Extraction of membrane protein from leaves: learnings from proteomic protocols

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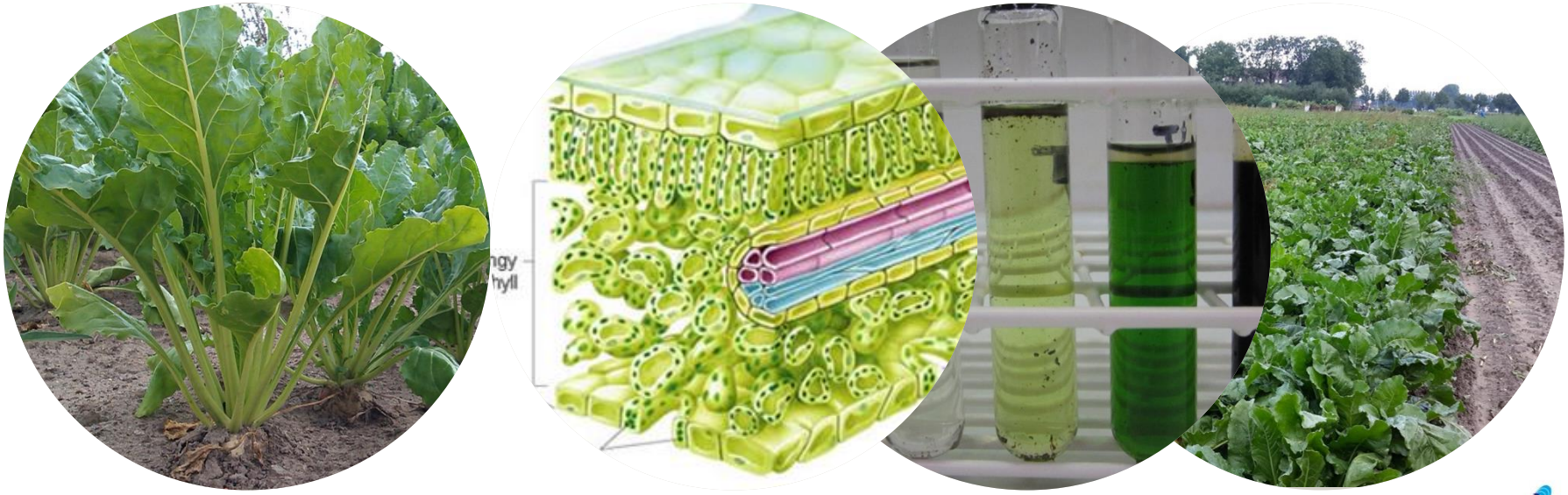
Remko Boom & Atze Jan van der Goot



WAGENINGEN
UNIVERSITY & RESEARCH

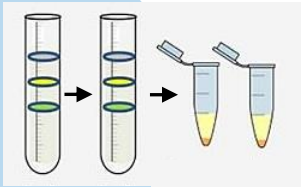
STW
Nieuwe technologie
mogelijk maken

Purification of leaf membrane proteins

WHAT? Understanding how to extract membrane proteins from leaves
Better use of resources, produce more food

HOW? Using extraction protocols designed for proteomics
Food process focused on soluble proteins, rubisco

- Specific role of each step (purification, fractionation).
- Selective extraction, single protein or small group
- Purity compromises yield
- Food-grade options depending on application



