Lise Friis Christensen
Astrid Lund Paulsen

REFINES

Remove off-flavors in plant protein side-streams

(January 2025 to December 2027)

Photo from iSTOCK

novo nordisk foundation
PLANT2FOOD
A Novo Nordisk Foundation Sponsored Initiative
AARHUS UNIVERSITY
DTU
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OUTLINE

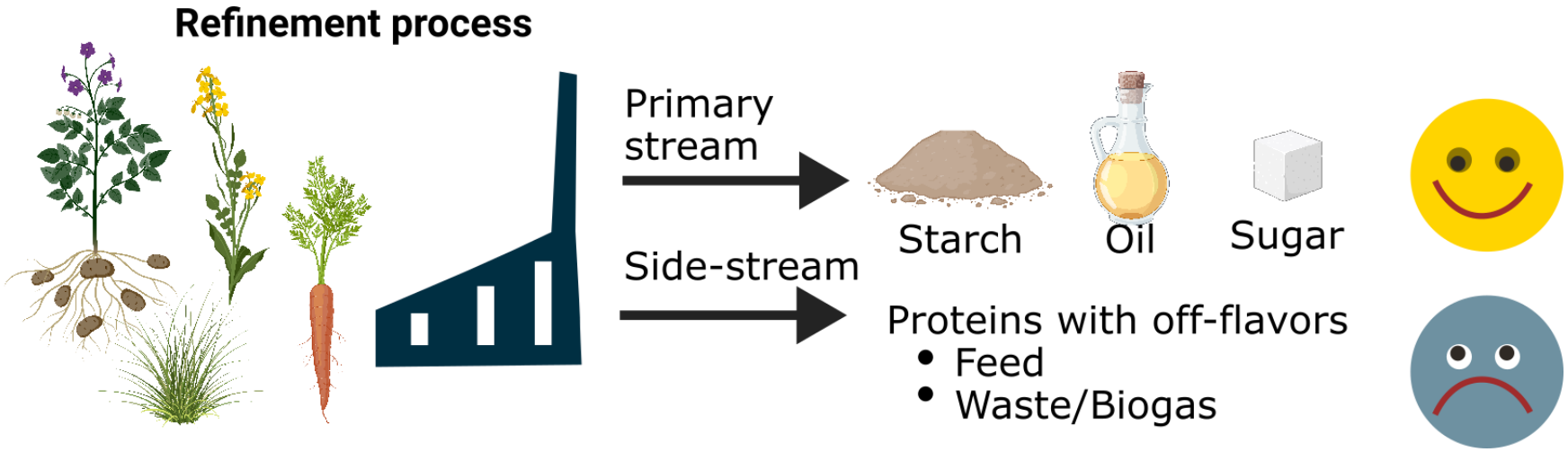
Background of the REFINES Project

Understanding the Origin of Off-Flavors (Part 1)

Screening for Off-Flavor-Removing Enzymes (Part 2)

Future Impact and Applications

THE CHALLENGE
Loss of quality food-grade protein



THE CHALLENGE
Loss of quality food-grade protein

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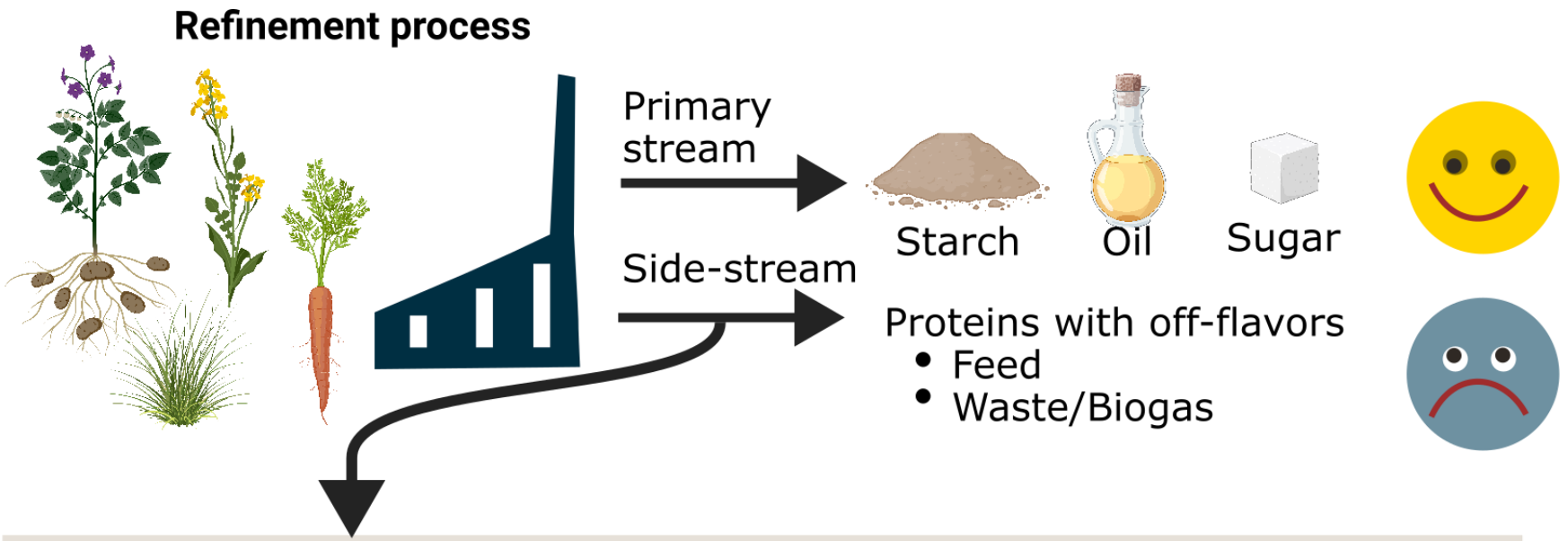

ID of off-flavor molecules
+ Detection methods
+ Understanding of origins
+ Enzymatic removal

→ Food-grade protein for human consumption

Some illustrations sourced from Biorender.com

CASE AND PARTNERS

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Open-innovation platform

Pre-competitive frame

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DRIVING QUESTIONS
For academia and industrial partners

