CARINA Project: Glucosinolate extraction and characterization

CARINA is a transformative initiative funded under Horizon Europe, dedicated to advancing agricultural sustainability across Europe for bioeconomy development. By integrating camelina and carinata into local farming systems, CARINA aims to enhance economic, environmental, and social outcomes for European farmers.

OUR MISSION

CARINA's mission is to study diversified and low ILUC cropping systems including camelina and carinata as bioresources and their impact on productivity and ecological benefits (e.g. by increasing soil cover and biodiversity).

A central portion of the CARINA project focuses on the valorisation of camelina and carinata crops into bio-based products. A key output is the extraction of glucosinolates (GSLs) - sulphur-containing secondary metabolites found in Brassica species - from the press cake remaining after oil extraction. These bioactive compounds have potential applications in biocontrol and biostimulation.

This booklet reports on the experimental work, covering both the destruction of glucosinolates under mild thermal treatment and the development of a pilot-scale extraction protocol from Brassica carinata press cake.

LAB-BASED ASSESSMENT

Glucosinolate Destruction

Thermal treatment trials on Brassica carinata meal and press cake aiming to identify a detoxification pathway capable of reducing the antinutritional effect of GSL while preserving protein quality. The thermal experiments demonstrated how difficult this compromise was and why this approach was ultimately superseded by an industrial solution.

Pilot-Scale Extraction

Development and optimisation of a hydro-ethanolic extraction protocol, from the first production trial through lab-scale investigations to a second improved pilot-scale campaign. Key challenges: myrosinase activity and percolation limitations.

Concentration & Supply to Partners

Investigation of glucosinolate losses during miscella concentration, an optimised protocol to prevent enzymatic degradation, and the supply of crude GSL extracts to downstream CARINA partners for bioactivity testing.

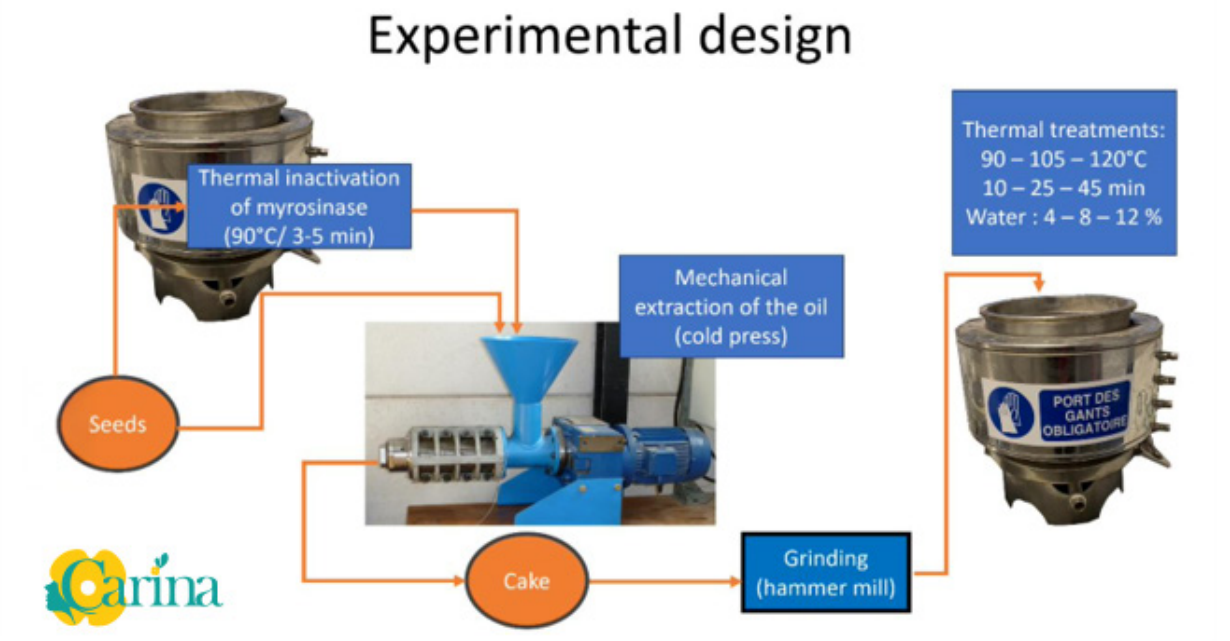
GLUCOSINOLATE DESTRUCTION

METHODOLOGY

Thermal degradation of glucosinolates in Brassica carinata meal was investigated using a bench-scale cooker. Seeds were either cold-pressed or pre-heated (90 °C, 3–5 min) to inactivate myrosinase, then the resulting press cake was subjected to a Box–Behnken experimental design varying:

- Temperature: 90 – 105 – 120 °C
- Duration: 10 – 25 – 45 minutes
- Initial moisture: 4 – 8 – 12 %

Both glucosinolate content (µmol/g) and protein solubility (KOH method) were measured as outputs.



