

Comparative proteomic profiling of fava bean tissues using an optimized extraction method and DIA-MS

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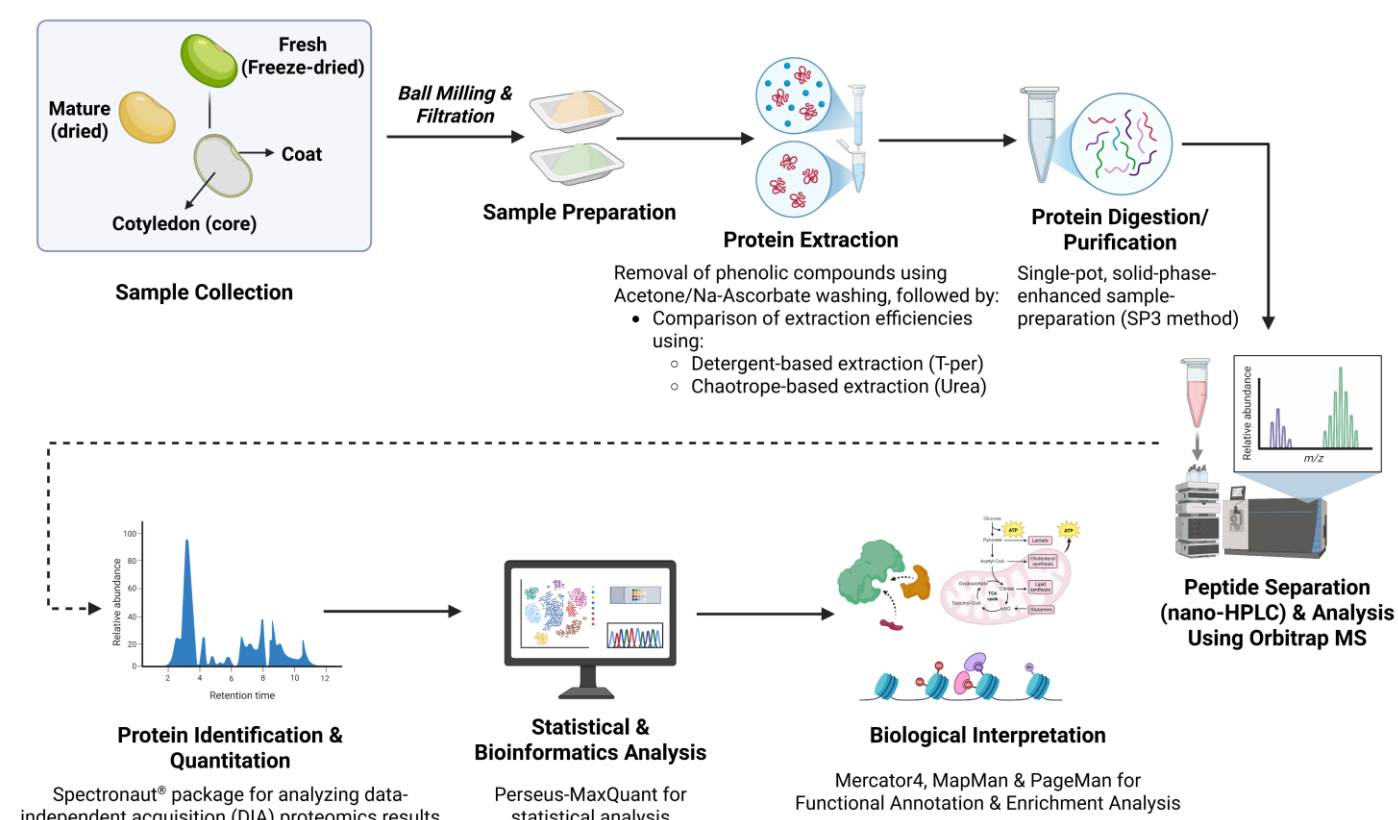
Towards High Protein Fava beans

Why?

Global demand for plant-based protein is rising, and faba beans provide high protein and functional compounds but their tissue-specific proteome and optimal extraction methods are poorly defined. Establishing a robust workflow will support better use of faba beans in food, breeding and sustainability applications.

How?

We compared a detergent-based (T-PER) and a chaotrope-based (8 M urea) extraction on freeze-dried faba bean tissues (fresh whole, dried whole, seed core and seed coat) from a controlled glasshouse experiment. Proteins were processed using SP3, analysed by DIA-MS on a Q-Exactive HF, and quantified against a new *Vicia faba* spectral library and annotated proteome using Spectronaut, Perseus, Mercator4, MapMan and PageMan.



What?

