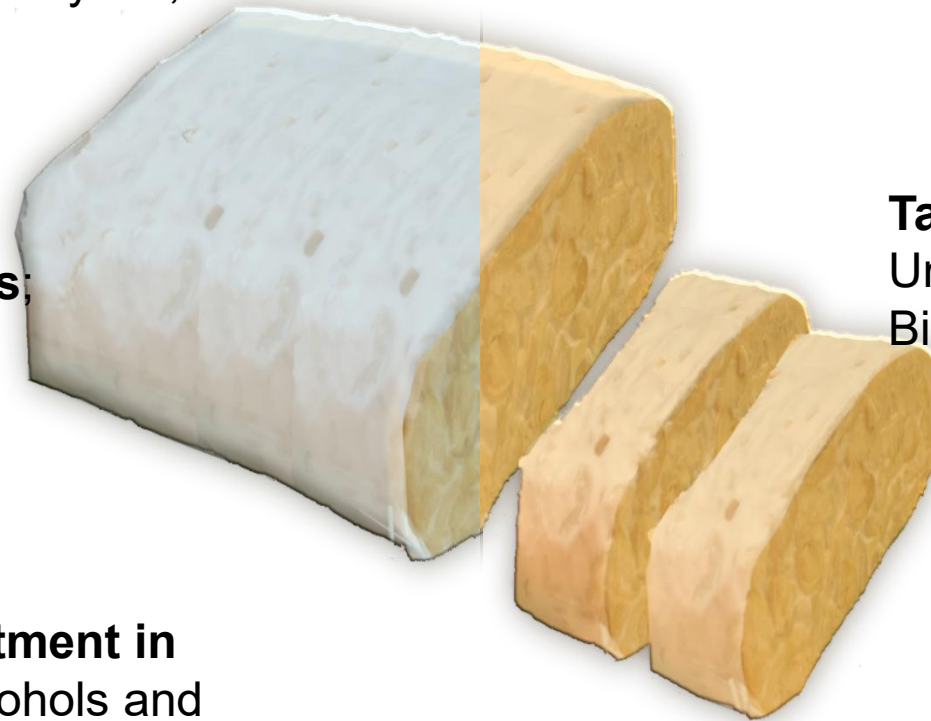


Volatile Organic Compounds profile

65 VOCs identified, grouped into **7 chemical classes** (e.g., esters, alcohols, aldehydes, acids).

Cooking increased **pyrazines**;
decreased alcohols.

***L. plantarum* pre-treatment in SOAKING** reduced alcohols and aldehydes, and increased pyrazines in TEMPEH



Sensory profile

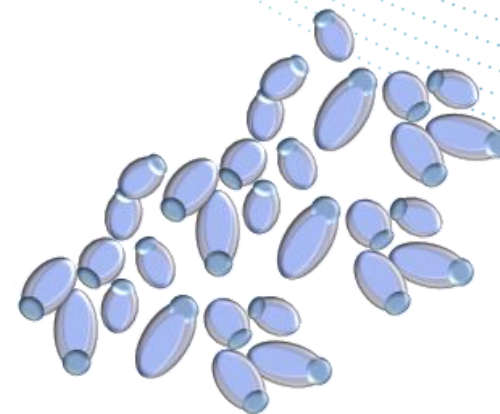
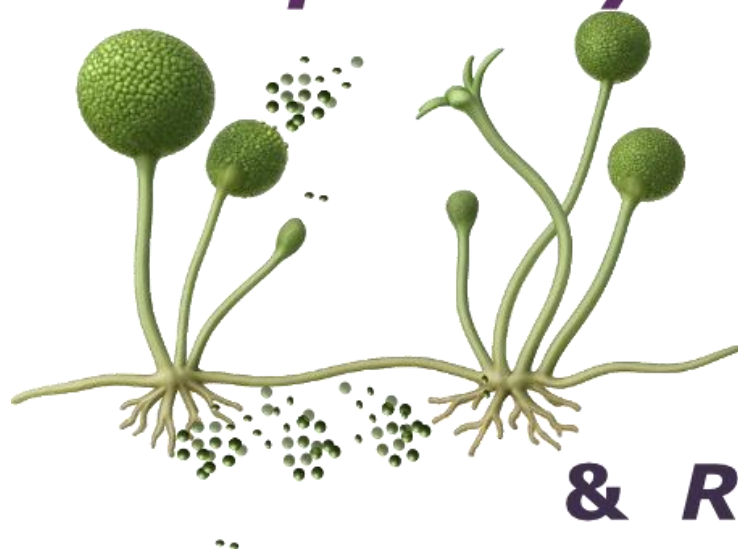
Aroma: nutty, buttery, sour and roasted notes