Graphical presentation of the experiment design

KEY FINDINGS

≤45%

Maximum GSL reduction achieved (120 °C, 25 min, 8% moisture)

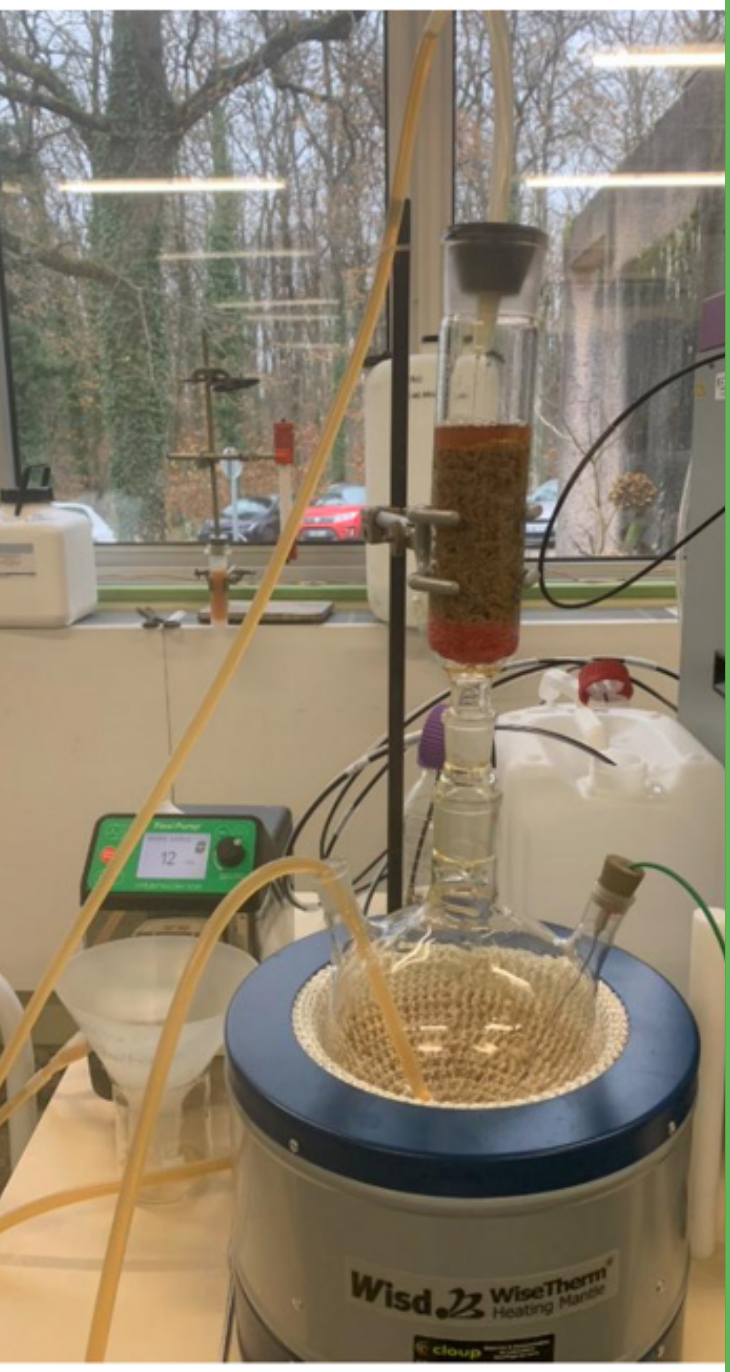
−33 pts

Drop in KOH protein solubility (from 82% to 55%) at most severe conditions

Even under the most stringent conditions tested, total glucosinolate reduction did not exceed 45% and was accompanied by severe deterioration of protein quality, making it unsuitable for high-value feed applications.

The objective of simultaneously achieving deep GSL reduction and preserving protein functionality could not be met under mild processing constraints.

Outcome: this task was discontinued after industrial partner SAIPOL independently validated its ability to adapt its industrial process at plant scale to reduce the glucosinotlate content in the meal. The lab results nonetheless confirm the technical challenge and provide a useful baseline for future process benchmarking.



Experimental extraction setup

TRIALS 1 & 2: PILOT-SCALE EXTRACTION

EXTRACTION PROTOCOL
Inspired by a mustard biorefinery concept (Hébert 2020). Key steps:

1. **Seed drying** (4–5% moisture)
2. **Myrosinase inactivation** (heat treatment)
3. **Mechanical pressing** (MBU20 / Komet press)
4. **Hydro-ethanolic extraction** (50–60% ethanol, 40–50 °C) in percolation column
5. **Miscella concentration** (vacuum evaporator)

Trial 1: no pre-heat — ethanol assumed sufficient to inactivate myrosinase.

Trial 2: explicit thermal inactivation at 100 °C for 20 min; smaller die (4 mm Komet press) for better cell-wall disruption; Higher ethanol content was used to limit residual myrosinase activity, while weak acidification was introduced to favour the extraction of ionized glucosinolates.

