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Report on glucosinolate extraction and characterization

TERRES INOVIA

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ABBREVIATIONS

BCPC	Brassica carinata press cake
GLS	Glucosinolates
DDM	Dry defatted matter

1 Executive summary

Within Task T2.3.2, Terres Inovia investigated the thermal destruction and solvent extraction of glucosinolates (GSLs) from *Brassica Carinata* press cake, with the dual objective of (i) identifying mild processing conditions compatible with industrial constraints for feed applications, and (ii) producing GSL-rich extracts for biocontrol evaluation. Thermal treatments were first assessed on meal and press cake to reduce GSL content while preserving protein quality. Even under the most severe conditions tested (up to 120 °C, 25 min, intermediate moisture), total GSL reduction did not exceed about 45%, and was accompanied by a marked loss of protein solubility. These results highlighted the difficulty of achieving sufficient GSL destruction under mild conditions compatible with high-value feed uses.

In parallel, a series of pilot- and lab-scale extraction trials was conducted to adapt a mustard-based biorefinery protocol to Brassica carinata. Extraction with hydroethanolic solvents (typically 50–60% ethanol, 40–50 °C) on press cake generated from mechanical pressing was optimised with respect to solvent composition, solvent-to-solid ratio, extraction time and percolation behaviour. Lab-scale experiments on milled press cake confirmed that near-quantitative recovery of GSLs is achievable when mass transfer limitations are minimised. However, pilot-scale percolation trials revealed (i) strong sensitivity of GSL extraction to press-cake structure and percolation quality, and (ii) incomplete GSL recovery (often ≤70%) even under extended extraction times and high solvent ratios. Additional lab work showed that diffusion limitations and poor bed irrigation could explain the residual GSL fraction in spent cakes.

A second major finding concerns the role of myrosinase during extraction and concentration. Contrary to initial assumptions, myrosinase activity was shown to persist in 50% ethanol and to cause significant GSL hydrolysis when enzyme inactivation was incomplete. Heat treatments specifically designed to inactivate myrosinase (bench cooker and Spirajoule screw) reduced but did not always eliminate enzymatic degradation. Moreover, miscella concentration proved to be a critical step: under some vacuum-evaporation conditions, up to 30–60% of initially extracted GSLs disappeared from the liquid phase. Subsequent trials demonstrated that GSL losses during concentration depend on the combination of temperature, pressure (and thus ethanol content), and the presence of suspended enzymatic material. When extracts were clarified by centrifugation and concentrated at moderate temperature under appropriately controlled vacuum, GSL degradation could be almost completely avoided.

Building on these insights, a new pilot-scale extraction protocol was implemented combining: (i) controlled thermal treatment for myrosinase inactivation, (ii) pressing conditions maximising oil removal and cell-wall disruption, (iii) an optimised hydro-ethanolic solvent system (higher ethanol content plus weak acidification) to favour GSL solubilisation while limiting enzymatic activity, and (iv) a more cautious concentration strategy. Despite these improvements, GSL extraction remained only moderately efficient at scale, mainly due to percolation and diffusion limitations inherent to industrial-type press cakes.

A final production campaign was carried out in 2024 to supply crude GSL extracts to project partners for downstream purification and bioactivity testing. Several batches of carinata press cake were extracted at pilot scale and the miscellas concentrated prior to shipment. Overall mass balances indicated that roughly 40–45% of the initial GSLs could be recovered in the liquid extract, while a comparable fraction was hydrolysed and the remaining part stayed in the solid residue. The concentrated extracts obtained nonetheless met the quantitative needs of the receiving partners and provided a basis for assessing the biocontrol potential of carinata GSLs.

In summary, the work conducted under Task 2.3.2 demonstrates that: (i) mild thermal treatments cannot simultaneously ensure deep GSL destruction and preservation of protein functionality in carinata meal; (ii) hydro-ethanolic extraction of GSLs from industrial-type press cakes faces both mass-transfer and enzymatic constraints; and (iii) miscella concentration is a sensitive step requiring careful control of temperature, ethanol content and solid removal. Although a robust, high-yield industrial process for GSL recovery from carinata could not be fully established within the project, the experimental data generated clarify the main technical bottlenecks and provide operational guidance for future process development and for the downstream partners who received pilot-scale extracts for further valorisation.

2 Task 2.3.2 (Glucosinolates destruction)

In the frame of Task 2.3.2, Terres Inovia investigated the thermal degradation of glucosinolates in Brassica carinata meal under mild conditions, with the objective of identifying a process compatible with industrial constraints (limited residence time, moderate temperature, preserved protein solubility). A first design of experiments was carried out in 2023 on press cake, exploring the combined effects of temperature, residence time and initial moisture content.

This experiment was conducted using a bench-scale cooker consisting of a vessel 250 mm in diameter and 200 mm deep. The bottom was heated by a 1 kW electrical resistance, and the material was stirred by a helical belt and a sweeping blade, providing mixing at a rotational speed of 60–100 rpm. The temperature was adjusted by modulating the voltage at the resistance terminals and was monitored by four thermocouples connected to a data logger. Oil extraction was performed with an OLEANE 2 kW screw press (Olexa). Half of the seeds (50%) were preheated prior to pressing to inactivate myrosinase, by heating at 90°C for 3-5 minutes, while the remaining 50% were cold-pressed. The resulting press cake was then heated in the bench cooker according to a two-factor Box–Behnken design, with temperature levels of 90-105-120°C, cooking times of 10-25-45 minutes, and initial moisture contents of 4-8-12% (one set produced from preheated seeds, the other from cold-pressed seeds).

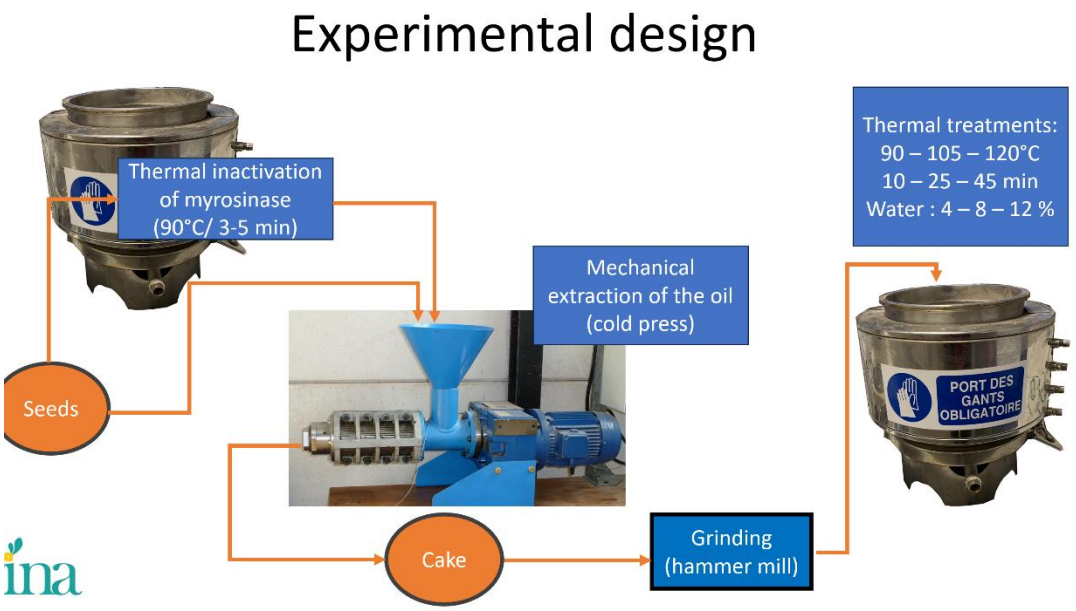

Figure 1: Graphical presentation of the experiment design

Under the most stringent conditions tested (120 °C, 25 min, initial moisture 8 %), the maximum reduction in total glucosinolate content was only about 45 %, and it was accompanied by a marked deterioration in protein quality, with KOH protein solubility decreasing by 33 points (from 82 % to 55 %). These results were considered unsatisfactory, as they did not allow the targeted glucosinolate level to be achieved while maintaining meal quality suitable for feed use.