Urea extraction consistently produced higher protein yields, identified more protein groups (2,896 vs 2,071 for T-PER) and showed lower variability and stronger replicate correlations, making it the more reliable method. Across tissues we detected 2,661–3,126 proteins, with dried tissue giving slightly higher recoveries and the seed core enriched in storage proteins, while the coat showed a distinct proteome enriched for cell-division and DNA-damage response pathways. This is the first DIA-MS study of faba bean using an annotated species-specific proteome, providing deep and functionally resolved coverage.

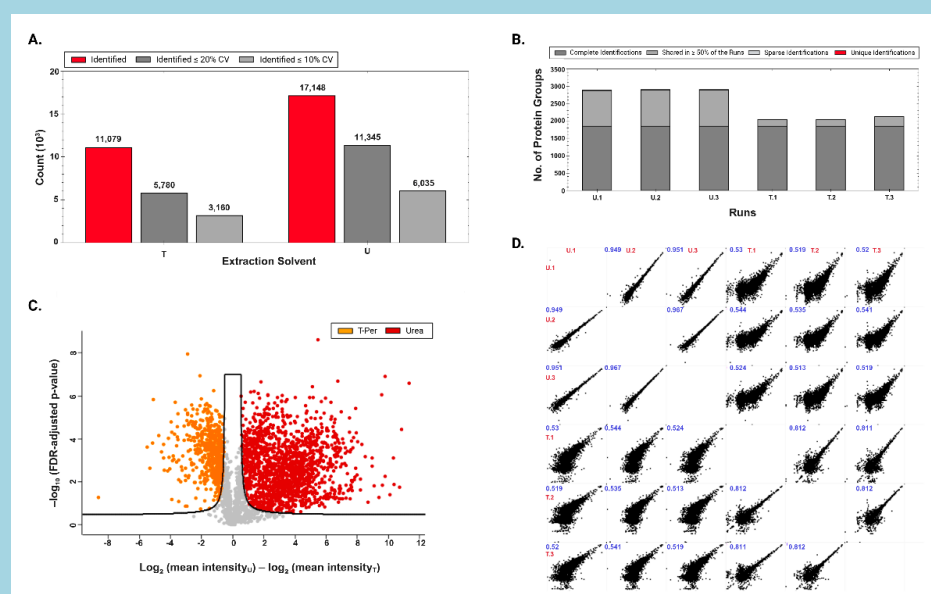


Figure 1: Comparison of detergent-based (T-PER) and chaotrope-based (urea) extraction for faba bean tissues, showing peptide CV distributions, numbers of protein groups, differential protein abundance and replicate correlations. Urea yields more peptides and protein groups with lower CVs, a higher proportion of high-abundance proteins and stronger run-to-run correlations, indicating superior extraction efficiency and reproducibility.

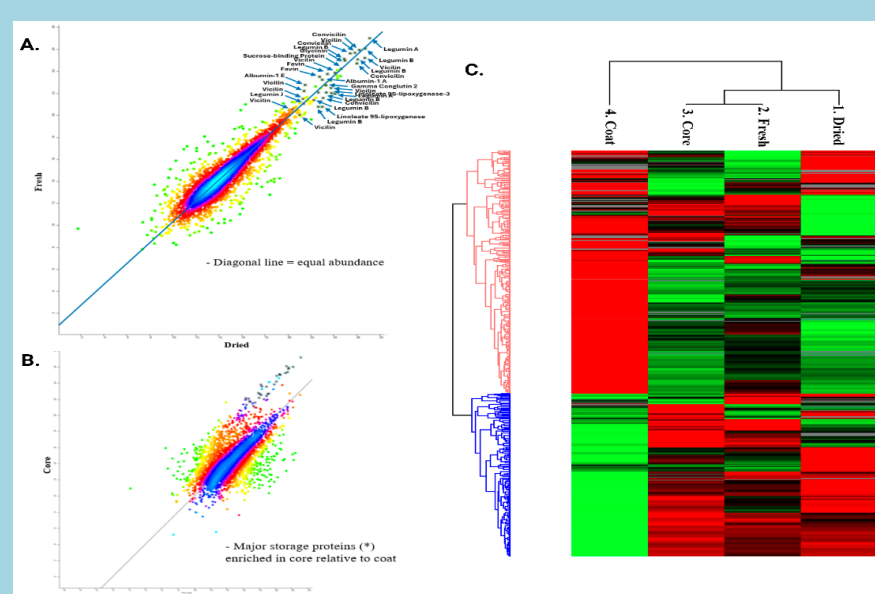


Figure 2: Proteomic comparison of faba bean tissues, showing protein abundance distributions for fresh vs dried whole beans and core vs coat by density plots, and a heat map of abundance across all four tissues with hierarchical clustering. Core tissue shows higher levels of major storage proteins, whereas the coat displays a distinct proteome consistent with a more specialised protective role.