Taste:
Umami and Sour
Bitterness remain but it is masked

Texture:
Chewiness, compact and slices

Appearance:
After cooking Golden and Grey

Co-culture of *Rhizopus oryzae*



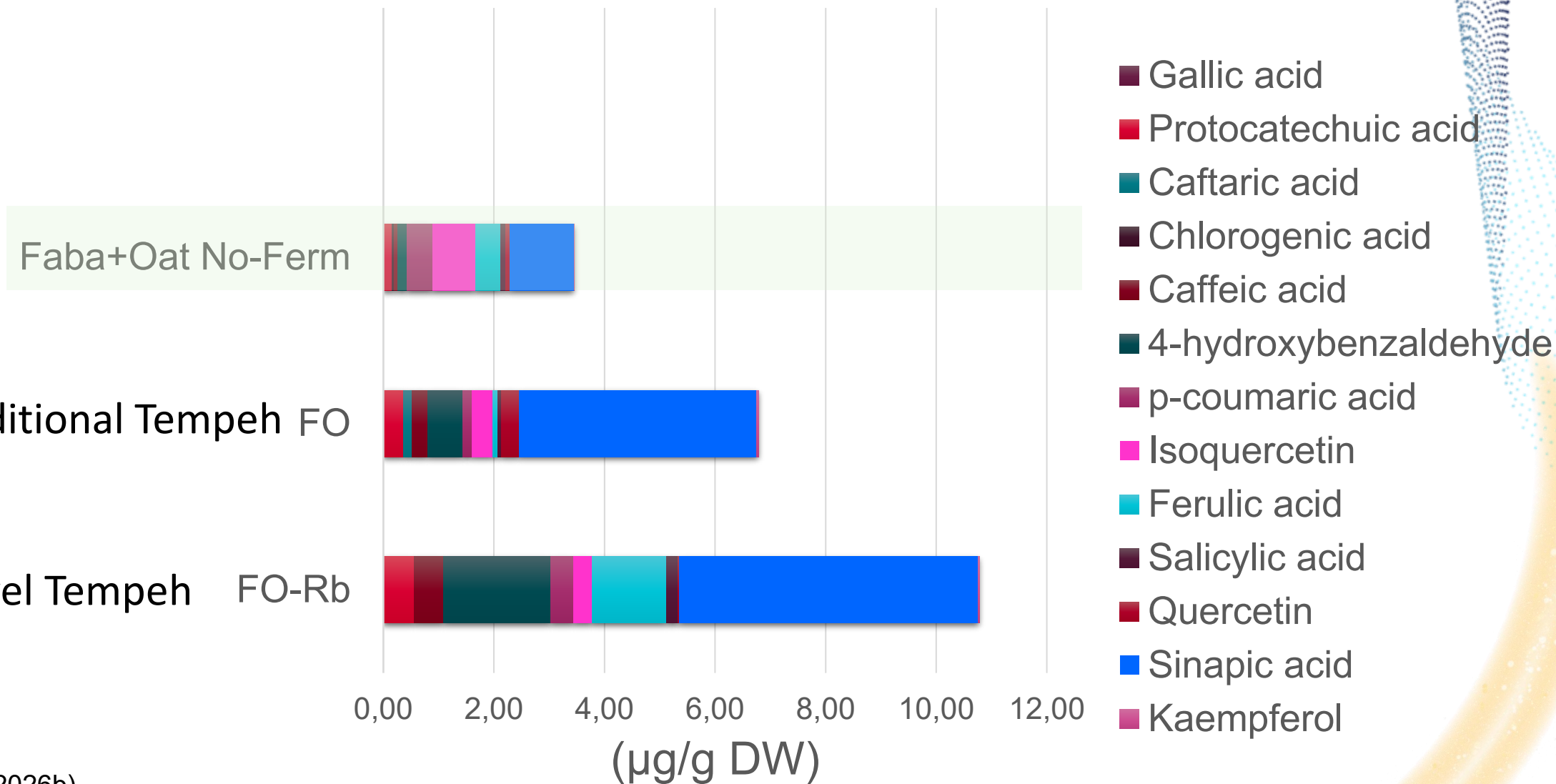
& *Rhodotorula babjevae*



**Novel
co-fermentation**

Faba-Oat Tempeh

Profiling of free phenolic compounds



CONCLUSIONS



Legumes and cereals contribute to a healthy diet.
Fermentation can further **improve** their nutritional quality