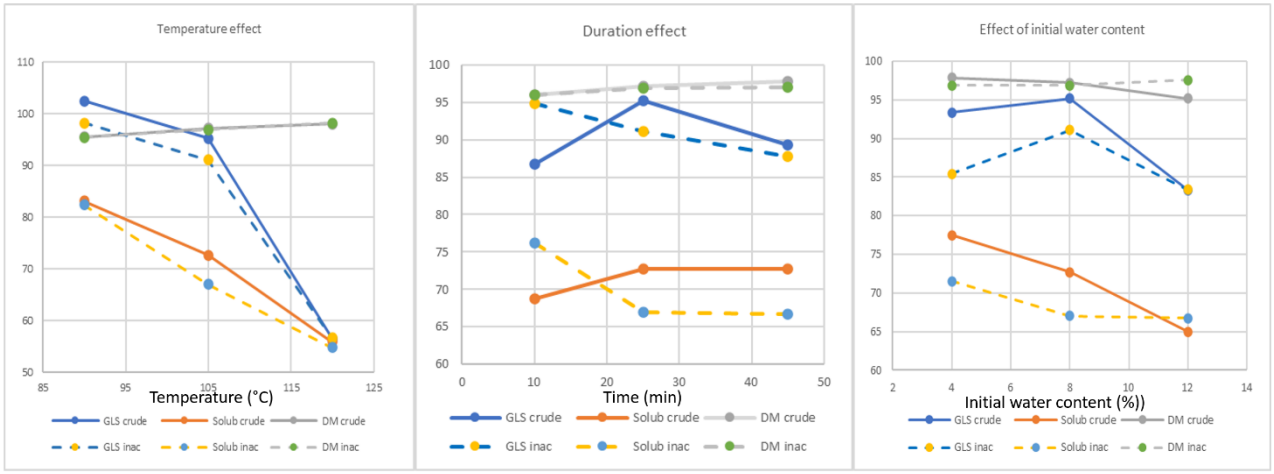

Figure 2: Results. The vertical axis give the glucosinolates concentration in µMol/g, the protein solubility in KOH (g/100g of proteins) and the dry matter in g/100 g of press-cake.

At that time, further investigations (additional experimental designs and potential process optimisations) could have been envisaged. However, during a subsequent WP2 meeting, it was reported that SAIPOL had already identified and implemented industrial toasting conditions capable of reducing glucosinolate content in carinata meal to an acceptable level at plant scale, with performance meeting their requirements. In view of this industrial solution, the continuation of Task 2.3.2 was no longer considered necessary by the industrial partner, while Terres Inovia was facing staff limitations that prevented the allocation of additional resources to this topic within the project timeframe.

Consequently, the work conducted in 2023 remains the only experimental dataset generated for Task 2.3.2, and no further trials were performed thereafter. The initial version of deliverable D2.4 (v1.0) mentioned that additional investigations would be carried out; this statement reflected the situation at the time of writing and has since been superseded by the availability of an industrially validated toasting process at SAIPOL.

The present revision of D2.4 therefore aligns the description of Task 2.3.2 with the actual status: preliminary trials have demonstrated the technical challenge of achieving sufficient glucosinolate degradation under mild conditions without impairing protein solubility, and the task was discontinued once an alternative industrial solution became available.

3 Task 2.4.1 glucosinolates extraction

3.1 First production trial

3.1.1 Objectives

The operation was intended to produce a large glucosinolate extract from Brassica carinata for Kimitec, who required this material to assess its potential as a biocontrol agent. It was designed according to a protocol inspired by a PhD thesis on the extraction of glucosinolates from Brassica juncea (Hebert, 2020).In this work, an integrated, eco-friendly process was developed for the selective recovery of glucosinolates from mustard seeds within a biorefinery concept. Mustard seeds were first subjected to a mild heat treatment at 80 °C to inactivate myrosinase while preserving both glucosinolates and protein functionality, then mechanically pressed to remove most of the oil and generate a protein- and glucosinolate-rich press cake. Glucosinolates were extracted from this press cake using a 40% (v/v) ethanol-water solvent at 40 °C, with a liquid-to-solid ratio of 10:1 and an extraction time of approximately eight minutes.

The crude glucosinolate extract was subsequently purified either by ion-exchange chromatography on strongly basic resins or by ultrafiltration using low-molecular-weight cut-off membranes, both approaches yielding fractions highly enriched in sinigrin and related glucosinolates. This sequence of operations produced, on the one hand, a concentrated glucosinolate solution suitable for phytobiotic or biofumigation applications and, on the other hand, a detoxified, protein-rich solid co-product with potential as an animal feed ingredient, thereby demonstrating the feasibility of a mustard-based biorefinery.

3.1.2 Pilot equipment

Drying was carried out in double-deck trolleys in which air heated to 70 °C was blown through the material. Seed oil extraction was performed using an MBU 20 screw press (OLEXA, France; 7 kW; 80 mm screw diameter; 850 mm screw length). Extraction was then conducted in a percolation column with a diameter of 130 mm and a depth of 600 mm. This unit corresponds to a single extraction stage in which the solvent percolates through a 400 mm bed of oil-bearing material (Fig. 3).

The solvent is circulated by a membrane pump and passes through a heat exchanger for temperature control. A double jacket around the extraction chamber allows the circulation of hot water to regulate the extraction temperature. The setup is complemented by glass tanks that enable visual observation of solvent flow and measurement of percolation rate. The liquid extract is

concentrated in a 50 L pilot evaporator heated by steam in a double jacket at atmospheric pressure. This unit includes a distillation column and a heat exchanger for solvent vapor condensation. The system operates under vacuum (−0.5 to −0.9 bar) to lower the boiling temperature of the oil/solvent mixture and thereby prevent thermal degradation of heat-sensitive compounds. A laboratory-scale rotary evaporator equipped with a 5 L flask and a vacuum pump is used for small-scale evaporation.

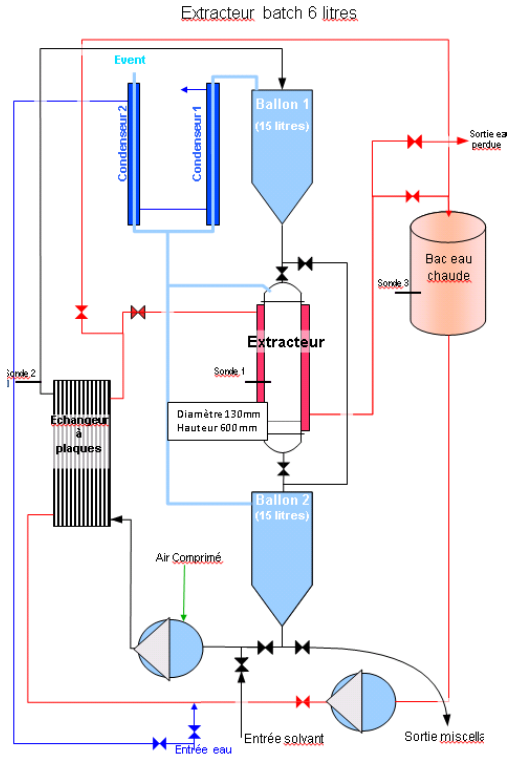

Figure 3: Diagram of the 6L batch extractor

3.1.3 Protocol

Since TERRES INOVIA's initial hypothesis was that ethanol would inactivate the endogenous myrosinase in the seeds, a thermal treatment prior to extraction was not considered necessary, except in the case of cold pressing. Based on our experience with mustard seeds, myrosinase activity is still possible when seed moisture content exceeds 7% (wet basis).

A 25 kg batch of seeds was dried to a moisture content of 4-5% to prevent glucosinolate losses during extraction. After drying, the seeds were passed through the MBU 20 press at low screw speed. This step aimed to remove as much oil as possible while keeping the temperature below 60 °C to avoid damaging heat-sensitive compounds.

From the resulting press cake, 12.5 kg produced under steady-state conditions were manually broken into pieces of approximately 1-2 cm² in order to increase the specific surface area and improve extraction efficiency in the subsequent steps. A sample of this press cake was analysed to determine its glucosinolate content, and once validated, extraction was carried out in a mode simulating continuous counter-current operation using the 6 L batch extractor. The extraction process used 50% (m/m) ethanol as solvent at 40 °C, with a solvent-to-solid ratio of 6 L per 2.5 kg of plant material. A sequential extraction scheme was applied in which the miscella (solution of extracted compounds in the solvent) was reused as wash solvent for subsequent batches, thereby limiting the volume of solvent requiring distillation.

The process, summarised in Fig. 4, began with approximately 10 L of solvent for the first batch and washing step (L1: E1 + E2 = 10 L). To prevent cake swelling due to the presence of water, the press cake (2.5 kg per batch) was first immersed in solvent at room temperature for 10 minutes. The liquid was then poured into a second container, and the soaked solid was recovered and transferred into the extractor.