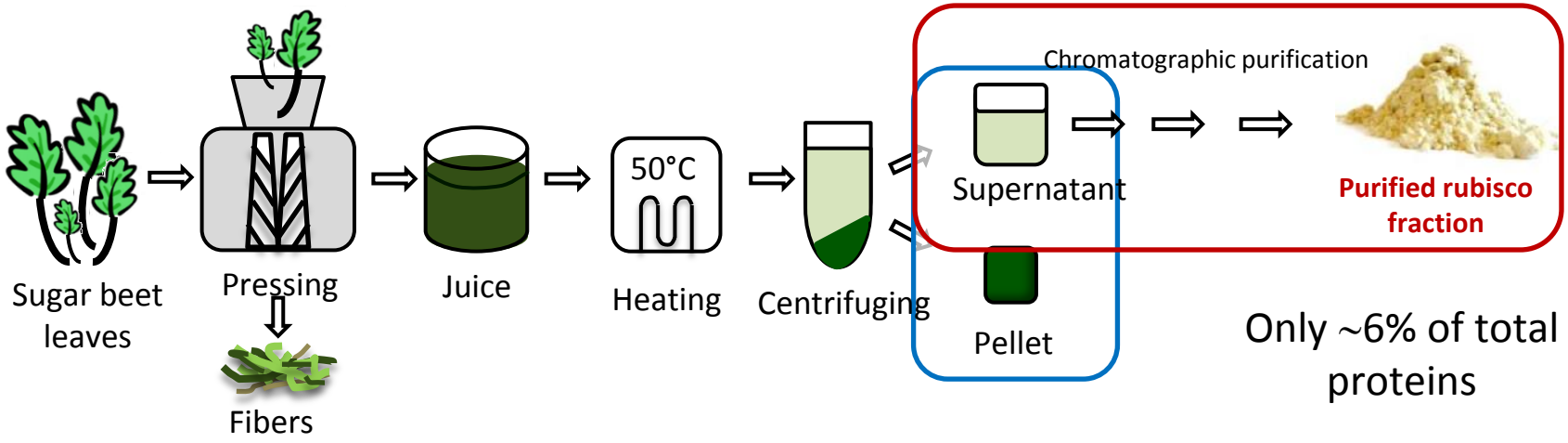
CONCLUSION

WHY?

- Multiple steps to break interactions or remove interfering compounds
- Heterogeneity of membrane proteins

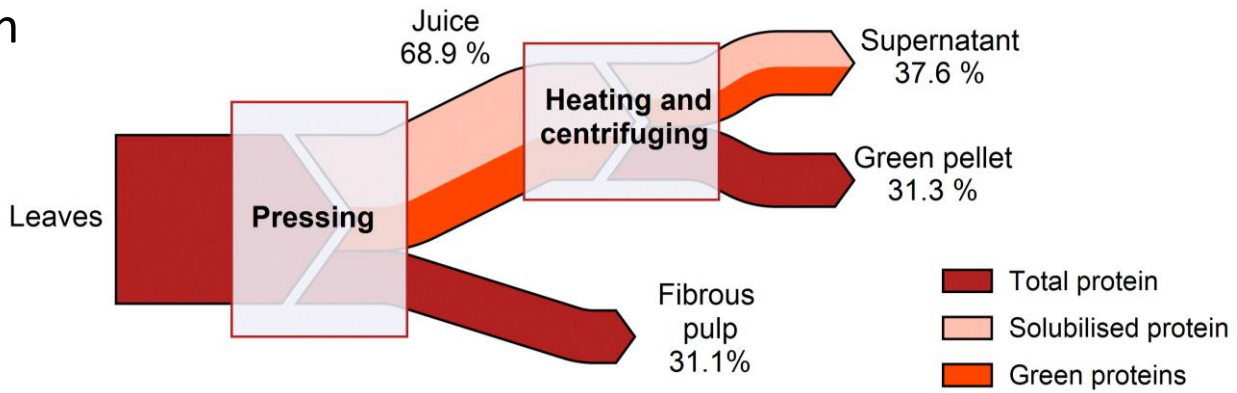
Leaf processing

- Optimization of resources

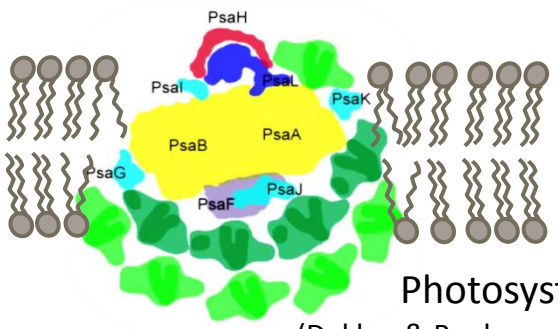


Only ~6% of total proteins

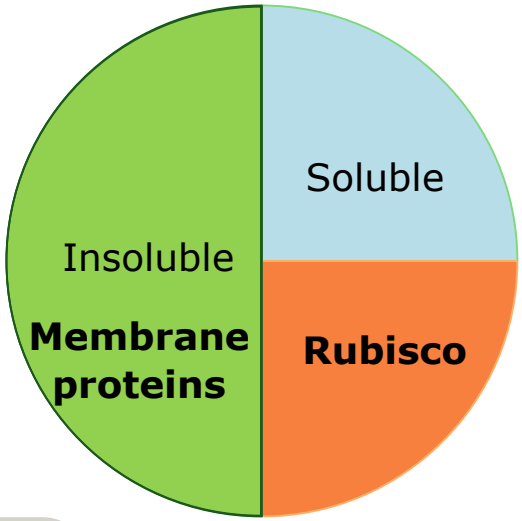
Protein distribution during processing

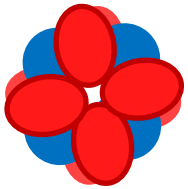


Leaf proteins



Photosystem I
(Dekker & Boekema, 2005)





Soluble and white
540 kDa, 2 subunits
Chromatographic methods!