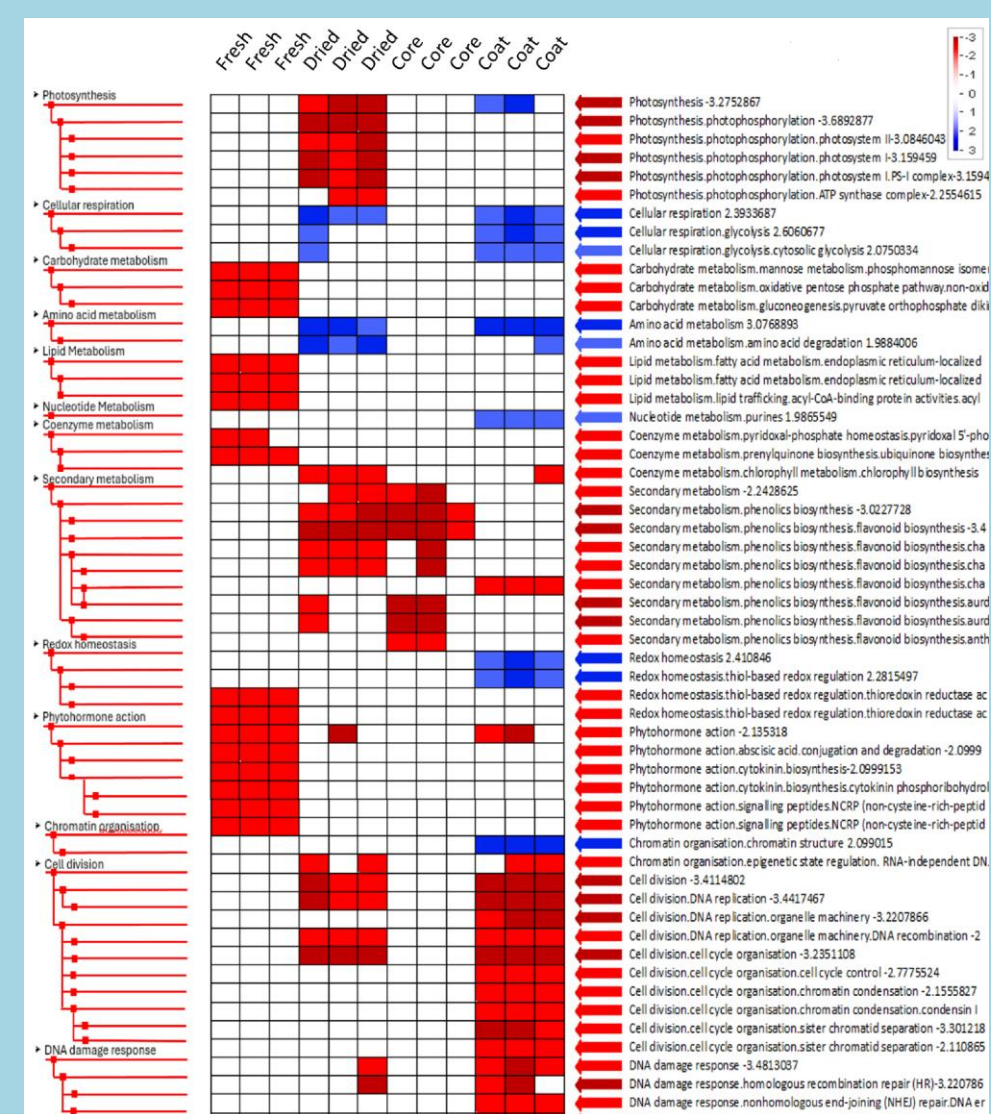


Figure 3: PageMan summary of tissue-specific metabolic pathways in faba bean, showing functional enrichment of proteins up-regulated in fresh, dried, core and coat tissues relative to their across-tissue average. Significantly enriched or depleted pathways (Fisher's exact test, $p < 0.05$) highlight photosynthesis/respiration in fresh tissue, secondary metabolism and phytohormone action in core, and cell-division and DNA-damage response in the coat.

What's Next?

Next, we plan to validate these tissue-specific patterns using biologically replicated plants and to extend the workflow to diverse faba bean genotypes and environmental conditions. The optimised DIA-MS pipeline and annotated proteome will then be used to link protein profiles to nutritional traits, processing performance and stress resilience, supporting breeding and product development in sustainable plant-protein systems. We are also developing NIR-based model to predict nutritional characteristics of Fava beans at the field-scale.

KEY POINTS

- Urea extraction yielded higher protein amounts and identified more protein groups than T-PER, with lower CVs and higher replicate correlations.
- Across tissues, 2,661–3,126 proteins were detected; dried tissue gave slightly higher yields, while coat tissue gave the lowest.
- Core tissue was enriched in storage proteins and pathways linked to secondary metabolism and phytohormone action; coat tissue was enriched in cell division and DNA-damage response pathways.
- The optimised DIA-MS workflow and annotated faba bean proteome provide a reusable resource for legume proteomics and sustainable plant-protein research.

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