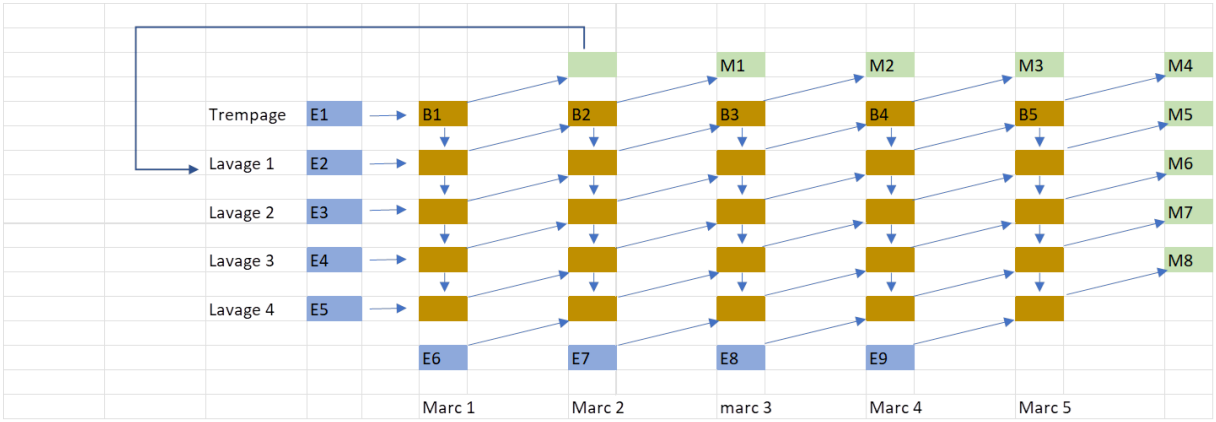

Figure 4: Diagram of the operations. Brown = solid (batches 1 to 5), new solvent blue (7 x 6L +10L = 52 L), final miscella green (8 x 5 L approximately). Down arrows = solid, right arrows: solvent and miscella.

The wet press cake was introduced into the extraction chamber, and the liquid was initially circulated in a bypass loop to allow heating. Once the temperature reached 40 °C, the liquid was directed through the percolation column and the flow rate was adjusted according to the percolation capacity of the material. The percolation time was set to 10 minutes, corresponding to the typical residence time per stage in an industrial extractor. The recirculation pump was then stopped and the liquid remaining in the extraction chamber was allowed to drain from the bed. The miscella was collected and stored in a container. A fresh batch of solvent was introduced into the system, and the procedure was repeated so that each batch of press cake underwent four successive solvent washes. The spent cake from the first batch was recovered, weighed, and spread on a large tray placed in a fume hood for solvent removal. The second batch of solid was then processed using, as solvent, the miscella M1 obtained from the first batch, starting with a 10-minute soaking step before introduction into the extraction column. The second wash was performed with the miscella resulting from the third wash of the previous batch, and so on.

This method minimises the amount of fresh solvent required for multiple extractions and reproduces the solute concentration profiles in the miscella observed in counter-current extractors. Only eight solvent loads were required to carry out 20 extraction steps.

All liquid extracts were concentrated in the pilot evaporator under vacuum, and the final concentration step was performed in the laboratory-scale rotary evaporator, targeting a final volume of 2 L of concentrate.

3.1.4 Results:

At the end of the extract concentration, 1.8 kg of a dark liquid was recovered. A sample of the liquid was sent to the laboratory for analysis.

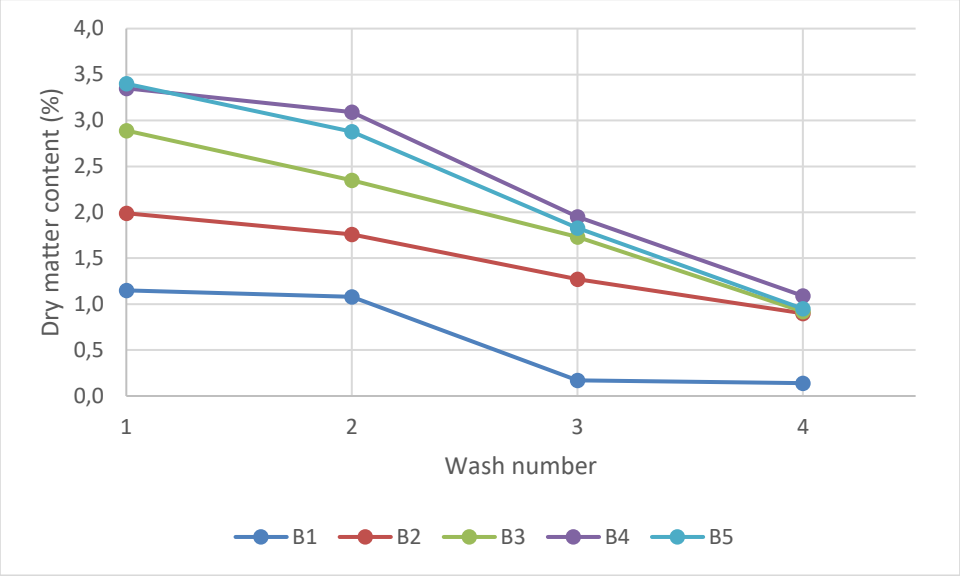

Figure 5: Evolution of the miscella content in dry matter for the extraction batches 1 to 5

Figure 5 shows how the dry-matter concentration in the miscellas evolved over the 20 extraction steps. As expected, the concentration increased with the batch number from B1 to B5, because the solvent used was the miscella from the previous wash of the previous batch.

In batch B1, where fresh solvent was used for each wash, the dry-matter concentration remained low in the third miscella, indicating that most solutes were extracted during the first and second washes. Miscellas from batches B4 and B5 showed similar concentrations, since the solvent they received had the same extraction history. The dry-matter concentrations in the fourth miscella of batches B2 to B5 converged to comparable values

Table 1: analysis of the materials before after extraction

	Water		Total GSL	Oil content	Proteins Kjeldahl
	DM %	Water %	µmol/g on DM	G / 100 g DM	g/100g DM
Seeds	95.6	4.4	66.1	45.3	23.67
Press cake	93.4	6.6	113.3	8.0	37.87
Spent cake	89.5	10.5	203.4	9.3	38.56
Concentrate	44.7	55.3	92.7	1.8*	24.48

According to the mass of press cake and recovered material, and from their glucosinolates concentrations, the glucosinolates mass balance can be assessed with Table 2.

Table 2: glucosinolates balance

	Mass (kg)	DM (%)	GLS/DM (µMol/g)	Amount of GLS (mMol)	% of initial GSL
BCPC	12.5	93.4	113.3	1322.8	
Extract (liquid)	1.8	44.7	203.4	163.7	12%
Spent cake	12.1	89.5	47.9	518.7	39%

Total GSL recovered				682.4	
Losses (GSL)		11.675		640.4	48%

The results showed that glucosinolate recovery was very low, since only 12% of the glucosinolates initially present in the press cake were recovered in the final concentrate, while 39% remained in the spent cake and 48% were lost during processing.

This outcome was disappointing and called for further investigation.

3.2 Lab-scale investigation on GSL Losses

3.2.1 Context and hypothesis

Three facts can explain the low rate of GLS recovery in the initial experiment.

The first relates to the high residual glucosinolate content in the spent cake (47.9 µmol/g), which may be explained by several factors: (1) glucosinolates are ionised molecules with limited solubility in ethanol, and the water content (40%) may not have been sufficient to ensure efficient extraction, although ethanol is necessary to prevent the co-extraction of proteins and other undesirable compounds; (2) the extraction time and solvent-to-cake ratio may have been insufficient to achieve maximal transfer of glucosinolates into the solvent; (3) solvent circulation in the percolation column may have followed preferential pathways, leaving some areas poorly irrigated. Laboratory-scale extraction trials were therefore required to clarify these points.

A second potential cause of poor recovery could be **insufficient myrosinase inactivation** by 50% ethanol. This was not initially expected, since, to our knowledge, enzymes are generally denatured in the presence of 20–30% ethanol. (Green, 1931; Li et al., 2021; Wilson et al., 2014; Oloyede, 2021).

Classic biophysical work on alcohol–protein systems shows that water-miscible monoalcohols, including ethanol, induce denaturation and precipitation of diverse proteins once alcohol content exceeds roughly 20–30% (v/v), reflecting a general solvent effect rather than an idiosyncratic response of a single protein. In whey protein isolate, multiple studies demonstrate that ethanol in the range 20–80% (v/v) causes “significant denaturation” and aggregation, with denaturation becoming clear already at ≥20% ethanol and functional properties (e.g., gelation) changing markedly as a consequence.

In the context of this work, it is possible that protein denaturation was incomplete or required some time to become fully effective. As a result, a residual enzyme activity may have persisted, potentially causing glucosinolate losses both in the cake during extraction and in the spent material while it was left to dry in a thin layer at ambient temperature. However, this residual activity appeared to be insufficient to achieve complete glucosinolate hydrolysis once ethanol had evaporated at ambient temperature.

A third factor likely to have contributed was the possibility of **losses during the concentration step**. Glucosinolates are not generally considered highly sensitive to heat. Doheny-Adams et al. (2017) directly assessed the thermal stability of sinigrin in water at 100 °C and in 70% methanol at 75 °C for up to 60 minutes and reported that sinigrin and glucotropaeolin did not decrease significantly over this period, indicating that boiling water or boiling methanol extractions do not appreciably degrade these glucosinolates. Nevertheless, the conditions applied during miscella concentration may have exceeded the thermal tolerance of glucosinolates. The steam-heated boiler, operating with wall

temperatures close to 100 °C, may have induced glucosinolate degradation upon contact, particularly as it was run continuously without removing the concentrated material and the initial extracts remained under these boiler conditions for several days.