~100 proteins, protein complexes
Subunits: between <5 and ~60 kDa
No water soluble, green
Membrane spanning

- No food-grade methods
- Understanding extraction conditions
- Type of interactions

Proteomics as learning tool

Large-scale **study** of proteins, pure form to understand biological systems

Protein extraction with proteomic protocols

- Selective extraction, complementary methods

Solvent extraction protocol

Multiple steps

Solvents for different conditions
- Trichloroacetic acid (TCA)
- Acetone
- Methanol
- Phenol/SDS

Surfactants, buffers

(Wang *et al*., 2008)

Phase partitioning protocol

Mild conditions

Based on hydrophobicity/ hydrophilicity

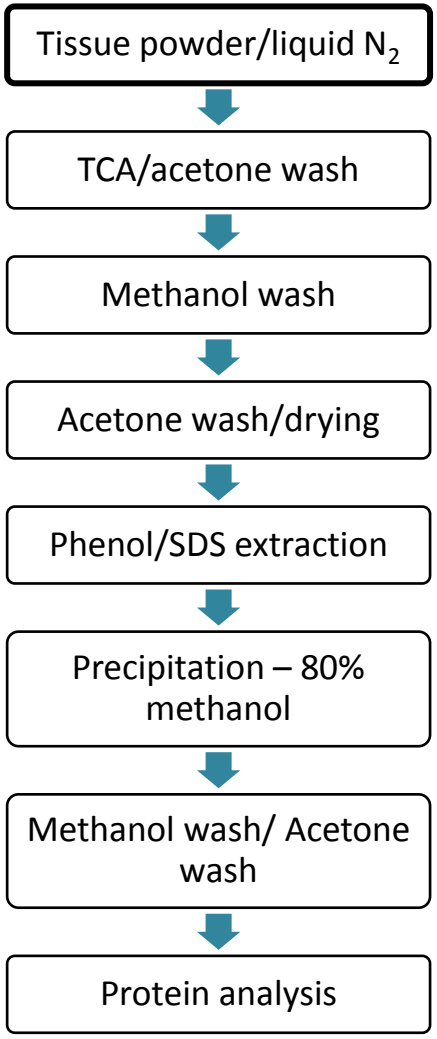
Phase separation of surfactant with low critical solution temperature: **Triton X-114**

Two phases (37°C):
- surfactant-rich (membrane proteins)
- Surfactant-poor (soluble proteins)

(Brusca & Radolf, 1994; Okamoto *et al*., 2001)

Tamayo Tenorio *el at*, 2016

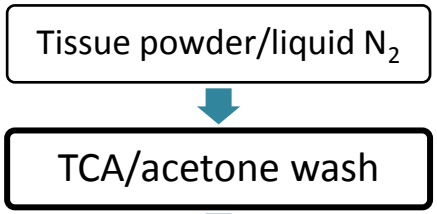
Learning from proteomic protocols



Open cell wall and release cell contents

Mechanical pressing of leaves

Learning from proteomic protocols



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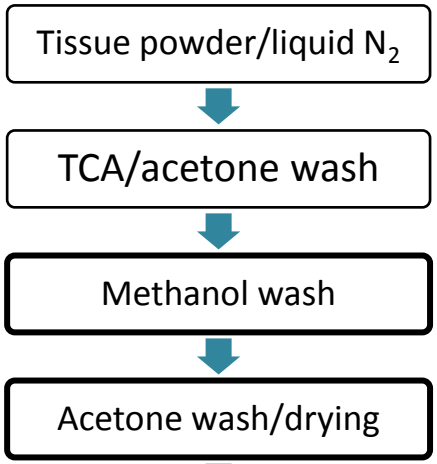
Mechanical pressing of leaves

Enzyme inhibition, protein precipitation

Learning from proteomic protocols



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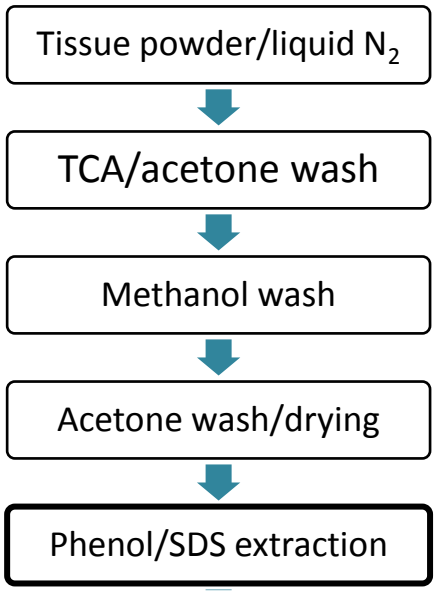
Mechanical pressing of leaves