REFINES

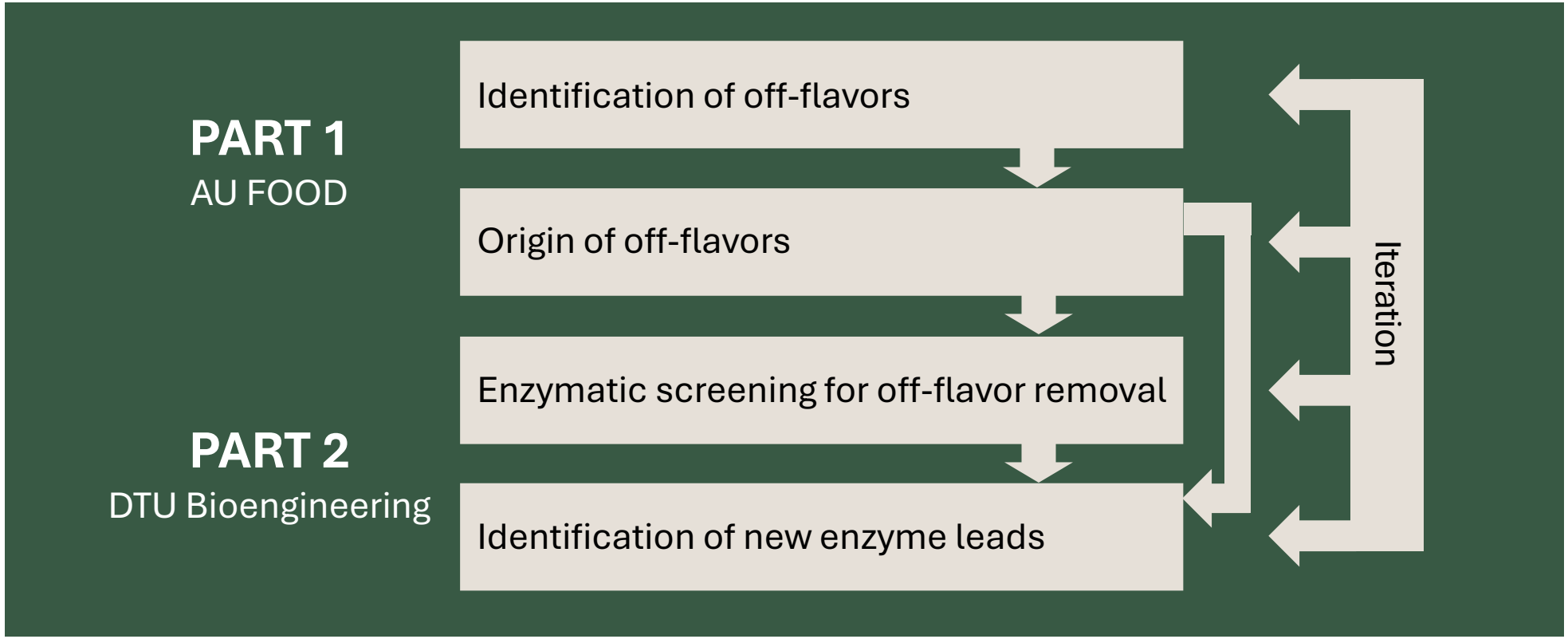
Pre-competitive frame

What is the origin and/or causing the off-flavors?
Understanding off-flavors at the molecular level

How can we circumvent the off-flavors in potato protein side-streams?
Tackel off-flavors or off-flavor precursors

PROJECT STRUCTURE
Key challenges

REFINES



PART 1
Understanding the Origin of Off-Flavors

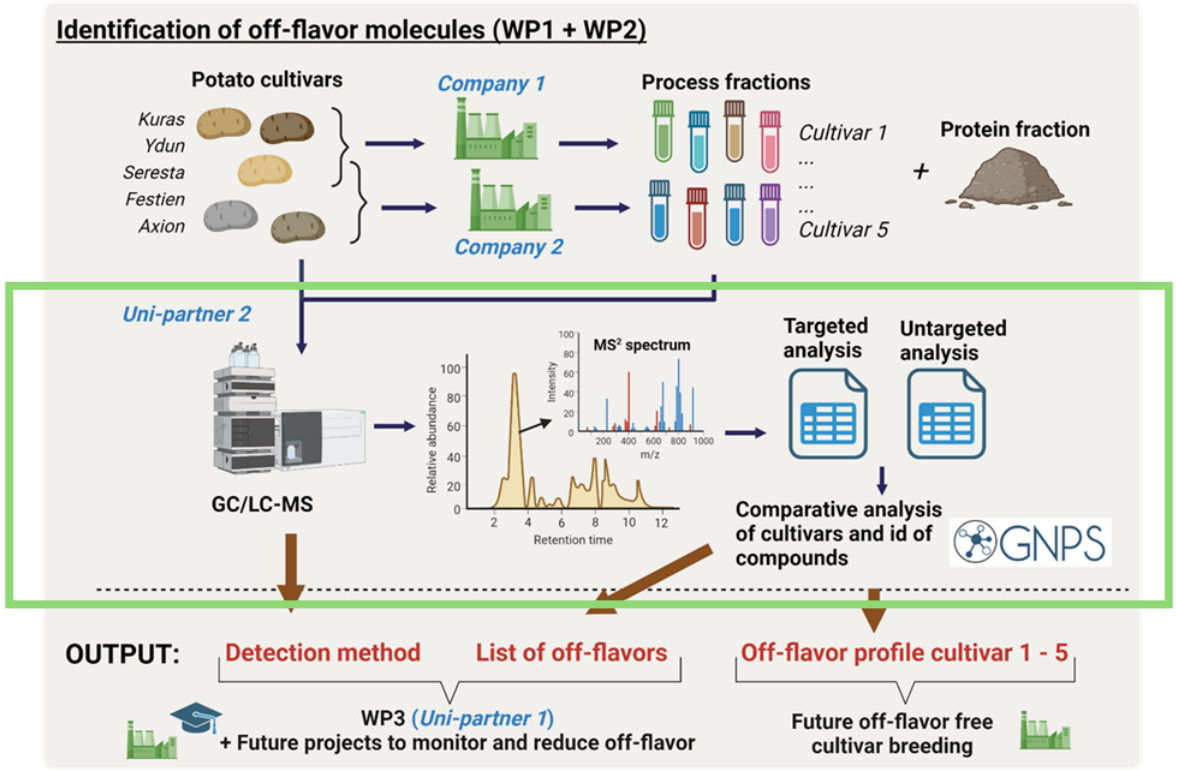
REFINES

UNDERSTANDING OFF-FLAVORS

AU overall **aim**: Understanding off-flavors in potato juice

- Screen and analyse for off-flavors
- Understand the mechanisms of how they are formed: Biological and process