The first trial revealed that myrosinase remains active in 50% ethanol, causing ~48% of glucosinolates to be hydrolysed during processing. Lab-scale experiments confirmed near-complete extraction is achievable when material is milled and immersed; but in percolation through intact press cake, diffusion is limited.

The second trial addressed these issues with heat inactivation and introducing a pellet-shaped press cake (Komet, 4 mm die), achieving much lower enzymatic losses. However, the concentration step emerged as a new bottleneck: both the rotary evaporator (36% loss) and the large boiler (61% loss) caused significant GSL degradation under vacuum.

	Trial 1	Trial 2
Myrosinase inactivation	None (ethanol assumed sufficient)	100 °C for 20 min (conditions designed in previous experimentation)
GSL recovery in extract	12% of initial GSL	~70% cumulative (3 washes)
GSL losses (hydrolysis)	~48%	~1% during extraction
GSL remaining in spent cake	39%	~30%
Concentration losses	Not measured (first trial)	36–61% depending on method
Main finding	Myrosinase active in 50% EtOH	Concentration step is critical

Summary of extraction steps results

FURTHER INVESTIGATIONS: CONCENTRATION & SUPPLY

WHY CONCENTRATION FAILS

Investigations at fixed temperature (60 °C) comparing high vacuum (<200 mbar) vs near-atmospheric (900 mbar) showed:

- High vacuum → 43–73% GSL losses
- No vacuum (900 mbar) → no losses

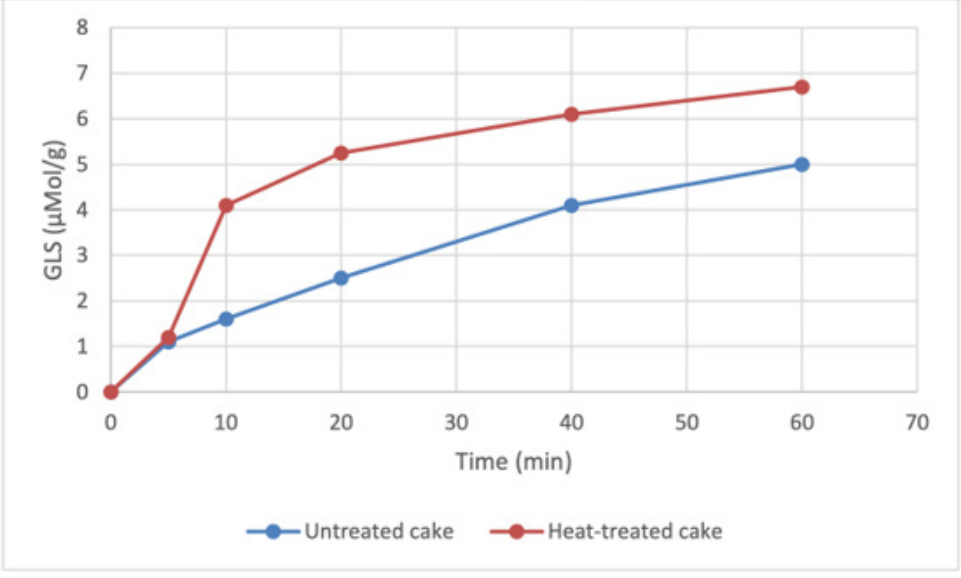
More likely explanation: as ethanol concentration decreases during evaporation, residual myrosinase associated with suspended material may recover sufficient activity to hydrolyse GSL.

Solution: centrifuge extracts before concentration to remove suspended enzymatic material. When applied, losses dropped to near zero even under effective vacuum.

Percolation Limitations
Four press cake types were tested (Oleane vs Komet press; untreated vs heat-treated seeds). Oleane flat-cake collapsed percolation to near-zero within 10 min. Komet pellets maintained acceptable flow (>0.7 mm/s) but still declined sharply after 20–45 min.

Untreated cakes: 46% GSL losses from myrosinase activity during extraction.

Heat-treated cakes: 25–35% losses — partial inactivation only at 90 °C; 100 °C needed for complete deactivation.



Evolution of the extracts concentration with time

Supply to FLANAT (October 2024)
Five batches of 2 kg carinata press cake were extracted at pilot scale to supply crude GSL extracts for downstream purification and bioactivity testing. Overall mass balance from the final campaign:

- 43% of GSLs recovered in liquid extract
- 42% hydrolysed (residual myrosinase)
- 15% remained unextracted in cake

~72 g total glucosinolates shipped (~12 kg liquid concentrate at 15.3 mmol/L).

Overall, the project demonstrated that recovery of intact glucosinolates from carinata press cake is technically feasible, but more challenging than initially anticipated. Efficient recovery requires simultaneous control of myrosinase activity, cake structure and mass transfer, extract clarification and concentration conditions. These constraints are likely to result in significant additional processing costs. Economic viability would therefore probably require high-value applications for the recovered glucosinolates or their derivatives, rather than relying solely on the increased feed value of the detoxified meal.

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