Enzyme inhibition, protein precipitation

Removal of interfering compounds: phenolics, pigments

Learning from proteomic protocols



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Mechanical pressing of leaves

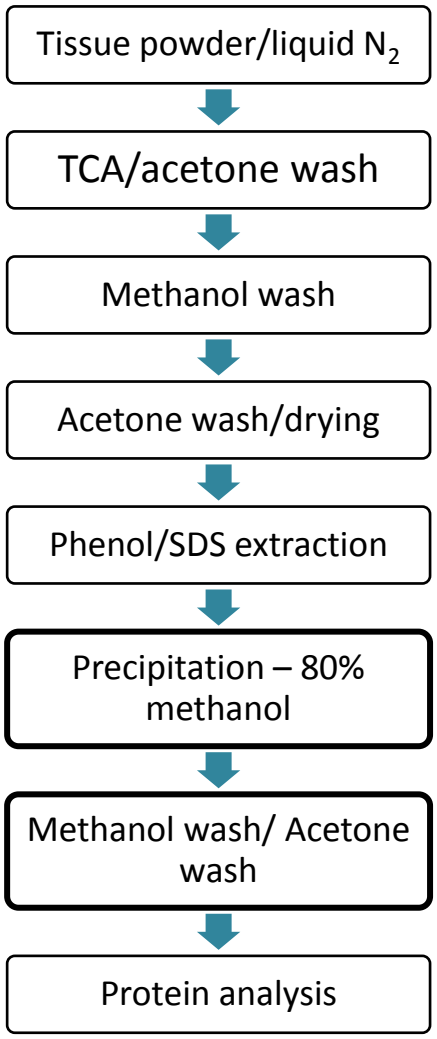
Enzyme inhibition, protein precipitation

Removal of interfering compounds: phenolics, pigments

Protein fractionation

- Weak electrostatic interactions
- Hydrogen bonds
- Hydrophobic interactions
- Trp anchor interhelical loops