Contribute with:
Analytic methods



UNDERSTANDING OFF-FLAVORS

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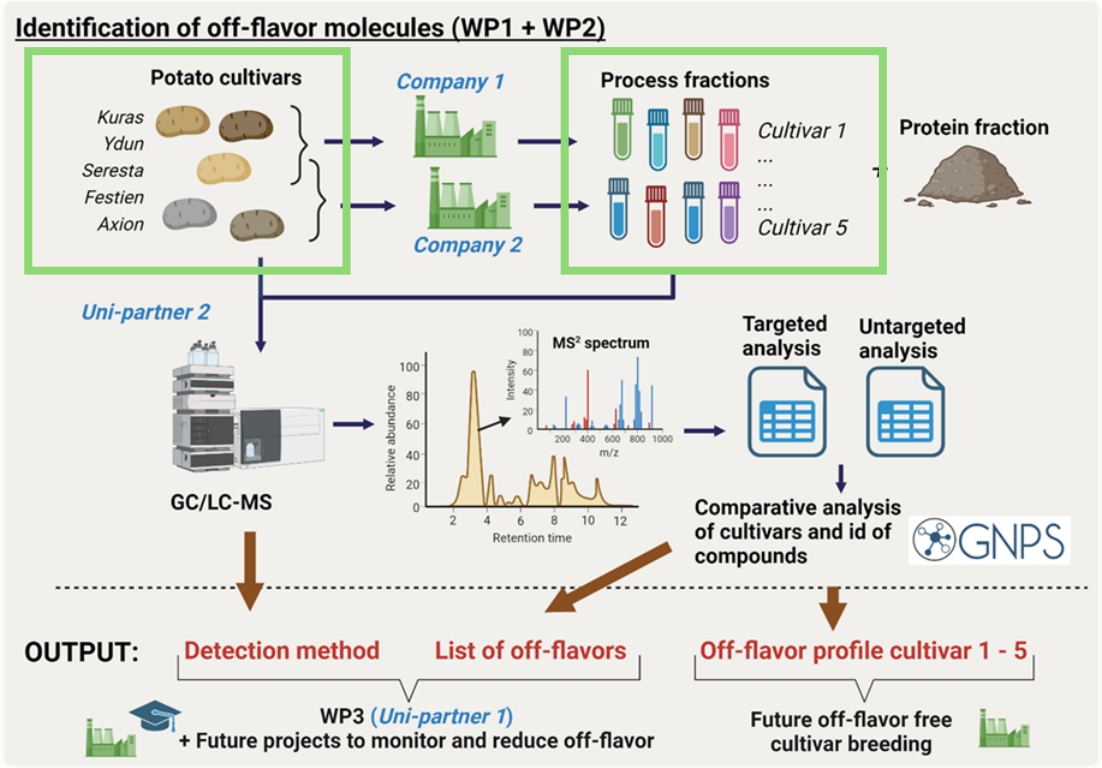


OFF-FLAVORS TO FOCUS ON

REFINES

Lipidomics and lipid oxidation

Metabolomics

OFF-FLAVORS TO FOCUS ON

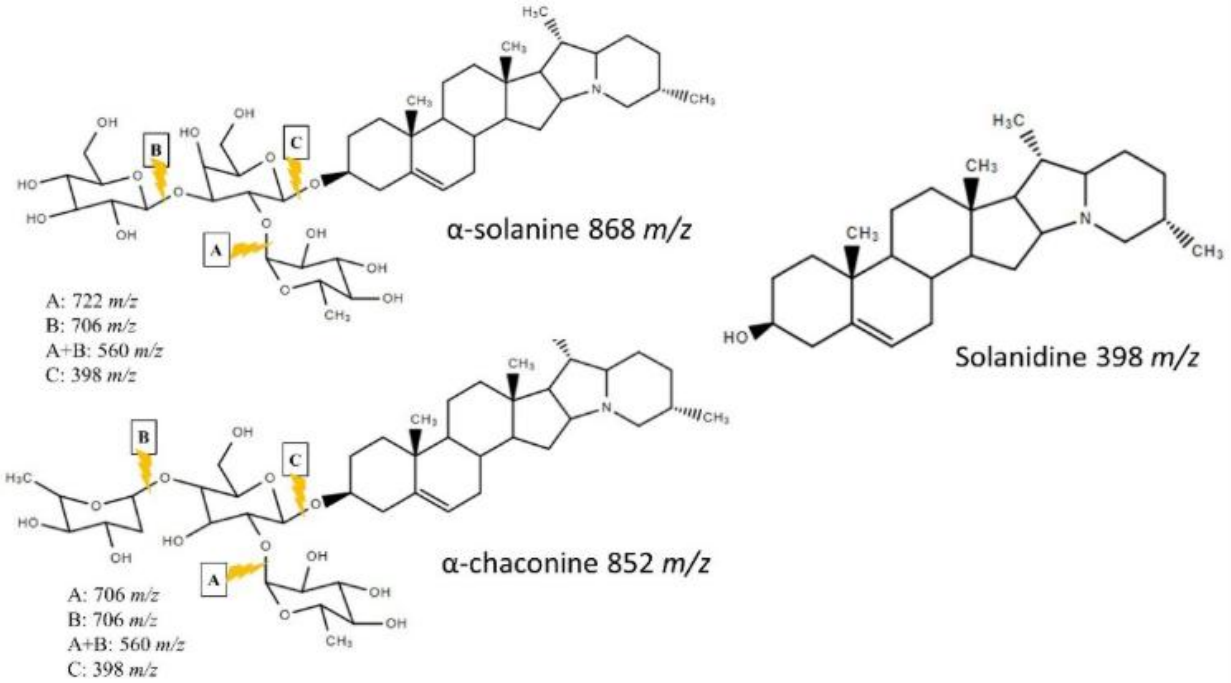


Figure: Nielsen, S. D et al. https://doi.org/10.3390/foods9040416

Metabolomics

Untargeted screening: Linked to known off-flavors

Tri-glycoalkaloids (TGAs): Bitter and toxic

Hydrophobic amino acids, Malliard products and strecker aldehydes

Methods: LC-timsTOF + GC-MS (TMS-derivatization)