3.2.2 Lab-scale investigations about extractability and myrosinase degradation

Lab-Scale Extraction Protocol

A detailed lab-scale extraction protocol was developed to investigate these hypotheses, using BCPC from the previous experiment. The material was milled with a hammer mill, and 15 g of the resulting powder were immersed in 75 mL of a 1:1 water–ethanol mixture (m:m) in a beaker. The beaker was covered with Parafilm to limit evaporation. Extraction was carried out in a water bath with magnetic stirring for 60 minutes at 40–50 °C.

The suspension was then centrifuged to separate the solid and liquid phases, which were both sampled to determine their glucosinolate contents. The pellet was resuspended in the same volume of solvent for a second and then a third extraction. The masses of liquid and sediment were weighed after each step. All operations were performed in triplicate.

Results and Discussion

Table 3 presents the results for each replicate, as glucosinolate losses differed somewhat between tests. The mass balance on recovered material was closed by attributing 1.7–3.7% of the mass to solvent losses.

Table 3: mass balances of GLS in lab-scale extraction in BCPC from experiment 1

	Rep1			Rep 2			Rep 3		
	Mass (g)	GLS (µMol/g)	µMol	Mass (g)	GLS (µMol/g)	µMol	Mass (g)	GLS (µMol/g)	µMol
Cake powder	**15.0**	**113.0**	**1695.0**	**15.0**	**113.0**	**1695.0**	**15.0**	**113.0**	**1695.0**
Pellet	32.0	1.5	**47.0**	39.2	1.5	**57.6**	40.4	1.5	**59.3**
E1	48.8	14.7	**714.9**	46.8	11.0	**512.5**	43.7	16.1	**704.2**
E2	66.0	4.4	**291.7**	65.9	1.5	**97.5**	66.8	5.7	**378.6**
E3	64.8	1.2	**79.1**	64.2	1.5	**93.1**	62.9	1.8	**114.5**
Sum of recovered GLS			**1132.7**			**760.7**			**1256.7**
Losses			33.17%			55.12%			25.86%

The experimental results confirmed the effectiveness of the extraction protocol using 50% ethanol in three 60-minute cycles for recovering glucosinolates from Brassica carinata press cake. The low residual glucosinolate content in the spent cake indicated a high extraction efficiency. However, the mass balance showed that, on average, about 38% of the glucosinolates were lost during the extraction process, with substantial variability between replicates: replicate 2 exhibited the highest losses (55%), whereas replicate 3 showed the lowest (26%). This high variability suggests that small differences in operating conditions may lead to pronounced changes in enzyme activity. An

additional trial was therefore carried out using the spent cake from the initial experiment, without replication.

Table 4: Mass balance of GLS in lab-scale extraction on spent cake from experiment 1

	Mass (g)	GLS (µMol/g)	µMol
Cake powder	**15.01**	**42.95**	**644.6795**
Pellet	50.22	0.63	**31.6386**
E1	46.23	7.46	**344.8758**
E2	64.66	2.46	**159.0636**
E3	64.00	0.75	**48**
Sum of recovered GLS			**583.578**
Losses			9.48%

Table 4 presents the results of this experiment. Glucosinolates were efficiently extracted, as evidenced by the low concentrations measured in both the pellet and the final miscella. Losses were substantially reduced, even though no thermal treatment was applied to the material.

3.2.3 Conclusion

Two clear conclusions can be drawn from these experiments. First, near-complete extraction of glucosinolates is achievable when the material is milled and fully dispersed in the solvent. Second, myrosinase is likely to remain active in 50% ethanol. A recent study by Albe-Slabi et al. (2025) supports this finding: the authors compared aqueous ethanol concentrations of 0, 20, 40, 60, 80 and 99.5% at temperatures from 20 to 60 °C for the extraction of rapeseed press cake and observed an almost complete disappearance of glucosinolates when ethanol concentration was below 60%, except at higher temperatures where this effect was attenuated. With 60% ethanol, degradation was still significant but less pronounced: at 20 °C, 45% of glucosinolates were preserved, mostly in the solvent phase, whereas at 60 °C, 82% were preserved but 44% remained unextracted.

The fact that losses observed during extraction of the spent cake were lower than those obtained with BCPC suggests that contact with ethanol substantially reduced enzyme activity without fully inactivating it. Overall, our results indicate that myrosinase activity in Brassica carinata press cake follows a similar pattern.

3.3 Second pilot-scale extraction

3.3.1 Objectives

Lab-scale investigations having shown that myrosinase can remain active in aqueous ethanol, a new pilot-scale trial was designed in which thorough enzyme inactivation was implemented to prevent glucosinolate losses during extraction. The experiment also aimed to improve extraction performance through better press-cake preparation, using a pressing strategy that maximised oil yield and generated a strongly comminuted material, thereby promoting extensive cell-wall disruption and improving solvent access to secondary metabolites. A third objective was to assess potential losses occurring during the concentration of the extract.

3.3.2 Materials and Methods

Thermal treatment of the seeds was carried out in the same bench cooker used for the glucosinolate destruction trial. Two batches of 3 kg each (total 6 kg) were processed. The cooker was preheated to 100 °C, then the seeds were introduced and maintained at 100 °C for 20 minutes under vigorous agitation, with direct steam injected throughout the treatment. This thermal protocol was defined in a preliminary step during which different conditions (temperature, duration, steam rate) were tested, and myrosinase activity was monitored by measuring the pH decrease associated with glucosinolate hydrolysis and sulphate ion release.

Immediately after heating, the seeds were transferred to a Komet CA 59 screw press (IBG Montforts) for defatting. The press was operated at very low speed (setting 1.5 on a 1–10 scale) and with the smallest die (4 mm) to maximise oil removal. The extraction process was then conducted on 2 kg of the resulting press cake, using the same apparatus as in the previous pilot-scale trial. The solvent composition was adjusted to ethanol:water:acetic acid (12:8:0.2 by mass). This modification had two objectives: the higher ethanol content further limited the risk of glucosinolate degradation by any residual myrosinase activity, and the addition of acetic acid was intended to enhance glucosinolate solubility.

As in the first pilot-scale extraction trial, the press cake was allowed to swell in the solvent for 10 minutes prior to extraction. Three sequential extraction steps were then performed, using 8 kg of solvent for the first step and 6 kg for each of the second and third washes. The extraction temperature was set to 50 °C, and the extraction time was substantially increased to 60 minutes for each wash. After the final extraction, the spent cake was spread on a large flat tray and dried under a fume hood. Liquid extracts from each step were sampled before being pooled for concentration.

Two concentration methods were compared: (1) a laboratory-scale rotary evaporator operated under vacuum, with a water-bath temperature of 80 °C and an internal pressure of 350 mbar; and (2) a larger-scale static evaporator with a heating temperature of 100 °C and an operating pressure of 400–500 mbar.

3.3.3 Results

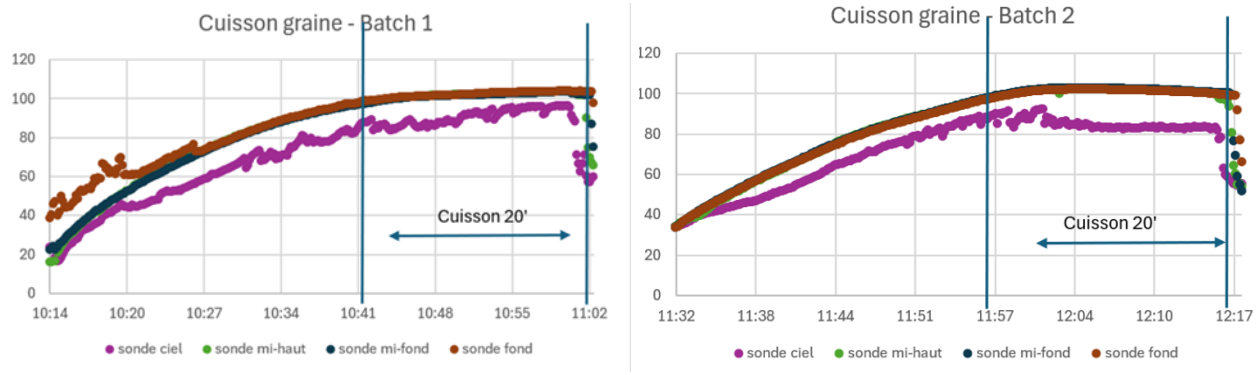

Figure 6: Temperature conditions during the myrosinase inactivation step. Sonde ciel: temperature probe of head space, sonde mi-haut: temperature of upper part of the cooker, sonde mi-fond: temperature in the middle part of the cooker, sonde fond: temperature in the bottom part of the cooker. Horizontal axis: time, vertical axis temperature in °C.

Figure 6 represents the temperatures registered during the thermal treatment. The average temperature of the seeds was slightly above 100°C during the final part of the conditioning.