Nutrient bioavailability

- ✓ ↑ protein digestibility & free amino acids
- ✓ Improved estimated iron and zinc availability,

Anti-nutrients ✓ Reduction of phytic acid & v-c

Microstructure ✓ Cell wall disruption → better nutrient release

Sensory characterization and Liking

CONCLUSIONS



- ✓ Tempeh fermentation increases umami & sourness, masking beany off-flavours.
- ✓ Texture

CONCLUSIONS



Novel fermentation with *Rhodotorula babjevae*

- ✓ Higher total free phenolic content.
- ✓ Higher content of total free amino acids, especially Tyr and Ser.

Faba-Oat Tempeh

- ✓ Soy-free
- ✓ Locally produced
- ✓ Made from whole legumes and cereals
- ✓ Minimally processed (NOVA classification)
- ✓ Easy to cook and easy to make at home

100 g of fresh tempeh

**14 g
protein**

**6 g of
fiber**

**Reduced
Antinutrients**

**Clean-
label**





Collaboration



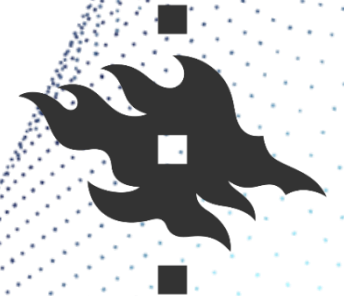
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HELSINGIN YLIOPISTO

ETH zürich



Universitas
Gadjah Mada

- Fernandez Castaneda, L.A., Auer, J., Leong, S.L.L., Newson, W.R., Passoth, V., Langton, M. and Zamaratskaia, G., **(2024)**. Optimizing soaking and boiling time in the development of tempeh-like products from faba bean (*Vicia faba* L.). *Fermentation*, 10(8), p.407. <https://doi.org/10.3390/fermentation10080407>.
- Fernandez Castaneda, L.A., Saini, S., Laaksonen, O., Kårlund, A., Leong, S.L.L., Newson, W.R., Passoth, V., Hanhineva, K., Langton, M. and Zamaratskaia, G., **(2025)**. Sensory and volatile compound profiles in tempeh-like products from faba bean and oats. *Current Research in Food Science*, 10, p.101029. <https://doi.org/10.1016/j.crfs.2025.101029>.
- Fernandez Castaneda, L.A.F., Lu, J., Auer, J., Hansson, H., Kravchenko, O., Pokorna, S., Tuccillo, F., Zehnder-Wyss, O., Edelmann, M., Katina, K. and Nyström, L., Zamaratskaia, G., and Langton, M.; **(2026a)** . Changes in the nutritional profile, anti-nutrients, microstructure, and *in vitro* digestibility of faba bean–oat tempeh during pre-treatment and fermentation. *Food Chemistry*, p.149050. <https://doi.org/10.1016/j.foodchem.2026.149050>.
- Fernandez Castaneda, L. A., Zamaratskaia, G., Leong, S.-L. L., Passoth, V., Viretto, C., Alabiden Tlais, A. Z., Di Cagno, R., Tuccillo, F., Edelmann, M., Katina, K., Zehnder-Wysse, O., Nyström, L., Rahayu, E. S., Mariyatun, M., Langton, M., & Newson, W., **(2026b)**. Synergistic co-culture of *Rhizopus oryzae* and *Rhodotorula babjevae* transforms faba bean and oat into nutrient-rich tempeh-like foods. *Food Research International*, p.119720. <https://doi.org/10.1016/j.foodres.2026.119720>.
- Auer, J., Duivenvoorde, L., van der Zande, M., Alminger, M., Fernandez Castaneda, L. A., Lu, J., Tizzanini, G., Zamaratskaia, G., Högberg, A., & Langton, M. **(2026)**. Effect of processing on the protein digestibility and mineral bioavailability of legumes. *Food Research International*, 232, 118938. <https://doi.org/10.1016/j.foodres.2026.118938>.

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Join us

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By

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Tempeh
By

Alejandra
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Faba bean
&
Oats



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