Data analysis: Chemometrics + Molecular networks (GNPS)

OFF-FLAVORS TO FOCUS ON

REFINES

Lipidomics and lipid oxidation

Formation

1) Endogenous enzymes

Lipidoxygenase (LOX) and Patatin

Juicing: Enzyme in contact with substrate (fast)

Methods: Enzyme assays

Methods

LC-timsTOF	Triglycerides Phospholipids
GC-MS + GC-FID	Poly-unsaturated fatty acids
LC-MS + Spectrometry	Hydroperoxides (LOOH)
SPME-GC-MS	Volatile oxidation products

OFF-FLAVORS TO FOCUS ON

REFINES

Lipidomics and lipid oxidation

Formation
1) Endogenous enzymes
Lipidoxygenase (LOX) and Patatin

Juicing: Enzyme in contact with substrate (fast)

Methods: Enzyme assays

2) Process
Conditions: Temperature, pH etc
Process strategies

Methods: Established GC- and LC-MS methods

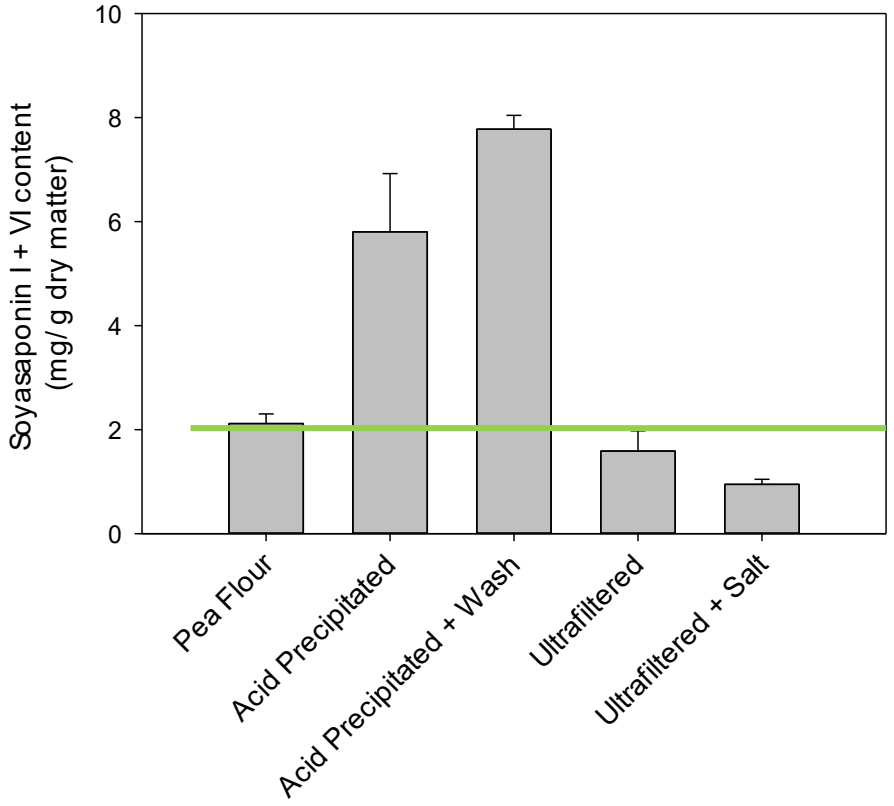
Methods

LC-timsTOF	Triglycerides Phospholipids
GC-MS + GC-FID	Poly-unsaturated fatty acids
LC-MS + Spectrometry	Hydroperoxides (LOOH)
SPME-GC-MS	Volatile oxidation products