Mechanical Extraction

The mechanical extraction was performed on 5 218 g of Brassica carinata seeds using a screw press. This operation produced 1 983 g of crude oil and 2 911 g of press cake, with process losses of 324 g. The barrel temperature during pressing ranged from 68 to 76 °C, and the press cake was discharged through a 4 mm nozzle. The low residual oil content in the press cake (7.4%) indicates an efficient mechanical extraction and suggests extensive cell-wall disruption, which is favourable for subsequent solvent extraction of glucosinolates (GSLs).

Glucosinolate concentrations, expressed on a dry defatted matter (DDM) basis, were measured in both seeds and press cake. Values were 132 and 111 µmol/g DDM for seeds and press cake, respectively, indicating that the thermal treatment resulted in an overall loss of about 16% of total glucosinolates.

Solvent Extraction Following mechanical extraction, 2 000 g of press cake underwent a three-stage solvent extraction. Before extraction, the pellets were soaked in the solvent to allow swelling prior to introduction into the percolation column. In the first step, 8 000 g of solvent (ethanol 58/water 42) was used at 47–50 °C for 60 minutes with recirculation, yielding 4 094 g of miscella with a dry-matter (DM) concentration of 15 g/L. Approximately 1 800 g of liquid was retained in the marc after draining, which impacted the overall mass balance. The second extraction step, again using 8 000 g of solvent, produced 6 066 g of miscella with a reduced DM concentration of 8.8 g/L. The final extraction, performed with 6 000 g of solvent, yielded 6 186 g of miscella at 4.5 g/L DM. The progressive decrease in DM concentration across successive extractions reflects the gradual depletion of soluble components from the press cake and is consistent with typical extraction kinetics, where most solubles are removed in the early stages and subsequent steps show diminishing returns.

Figure 7 shows the glucosinolate balance during extraction: 30.9% of the glucosinolates were recovered in the first wash, 24.2% in the second, and 14.3% in the third. Losses during extraction

were very low (around 1%) and within the analytical uncertainty. Approximately 30% of the initial glucosinolates remained unextracted, despite a total extraction time of 3 hours and an initial solvent-to-solid ratio of 10:1. Percolation was rapid during the initial stages but gradually slowed over time. This decrease was unexpected given the low oil content of the cake and the prior soaking step, although percolation rates remained sufficient to ensure effective solvent flow.

Using the mass balance for each extraction stage, it is possible to distinguish the fraction of glucosinolates solubilised in the residual miscella from the fraction remaining non-extracted in the cake (Fig. 8). After one hour of percolation in the first step, only 50.4% of glucosinolates had been extracted, which is relatively low compared with oil extraction using hexane. Under similar conditions, oil extraction typically reaches a plateau after about 5 minutes, with 80–90% of the oil extracted at a hexane-to-cake ratio of 1.75:1. After the second extraction period, the cumulative glucosinolate recovery reached 67.2%, and 76.0% at the end of the third.

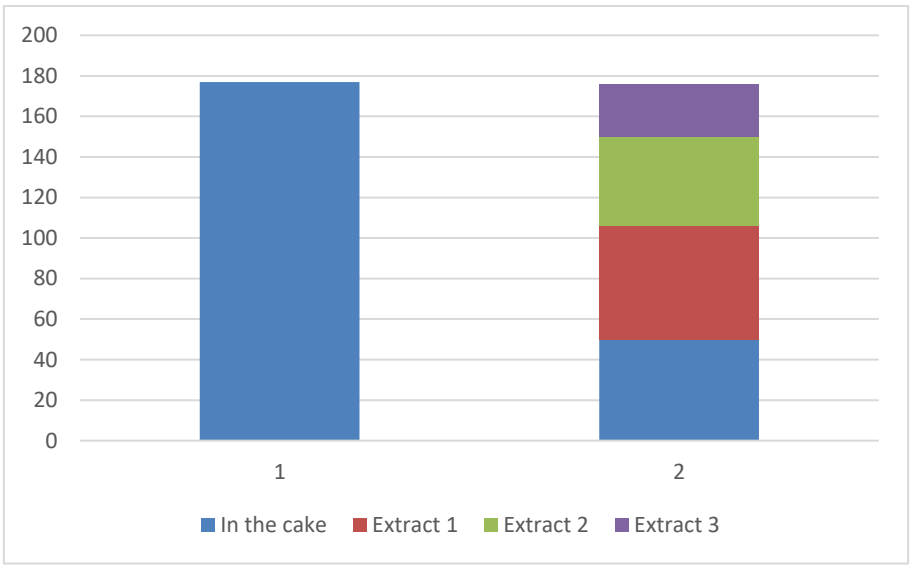

Figure 7: Percolation extraction (2kg) with 60% ethanol, glucosinolates balance

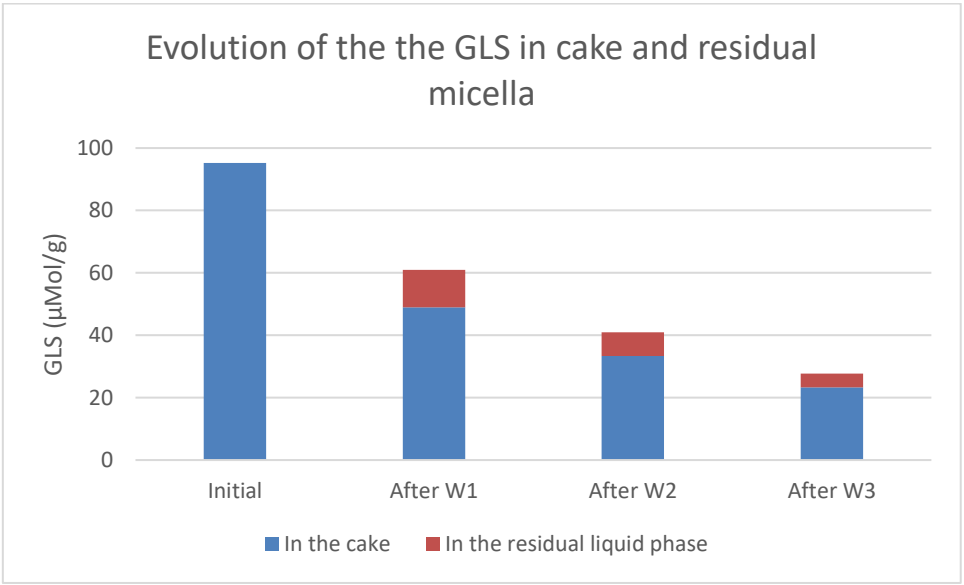

Figure 8: evolution of the GLS concentration in the marc after each extraction step.

Miscella concentration

The concentration step using the vacuum rotary evaporator (water bath at 80 °C, pressure 350 mbar) was carried out on 1 850 g of miscella. After 2 h 35 min, 180.6 g of concentrate were obtained, corresponding to a 10.2-fold concentration factor (mass out / mass in). Glucosinolate concentrations in the initial miscella and in the final concentrate were 7.1 and 46.3 µmol/g, respectively. The mass balance indicated the loss of 4 773 µmol of glucosinolates, i.e. 36% of the initial amount.

The large evaporator received 13 975 g of miscella. It was heated with steam, at a pressure of around 490 mbar for most of the process, decreasing to 420 mbar towards the end when the concentrate was almost free of ethanol (Fig. 9). The liquid temperature evolved accordingly with the ethanol content of the mixture. After 1 h 40 min, 495 g of concentrate were recovered with a glucosinolate concentration of 77.4 µmol/g. The concentration factor was 28, resulting in a loss of 61.4% of the initial glucosinolates.

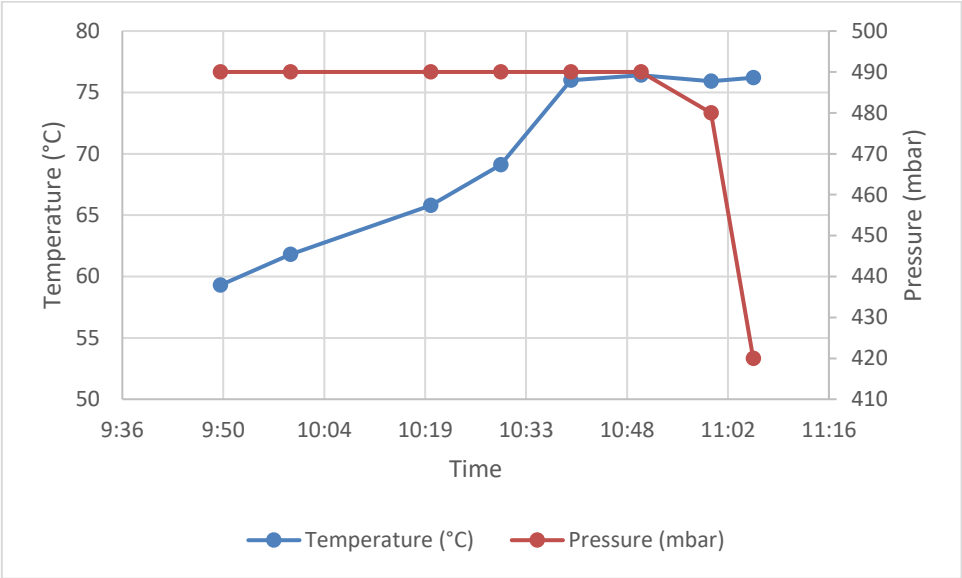

Figure 9: Evolution of the temperature and pressure during the concentration in the large boiler

3.3.4 Conclusions

This experiment showed that, when appropriately controlled, the heat treatment was able to fully prevent enzymatic degradation of glucosinolates, while limiting glucosinolate losses to about 16%. At the same time, despite a high solvent-to-cake ratio, extended extraction times and an intensive preparation of the material using small-diameter pellets, the results indicated that glucosinolates remained only moderately extractable, with overall recovery capped at around 70% under these ‘maximal’ conditions.