Learning from proteomic protocols



Open cell wall and release cell contents
Mechanical pressing of leaves

Enzyme inhibition, protein precipitation

Removal of interfering compounds: phenolics, pigments

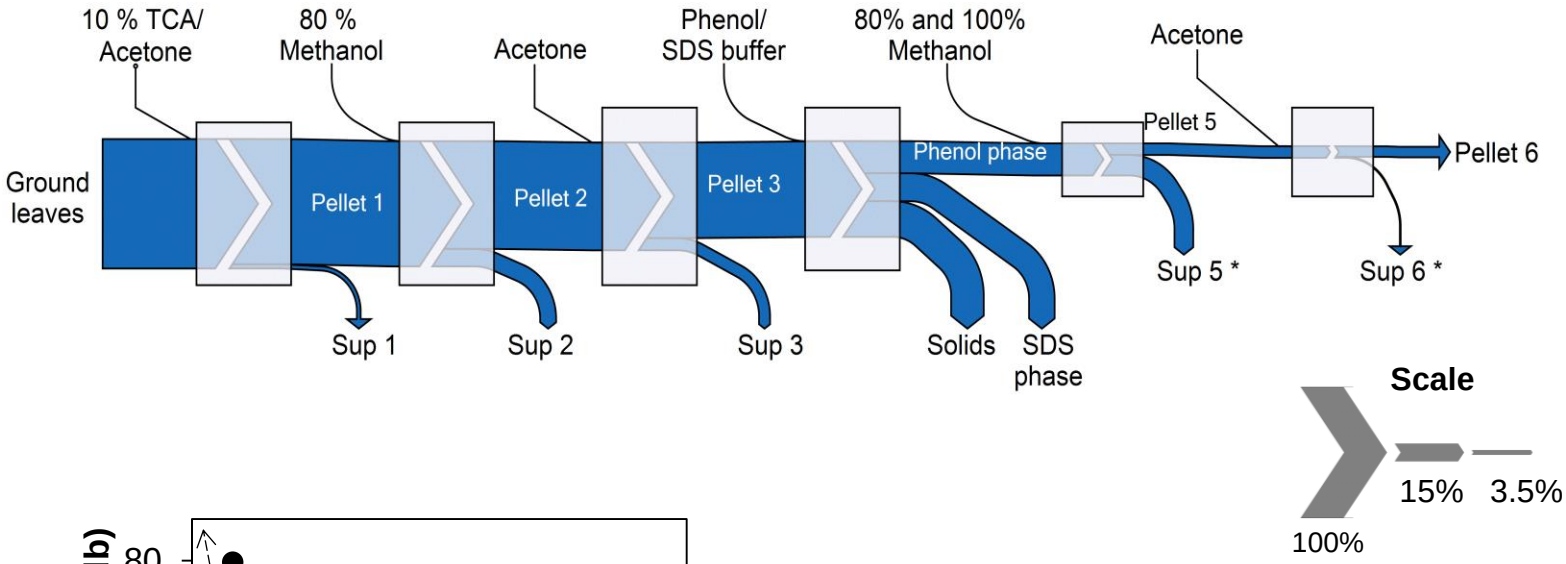
Protein fractionation

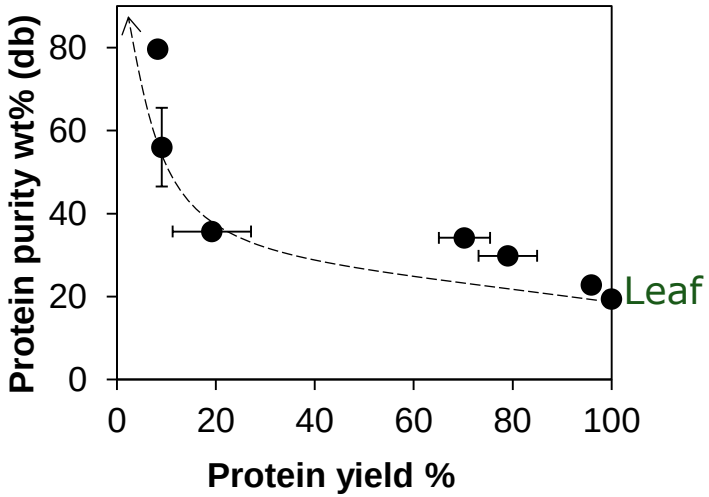
Recovery of the fractionated proteins
Removal of interfering compounds: phenolics, pigments
Multiple acetone washes, ethanol

- Specific role of each step (purification, fractionation).

CONCLUSION

Protein distribution



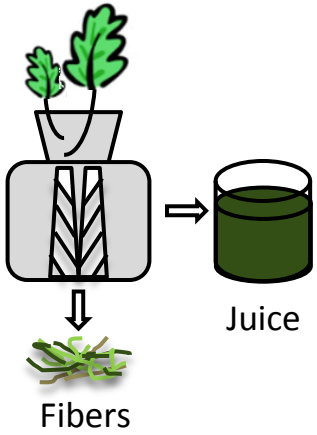


- Selective extraction, single protein or small group
- Purity compromises yield

CONCLUSION

Implications for food processes

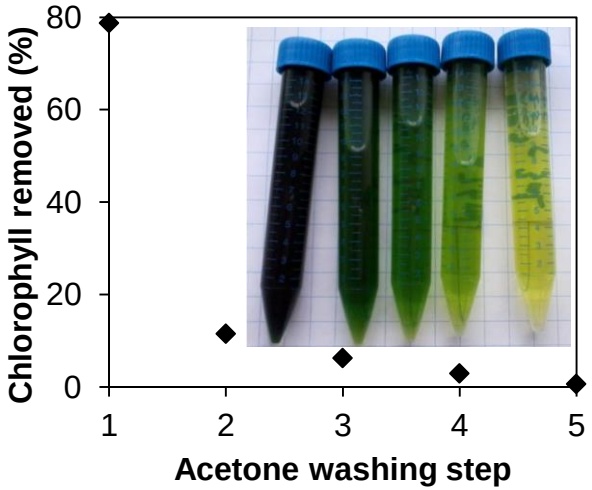
1. Tissue disruption
2. Enzyme inactivation
3. Protein fractionation
4. Removal of interfering compounds



2% metabisulfite

pH precipitation with HCl
Average IEP 4.5

Surfactants (Triton X-100)

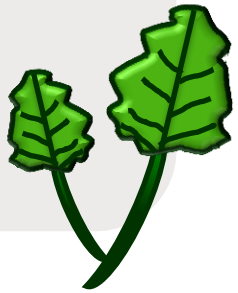


- Food-grade options depending on application

CONCLUSION

OPPORTUNITIES

- Heterogeneity of membrane proteins
- Different fractions with different physicochemical properties
- Total protein extraction, total leaf fractionation for better use of resources



Thank you!

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