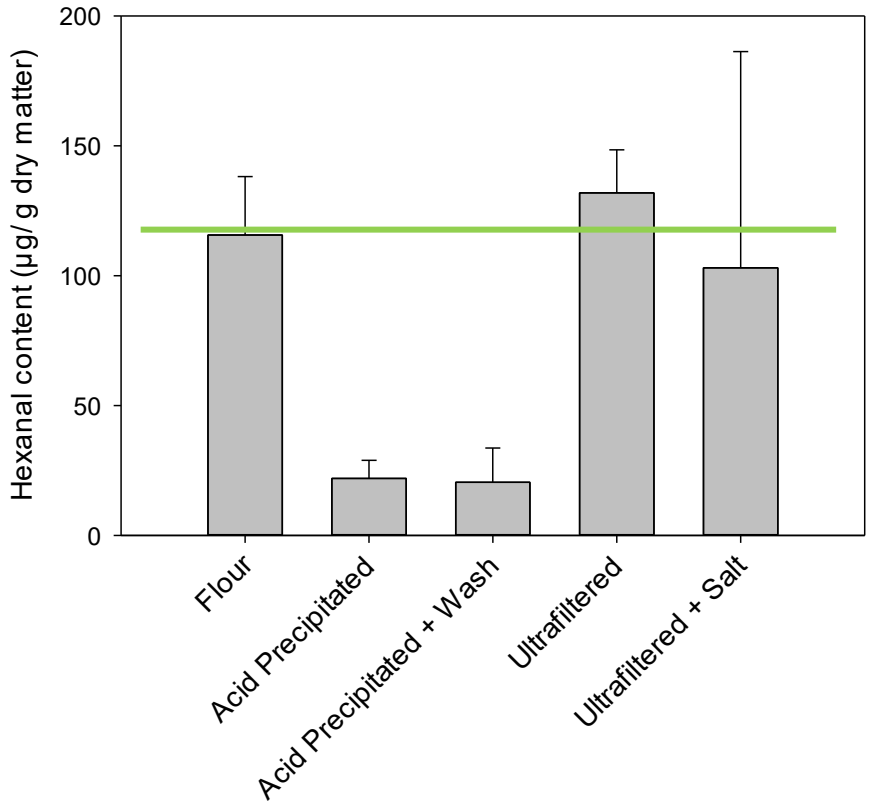
FATE OF OFF-FLAVORS UPON DIFFERENT PROCESSING STRATEGIES – LEARNINGS FROM PEA

REFINES

Pea saponins after acid precipitation and ultrafiltration

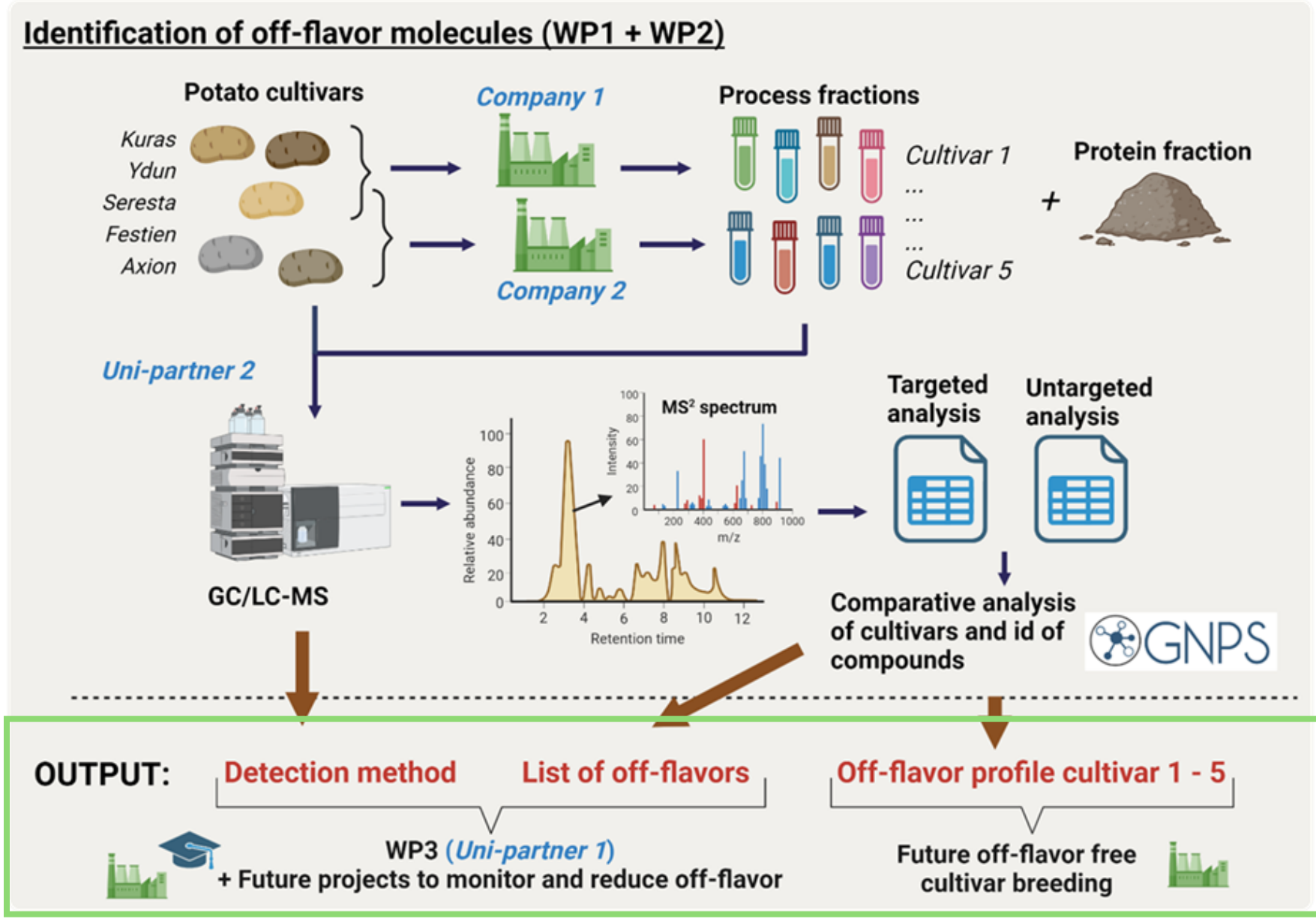


Hexanal after acid precipitation and ultrafiltration

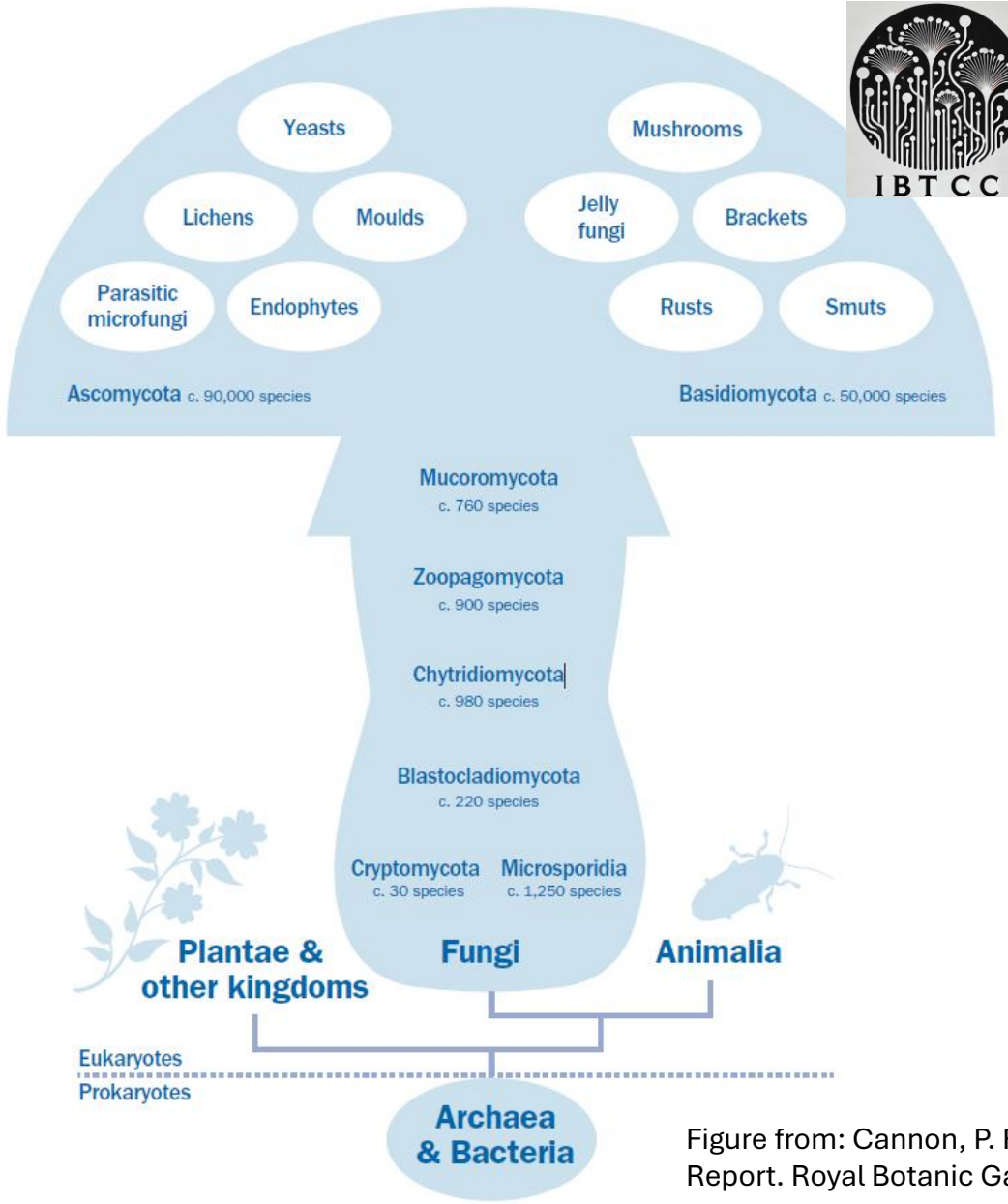


Conclusion:

Hexanal is eliminated and saponins are concentrated during acid precipitation.


Identification of off-flavor molecules (WP1 + WP2)
Potato cultivars
Kuras
Ydun
Seresta
Festien
Axion
Company 1
Company 2
Process fractions
Cultivar 1
...
...
...
Cultivar 5
Protein fraction
Uni-partner 2
GC/LC-MS
MS² spectrum
Intensity
m/z
Relative abundance
Retention time
Targeted analysis
Untargeted analysis
Comparative analysis of cultivars and id of compounds
GNPS
OUTPUT:
Detection method
List of off-flavors
Off-flavor profile cultivar 1 - 5
WP3 (Uni-partner 1)
+ Future projects to monitor and reduce off-flavor
Future off-flavor free cultivar breeding

PART 2
Screening for Off-Flavor-Removing Enzymes



FUNGI
An enzyme source

REFINES

IBT (Institute for BioTeknologi) Culture Collection

- DTU Bioengineering
- 40.000 fungal strains/isolates

Key Advantages of Fungal Enzymes

- Highly efficient secretion systems (large quantities of extracellular enzymes)
- Adaptation to diverse environments (robust, stable, and versatile enzymes)
- Ecological specialization (Enzymes evolved to degrade complex biopolymers)

Figure from: Cannon, P. F., et al. (2018). Definition and diversity. In: K. J. Willis (ed.), *State of the World's Fungi*. Report. Royal Botanic Gardens, Kew. pp. 4–11.

BITTER TASTING GLYCOALKALOIDS (GAs)