Unexpectedly, the concentration of the miscella proved to be a critical step for glucosinolate preservation, with both concentration conditions leading to losses, albeit to different extents.

3.4 Additional investigations

3.4.1 Losses during the concentration of the extracts.

To better understand the influence of vacuum level at a fixed, moderate temperature, a new series of trials was carried out at 60 °C, comparing a high-vacuum condition (<200 mbar) with a low-vacuum condition (900 mbar). For each condition, two replicates were performed with identical evaporation times, in order to isolate the effect of pressure.

The extracts were prepared in 50% ethanol and had an initial glucosinolate concentration of 6.4 µmol/g. Evaporation was performed in a rotary vacuum evaporator using approximately 800 g of solution. Under high vacuum, the first run was stopped when the residual mass reached 53.3 g, yielding a concentrate with 26.5 µmol/g glucosinolates; the second run was stopped at 148.5 g, with a glucosinolate concentration of 19.7 µmol/g. The corresponding concentration factors (based on liquid mass) were 15.0 and 5.4. Glucosinolate losses represented 73% and 43% of the initial content in batches 1 and 2, respectively. Analysis of the condensates revealed no detectable glucosinolates, which is consistent with the relatively high molar mass of sinigrin (approximately 400 g/mol), making its volatilization unlikely under the applied temperature and pressure conditions.

In the low-vacuum trials at 900 mbar, no solvent evaporation occurred at 60 °C, as this temperature was below the boiling point of the solvent mixture (>80 °C). With the same treatment time at 60 °C as for the second high-vacuum run, glucosinolate concentrations remained essentially unchanged (6.3 and 6.8 µmol/g at the end of the treatment).

A further experiment was conducted with smaller solvent volumes (182 and 176 g) at 100 mbar and 60 °C for 30 minutes, using an initial extract obtained with 50% water/ethanol and containing 20.1 µmol/g glucosinolates. This treatment produced 25.4 and 28.1 g of concentrate, with glucosinolate concentrations of 143.4 and 129.0 µmol/g, respectively. The concentration factors were 7.2 and 6.2, and glucosinolate mass balances showed no measurable losses (0.3% and −3%).

In conclusion, losses occurring during miscella concentration appear to result from a combined effect of temperature, ethanol content in the solution and, likely, the presence of enzymes. The absence of losses at 900 mbar, compared with the substantial losses observed under higher vacuum, suggests that ethanol concentration plays a key role in glucosinolate degradation. This behaviour is consistent with a residual myrosinase activity in the extract. In the final experiment, where no losses were detected despite effective concentration, the extracts were centrifuged before evaporation, unlike in the trials where glucosinolate losses occurred. This is consistent with the pilot-scale experiments, in which concentration was performed without a prior centrifugation step. It may also explain the variability of the results, since the amount of protein remaining in colloidal suspension depends on the time elapsed between extraction and concentration, if settling is slow.

The lack of losses when ethanol concentration remained constant can also be understood if hydrolysis activity depends on water availability: in excess ethanol, the enzyme is likely denatured due to disruption of hydrophobic interactions within the protein structure, whereas a decrease in ethanol concentration may allow the protein to partially refold and recover its activity.

3.4.2 Extraction performances

Objectives

The quality of percolation is a key factor governing extraction efficiency in industrial extractors. In the first extraction trial, 39% of glucosinolates remained in the spent cake after four 10-minute extraction steps. In the second pilot-scale trial, 30% of glucosinolates were still unextracted after

three 60-minute washes at 50 °C, despite the use of press cakes obtained by intensive mechanical extraction. These observations contrast with the lab-scale extractions, in which the cake was milled before immersion in the solvent, leading to very low residual glucosinolate levels in the spent material. This suggests that glucosinolate diffusion into the solvent was limited, most likely due to one or both of the following factors: (1) imperfect irrigation of the packed bed, with preferential flow paths in the material; and (2) very slow diffusion of glucosinolates within the press cake. The following investigations were therefore undertaken to clarify this issue.

Material and methods

Four press cakes were prepared using two thermal treatments and two pressing methods. For thermal treatment, a portion of the seeds was processed as-is, while another portion was treated in a heated screw conveyor. This device, called Spirajoule (ETIA, France), consists of a shaftless endless screw installed in a refractory or insulated casing, through which the bulk material is conveyed in continuous plug-flow. The screw (210 mm diameter, 3 000 mm length) is electrically heated by a low-voltage current, generating heat directly in the metal by the Joule effect and transferring it to the product mainly by conduction and convection. The apparatus has 60 flights and was operated at 19 Hz, corresponding to a residence time of 20 minutes, with an outlet temperature set at 90 °C.

Regarding mechanical extraction, one press was the Oleane, whose outlet is a 90 mm circular die producing a flat cake about 2 mm thick (as in the first pilot-scale trial), while the other was the Komet press, fitted with a 4 mm die and producing pellet-shaped cake.

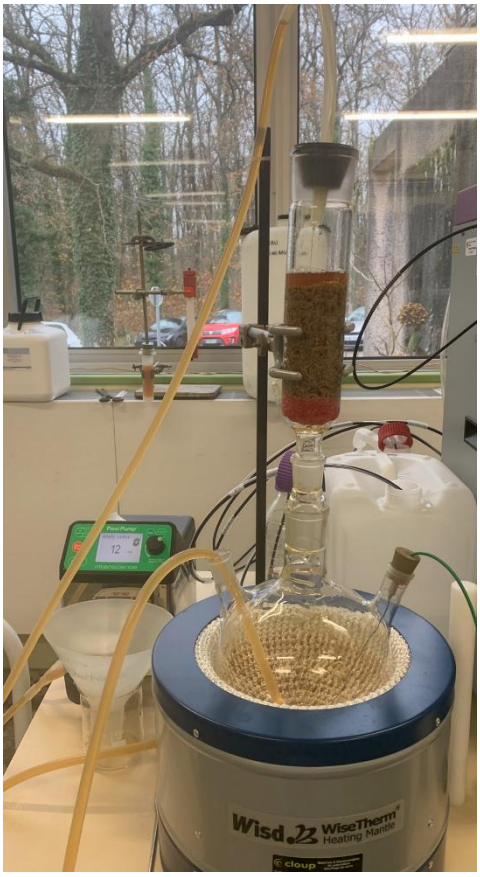

Figure 10: experimental extraction setup

Extractions were performed in a glass column with an internal diameter of 45 mm, in which a porous inert support placed at the bottom was used to retain the cake. The column was connected to a three-neck round-bottom flask placed on a heating device. The solvent was heated to 40 °C, with temperature controlled by a probe connected to a regulator, and circulated by a peristaltic pump delivering up to 1 000 mL/min. A 50 g sample of cake was loaded into the column, and 300 mL of solvent (ethanol–water 1:1, m/m) were placed in the flask. The four press cakes were compared to assess percolation behaviour and its evolution over time.

Results

For the Oleane cake, presoaking led to an initial percolation rate of around 100 mL/min during the first seconds, corresponding to approximately 1 mm/s. This flow rate is considered acceptable for a percolation extractor, with a lower limit of 0.7 mm/s reported by Laisney (1983). Over time, however, the percolation rate decreased and nearly dropped to zero after about ten minutes. Without presoaking, the initial percolation rate reached 600 mL/min but decreased to 300 mL/min after 5 minutes and 150 mL/min after 13 minutes. Under realistic multi-stage extraction conditions, percolation became disrupted and fell well below the 0.7 mm/s threshold.

With the Komet press cake, percolation was much easier and initially exceeded the pump capacity. This material was therefore selected for extraction trials comprising four successive 10-minute percolation steps with 300 mL of fresh solvent each. Figure 11 illustrates the evolution of percolation rates over time. The kinetics cannot be clearly attributed to the thermal pretreatment, as replicate behaviors were not consistent. Even in the least favorable case (K2T), the percolation rate remained above the critical threshold, indicating that percolation through pellets stayed acceptable despite a marked decline over time. Nevertheless, given the poor performance observed with the Oleane cakes, percolation limitations are likely to have contributed to the low efficiency of the first extraction trial.