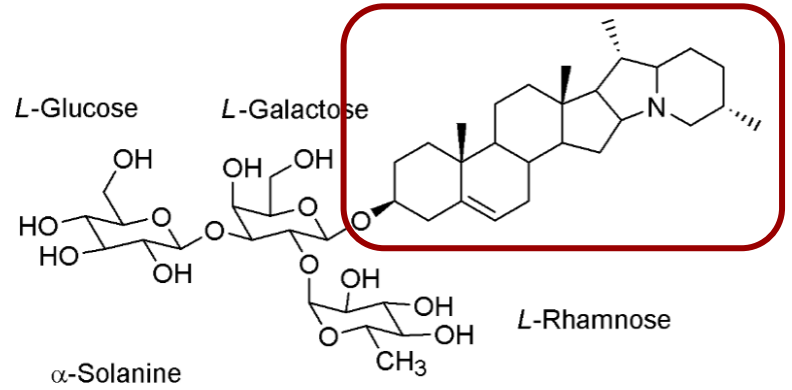
REFINES

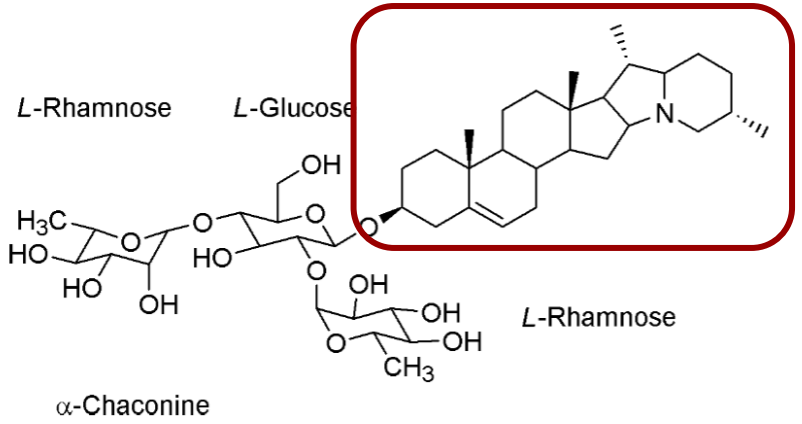
Potato defence systems

Highest GA levels in flowers and green plant parts, and during sprouting

- Biotic stress: Off-flavors and toxicity
- Abiotic stress: UV protection

Solanidine

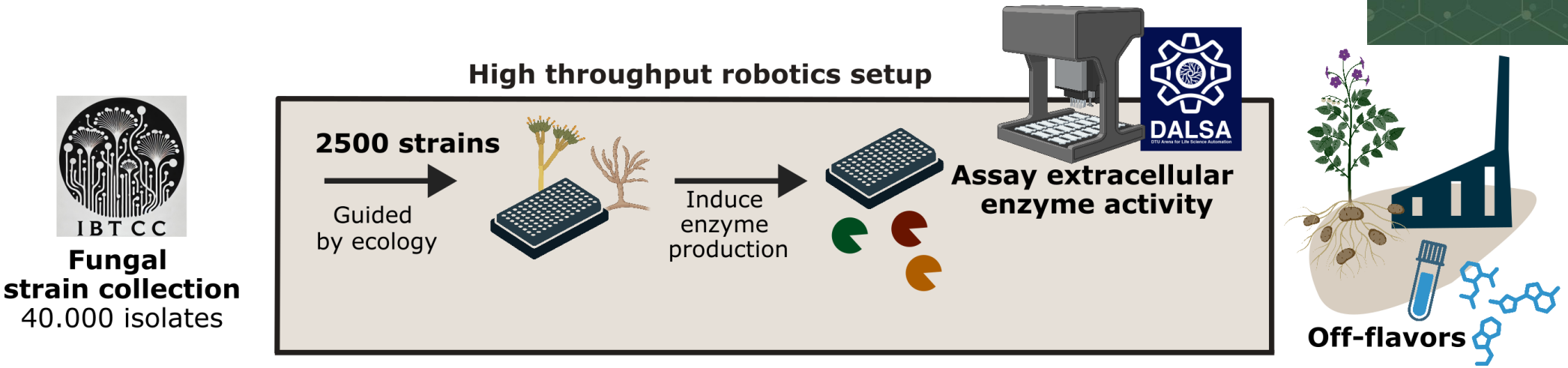




Fungi as potato pathogens: Detoxification of glycoalkaloids

- Secrete enzymes to degrade the GAs and solanidine
- First step involves glycosidases

DEVELOPING A SCREENING PLATFORM (I)



Screening challenge

- Large, repetitive workflow to screen a broad range of fungal strains
- Plate-based format: cultivation → enzyme production → colorimetric assay

Robotics-assisted enzyme screening enables

- Efficient sample handling (high throughput)
- Consistent conditions and reduced variability (high reproducibility)
- DALSA is the **D**TU **A**rena for **L**ife **S**cience **A**utomation to explore and develop new automated technologies within life sciences

DEVELOPING A SCREENING PLATFORM (II)

IBT CC

Fungal strain collection
40.000 isolates

High throughput robotics setup

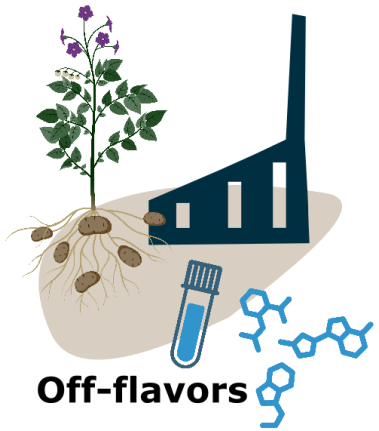
2500 strains

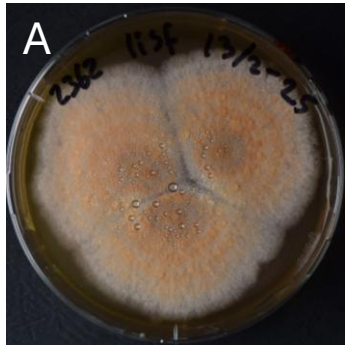
Guided by ecology

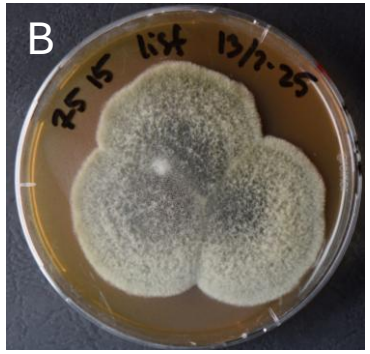
Induce enzyme production

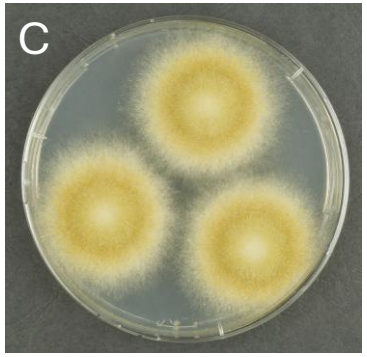
DALSA

Assay extracellular enzyme activity

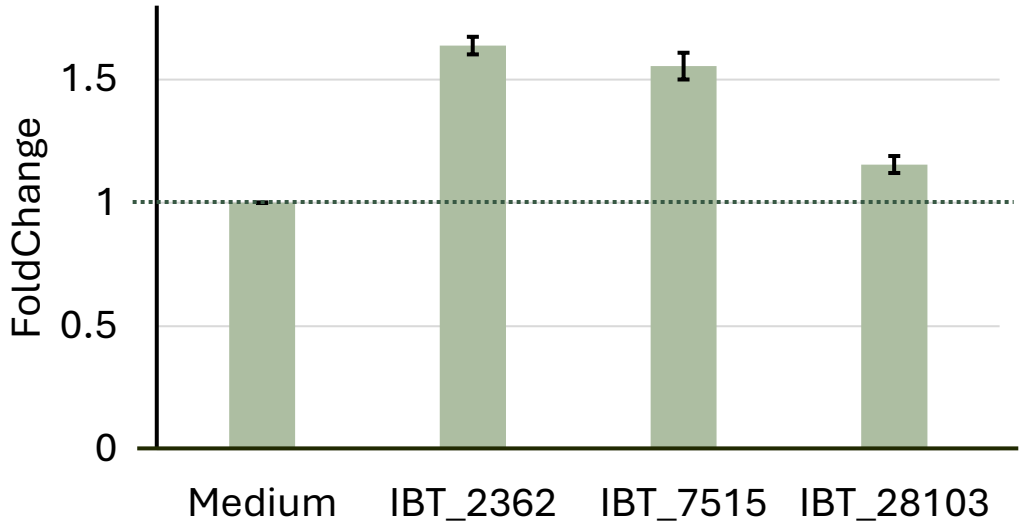






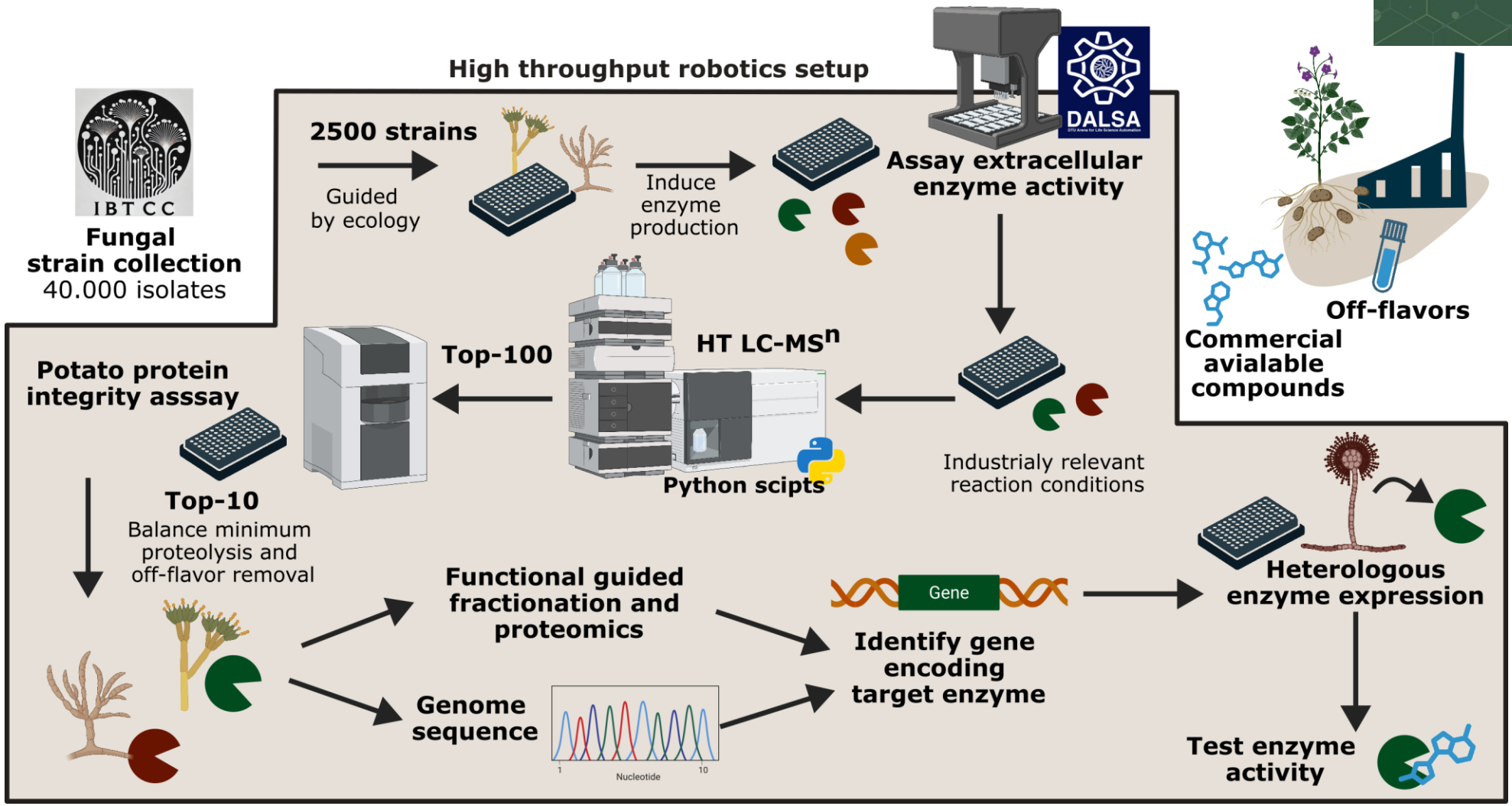


A) *Fusarium sambucinum* IBT 2362 (Potato tuber)
B) *Alternaria alternata* IBT 7515 (Potato)
C) *Aspergillus oryzae* IBT 28103 (Soy bean)



IDENTIFICATION OF NOVEL ENZYME LEADS

REFINES



Some illustrations sourced from Biorender.com

EXPECTED OUTPUTS

REFINES

Develop a robotics-assisted, high-throughput screening platform for efficient identification and comparison of fungal enzymatic activities

Discover novel enzymes with potential for industrial off-flavor degradation in potato protein side-streams

Uncover fungal metabolic pathways used by potato pathogens to bypass glycoalkaloid-based antimicrobial defenses

REFINES

THE REFINES PROJECT

Future Impact and Applications

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FUTURE IMPACT AND APPLICATION

REFINES

Output

Novel detection methods

Off-flavor ID

Off-flavor profiles of potato cultivars

Robotics-assisted enzyme screening platform

Enzyme leads for off-flavor removal

Future impact

Pre-competitive space

Chemistry on off-flavor formation
Predict off-flavor formation
Characterize new off-flavor removing enzymes

Competitive space

Off-flavor removal by new process steps, new plant cultivars, and new enzyme solutions

Other PLANT side-streams

Deploy learnings and tools to areas with similar challenges

Processed food sector

Use of potato proteins for new products
Replace existing animal-based proteins
New application areas for plant proteins

Consumers/Society

Consumer acceptace ↑
Better resource use

Animal-based proteins ↓
Environmental footprint
Food waste levels

Illustrations sourced from Biorender.com

THE REFINES TEAM

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AVEBE

Ronald Apeldoorn (Strategic manager)

Robin Spelbrink (Scientist)

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Trine Kastrup Dalsgaard (Prof.)

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Lise Friis Christensen (Postdoc)

REFINES at AU FOOD, 2025

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Please visit us at the Plant2Food stand

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THANK YOU FOR YOUR ATTENTION
Please visit us at the Plant2Food stand

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