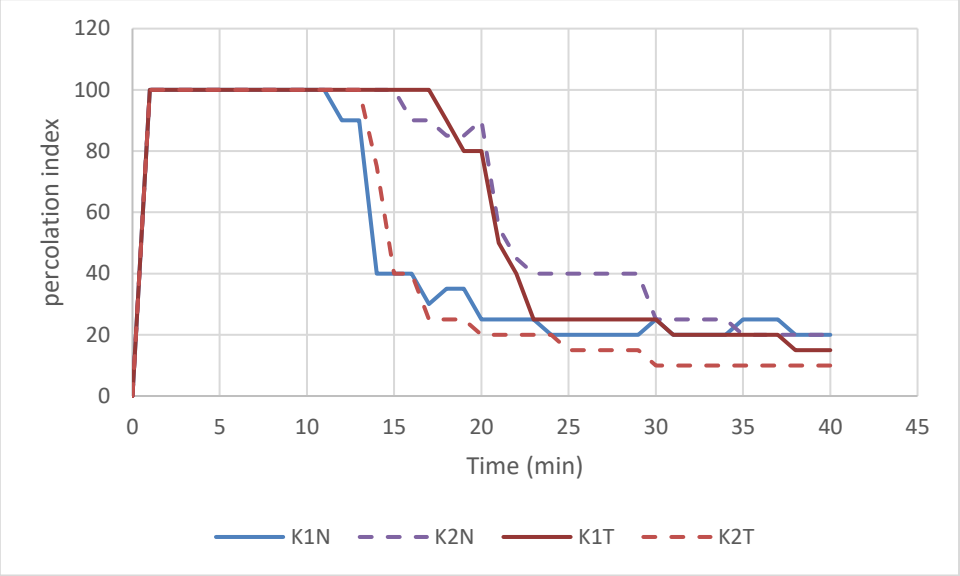

Figure 11: Evolution of the percolation in komet press-cake (KxN: untreated seeds, KnT: heated seeds)

Figure 12 shows the amount of GLS in the extract W1 to W4, in spent cake and the losses for each repetition of the komet press cakes from native or heat-treated seeds.

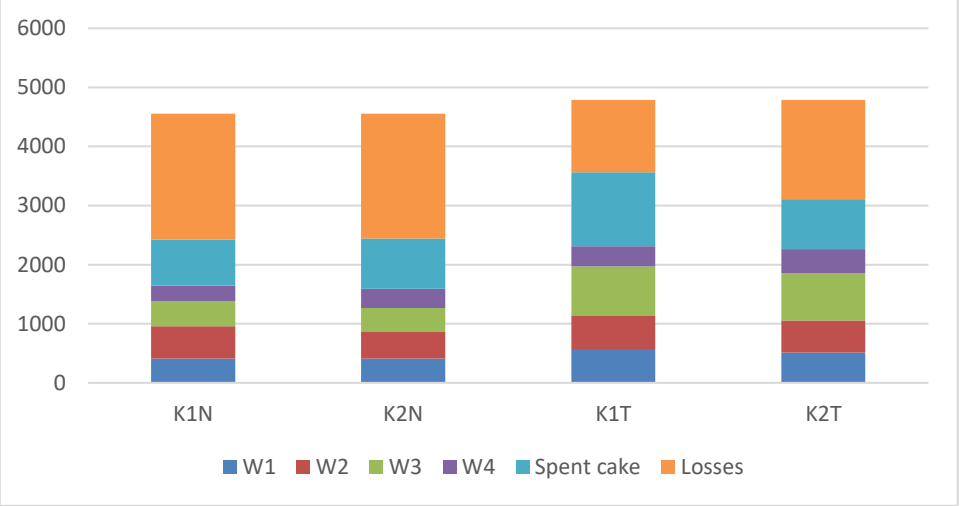

Figure 12: Repartition of the glucosinolates in extracts W1 to W4, spent cake and losses after Komet's cakes extraction.

In untreated cakes, glucosinolate losses were 46% in both replicates. Glucosinolates recovered in extracts W1 to W3 were relatively similar, with a decrease observed in W4, and unextracted glucosinolates accounted for only 17–18% of the initial amount. These results confirm strong myrosinase activity during extraction in untreated press cake. Regarding extraction kinetics, the slower percolation observed in K2N appears to have caused slower glucosinolate diffusion compared with K1N, an effect partly compensated by the higher glucosinolate concentration measured in W4 for K2N.

In heat-treated cakes, losses were markedly reduced, although the treatment was not sufficient to completely inactivate enzyme activity. Losses differed between replicates, at 25% in K1T and 35% in K2T. This discrepancy did not clearly affect glucosinolate recovery in the extracts, as compensation mainly occurred through differences in the fraction of unextracted glucosinolates. From a kinetic perspective, glucosinolate levels in W1 and W2 were relatively low compared with W3, suggesting that diffusion within the cake was slow and that efficient extraction required longer contact times.

An additional experiment was therefore conducted to monitor extraction kinetics over a longer percolation period for one untreated and one heat-treated cake. Figure 13 shows the evolution of percolation during this experiment. As in Figure 11, percolation rates dropped sharply after 20 minutes and, in the case of the heat-treated cake, fell below the acceptability threshold after 45 minutes.

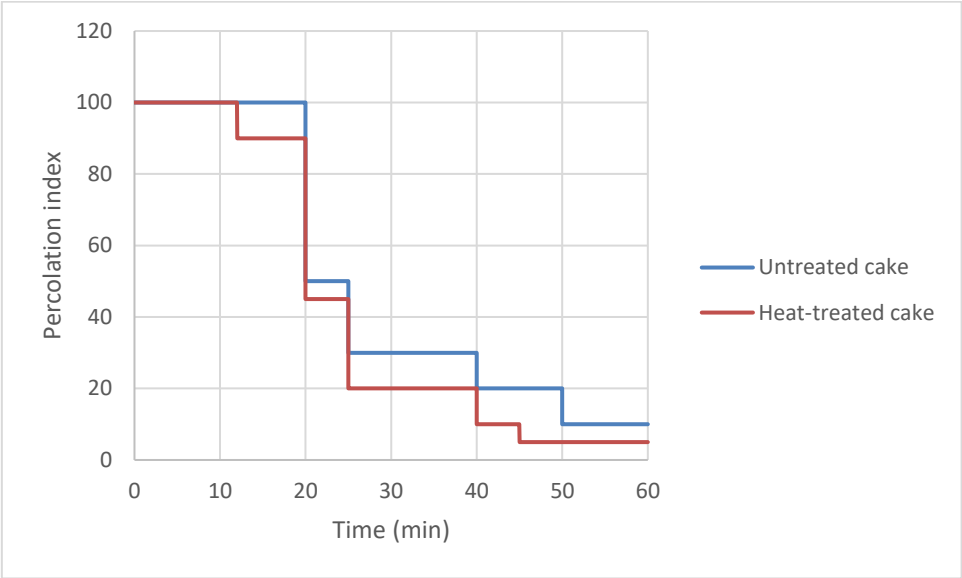

Figure 13: Evolution of the percolation during the long percolation tests

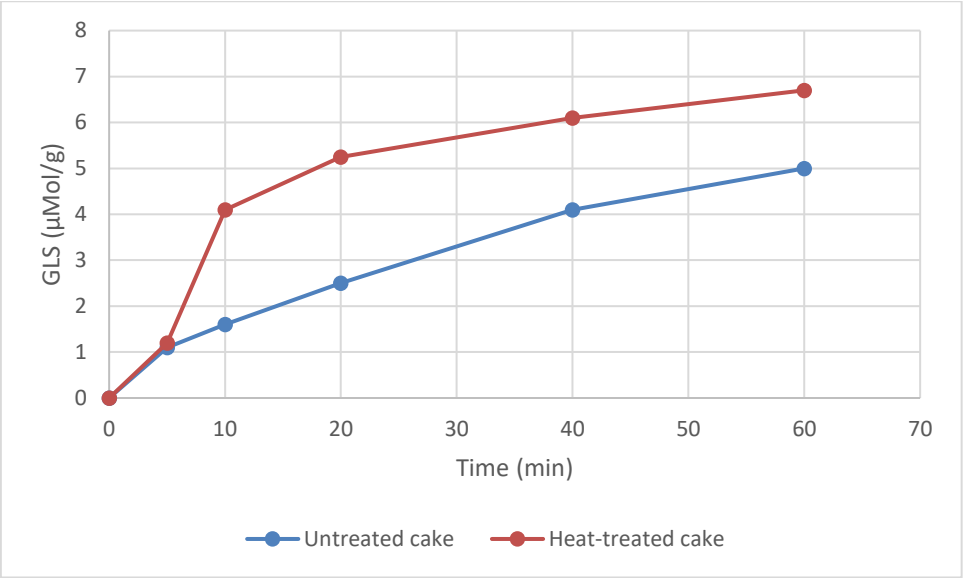

Figure 14: evolution of the extracts concentration with time

Figure 14 shows the evolution of glucosinolate concentrations. As expected, the profile differs in the untreated cake because of myrosinase activity. Classical diffusion curves can be described by an equation of the form:

$$C = C_{max} \times \left(1 - e^{(k \times t)}\right)$$

where C is the concentration at time t, $C_{max}$the concentration at infinite time, and k a mass-transfer coefficient. Such curves typically exhibit a very rapid increase in concentration during the first seconds of extraction, corresponding to the easy diffusion of solutes located at the particle surface into the solvent. In the case of glucosinolates, the kinetics display an initial delay that may reflect myrosinase activity, which could remove the most mobile glucosinolate fraction before it migrates into the solvent. The data point at 5 minutes, where both cakes show similar concentrations, suggests that myrosinase activity is mainly expressed during the first minutes of contact. Beyond this period, glucosinolate diffusion into the solvent follows a more conventional kinetic pattern.

3.5 Supply of a sample of glucosinolates to FLANAT

In order to provide material for downstream purification and bioactivity assessment, a dedicated production campaign was carried out in October 2024 to generate crude glucosinolate extracts for FLANAT. Five batches of 2 kg Brassica carinata press cake (5 × 2 kg) were processed using an extraction scheme representative of pilot-scale operation. The process was designed to supply 50 g of glucosinolates to Kimitec and therefore focused on recovering as much glucosinolate as possible via a rapid, practical protocol rather than maximizing extraction yield.

Press cake was extracted with 50% ethanol at ambient temperature for 60 minutes in 7 L batches, using the same equipment as in the other pilot-scale extractions. After each extraction step, the miscella was drained, filtered and pooled by batch. The combined miscella was then concentrated in a rotary evaporator operated at 60 °C under reduced pressure, with the aim of reducing liquid volume prior to shipment while preserving glucosinolate integrity as far as possible. The

concentrated extract was collected, weighed and sampled for dry-matter and glucosinolate analysis before being shipped to FLANAT.

Results

The pilot-scale campaign produced approximately 12 kg of liquid concentrate. Analytical results showed a glucosinolate concentration of about 15.3 mmol L^{-1}, corresponding to a total glucosinolate content of roughly 72 g (around 6 g L^{-1}). The initial miscellas (before evaporation) exhibited glucosinolate concentrations of the same order of magnitude (14.9, 12.5, 20.1, 15.3 and 12.9 µmol/g for batches 1 to 5, respectively). Although the solvent volume was reduced by approximately 50% during rotary evaporation at 60 °C under vacuum, the glucosinolate concentration in the final product remained essentially unchanged. Instead of the expected two-fold increase for a stable, non-volatile solute, the concentration remained close to its initial value.

This behavior indicates that a substantial fraction of glucosinolates disappeared from the liquid phase during concentration, despite the use of moderate temperature and reduced pressure, most likely due to residual myrosinase activity re-activated as ethanol was removed. In terms of extraction efficiency, 43% of the glucosinolates were successfully recovered in the extract, 42% were hydrolyzed, and 15% remained unextracted.

3.6 General conclusion about the extraction of glucosinolates from Brassica carinata

The work carried out in Task 2.4.1 shows that the recovery of glucosinolates from Brassica carinata press cake is technically feasible at pilot scale but would require substantial process intensification and investment to be implemented at industrial scale. In particular, robust inactivation of myrosinase demands relatively severe thermal treatment: our results indicate that heating at 100 °C for 20 minutes is necessary to ensure complete deactivation, whereas treatment at 90 °C leads only to partial inactivation, with significant enzymatic degradation of glucosinolates during subsequent processing.

Solvent extraction by percolation raises several additional constraints. Solvent uptake by the cake is unavoidable and markedly slows down percolation over time, so that extraction becomes increasingly inefficient. To maintain acceptable percolation rates for at least 60 minutes, the press cake must be textured as small-diameter pellets with sufficient mechanical strength to resist swelling and compaction. Such conditions appear difficult to achieve with deep-bed extractors at industrial scale and would more realistically require shallow-bed or thin-layer extractor designs. Moreover, solvent retention in the marc is high: the spent cake typically contains a solvent mass of the same order as its dry matter, which implies substantial drying requirements and an increased risk of damaging protein quality during solvent removal.

Alternative solid–liquid separation technologies could, in principle, alleviate some of these limitations. For example, combining strong mechanical comminution of the cake (flour-like particle size) with extraction under agitation followed by horizontal decanter centrifugation would improve mass transfer and reduce percolation issues. However, such technologies are considerably more complex and costly than simple percolation in fixed beds, and may be difficult to justify for a secondary coproduct.

If one remains within a process architecture similar to conventional hexane oil extraction, the overall energy demand for glucosinolate recovery would be high. Extraction times need to be significantly

longer because of the slow diffusion of glucosinolates in hydro-ethanolic solvent, and the latent heat of vaporisation of ethanol–water mixtures is about 4.6 times higher than that of hexane. Combined with the strong solvent retention in the marc, this leads to an estimated thermal energy requirement roughly triple that of a standard hexane process for the same throughput.

On a rough economic basis, taking a typical oil extraction cost of around 40 €/t seed, of which one third is attributed to thermal energy, a three-fold increase in thermal energy consumption for glucosinolate recovery would translate into an additional cost on the order of 120 €/t of treated press cake. In theory, this extra cost should be compensated by an improvement in the nutritional value and market price of the resulting, low-glucosinolate protein meal. However, even if glucosinolates are efficiently removed, the potential value of Brassica carinata meal remains modest when benchmarked against soybean meal. Assuming a protein content of about 38%, and using soybean meal 48% protein at 338 €/t and rapeseed meal 32% protein at 226 €/t as references (La dépêche, January 2026), the estimated value of carinata meal would be around 268 €/t. This is insufficient to offset a process cost increase of approximately 120 €/t of meal, even if part of this cost could be recovered through valorisation of the glucosinolates themselves.

The economic potential of glucosinolate recovery must therefore be considered carefully. As a rough benchmark, biofumigation applications are often cited as requiring at least 5 t/ha of dry biomass at 100 µmol/g glucosinolates, that is, about 200 kg of glucosinolates per hectare. In our process conditions, the potential glucosinolate yield per ton of treated press cake is on the order of 30–35 kg. Bridging the gap between these scales would require either very high added value for purified glucosinolates (e.g. specific biocontrol or fine-chemical applications) or the integration of glucosinolate extraction into processing chains where the additional capital and energy costs can be amortised over multiple high-value products.

Overall, the present work indicates that glucosinolate extraction from carinata press cake can be envisaged at industrial scale, but only at the price of significant thermal treatment, intensified extraction and concentration steps, and higher energy consumption. The practical “window” for an efficient and economically viable process appears narrow when constrained by feed quality requirements and the use of solvent systems compatible with current oilseed processing practice. Future developments could focus on: (i) alternative process configurations (e.g. short-path or membrane-based concentration, intensified solid–liquid contactors); (ii) upstream design of press cakes better suited for percolation; or (iii) targeting niche, high-value applications for glucosinolates where the additional processing cost is justified.

4 References

Albe-Slabi, S., Tollitte, A., Burgel, P. O. F., Beau, L., Galet, O., Mutelet, F., & Kapel, R. (2025). Rational Hydroalcoholic Extraction Strategy for the Recovery of Intact Glucosinolates from Rapeseed Cold-Pressed Cake. Applied Food Research, 101603.

Doheny-Adams, T., Redeker, K., Kittipol, V., Bancroft, I., & Hartley, S. E. (2017). Development of an efficient glucosinolate extraction method. Plant Methods, 13, 17.

Green, A. A. (1931). Denaturation of proteins in the presence of alcohol. Journal of Biological Chemistry, 93(1), 495–516.

Hébert, M. (2020). Nouvelles approches pour la valorisation des graines de moutarde riches en glucosinolates dans un concept de bioraffinerie (Thèse de doctorat, Université de Technologie de Compiègne, France)

La Dépêche. (2026, 29 janvier). Market price list n° 4663. La Dépêche – Le Petit Meunier, (4663), p. 8

Laisney, J. (1984). L'huilerie moderne. Art et techniques. Paris, France : Compagnie Française pour le Développement des Fibres Textiles (CFDT)

Li, X., Luo, S., Zhang, B., Wang, Q., & Li, Y. (2021). Ethanol induced changes in structural, morphological and functional properties of whey protein isolates: Influence of ethanol concentration. Food Chemistry, 345, 128809

Oloyede, O. O., et al. (2021). The impact of domestic cooking methods on myrosinase stability, glucosinolates and their hydrolysis products in cabbage. Foods, 10(11), 2872

Wilson, A. E., Hanlon, P. R., & Barnes, D. M. (2014). Purification of active myrosinase from plants by aqueous two-phase counter-current chromatography. Molecules, 19(8), 11832–11846.