



Liquid-liquid extraction of sinapic acid from a mustard seed by-product using a hollow fiber membrane contactor

Valentin Reungoat, Morad Chadni, Louis M.M. Mousterde, Fanny Brunissen, Florent Allais, Hélène Ducatel, Irina Ioannou

► To cite this version:

Valentin Reungoat, Morad Chadni, Louis M.M. Mousterde, Fanny Brunissen, Florent Allais, et al.. Liquid-liquid extraction of sinapic acid from a mustard seed by-product using a hollow fiber membrane contactor. Separation and Purification Technology, In press, 331, pp.125615. 10.1016/j.seppur.2023.125615 . hal-04287538

HAL Id: hal-04287538

<https://hal.science/hal-04287538>

Submitted on 15 Nov 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Liquid-liquid extraction of sinapic acid from a mustard seed by-product using a hollow fiber membrane contactor

Valentin Reungoat^{a,b}, Morad Chadni^{*}, Louis M. M. Mouterde^a, Fanny Brunissen^a, Florent Allais^a,
Hélène Ducatel^b, Irina Ioannou^a

^a URD Agro-Biotechnologies Industrielles (ABI), CEBB, AgroParisTech, 51110, Pomacle, France

^b Extractis, 33 avenue Paul Claudel 80480 Dury, France

* Corresponding author: morad.chadni@agroparistech.fr

Abstract

This study focuses on the recovery of sinapic acid using liquid-liquid extraction (LLE) assisted by a hollow fiber membrane contactor (HFMC) from an aqueous feed obtained through the hydrolysis of mustard bran. The hydrolyste contains 230 ± 36 mg/L of sinapic acid, with a natural pH of 4. A screening was performed with solvents of different chemical nature (alcohol, ester, ketone, ether, cyclic ether). Data showed that all the solvents tested gave an extraction efficiency of more than 80% for $\text{pH} < 5$, whereas the initial concentration of sinapic acid in the aqueous feed has little impact on the extraction efficiency. Four of the solvents tested were selected for use in the HFMC: two volatile (CPME, MIBK) and two non-volatile (octanol, octyl acetate). The solubility of the volatile solvents in the feed phase was found to be an important factor to consider in evaluating the HFMC (2.6 ± 0.5 % and 1.07 ± 0.05 % v/v with MIBK and CPME, respectively). Mass transfer coefficients with volatile solvents ($25 \pm 1 \times 10^{-6}$ and $15.3 \pm 0.5 \times 10^{-6}$ m/s for MIBK and CPME, respectively) exceeded those of non-volatile solvents ($4.1 \pm 0.2 \times 10^{-6}$ and $4.5 \pm 0.5 \times 10^{-6}$ for octanol and octyl acetate, respectively) by 4- to 6-fold. Extraction was intensified by increasing the initial concentration of sinapic acid in the feed phase and by increasing the feed-to-solvent ratio. CPME demonstrated optimal recovery efficiency at a phase ratio of 8:1 (v/v), yielding 0.9 g of sinapic acid per liter of CPME used.

Keywords: Sinapic acid, hollow fiber membrane contactor, solvent extraction, mustard seed by-product, liquid-liquid extraction

1. Introduction

Researchers are increasingly interested in the potential uses of sinapic acid (a member of the *p*-hydroxycinnamic acids family) in various sectors, including health and cosmetics due to its antioxidant [1]. In addition, it holds promise in areas such as materials, serving as a fundamental component for monomers materials [2], and in pharmaceuticals and food products as a preservative [3]. Sinapic acid can be readily synthesized through green synthetic routes [4–6], using lignin-derived syringaldehyde. Alternatively, a more environmentally-friendly production approach could involve extracting and hydrolyzing sinapic acid derivatives present in *Brassicaceae* seeds, such as rapeseed and mustard seeds, along with their processing by-products [7–10]. However, this approach yields an extract containing various metabolites (e.g., proteins, sugars, lipids) that must be separated from sinapic acid. Consequently, the availability of affordable, sustainable, and highly pure sinapic acid is currently limited as the relatively low purity of sinapic acid obtained from the extraction/hydrolysis process highlights the necessity for the implementation of purification procedures (Figure 1).

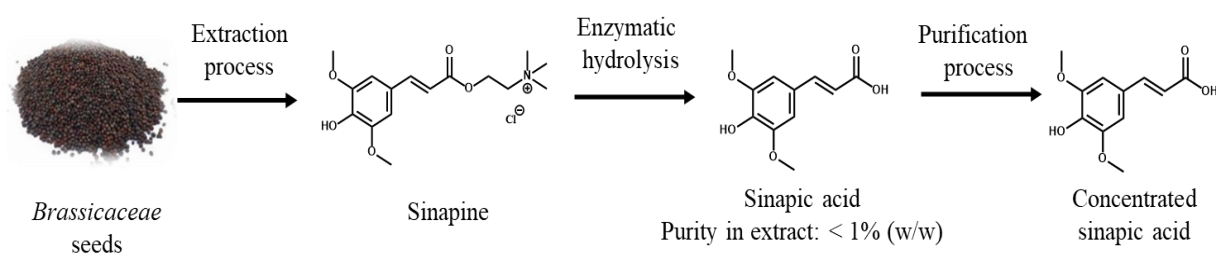


Figure 1: Recovery flow chart of concentrated sinapic acid [11]

Currently, only a limited number of studies are dedicated to the purification of sinapic acid extracted from rapeseed and mustard seeds [12]. Conventional methods employ purification technologies such as classical liquid-liquid extraction (LLE) [13], membrane filtration [14], or adsorption [15,16]. However, most of these investigations lack comprehensive sinapic acid purity data, and the scaling-up of these processes remains uncommon. Among the three technologies mentioned, LLE stands out as a promising avenue for recovering *p*-hydroxycinnamic acids using diverse bio-based solvents [17,18]. Nonetheless, studies involving LLE have largely been conducted at the laboratory scale, using ethyl acetate/diethyl ether (1:1, v/v) as a typical solvent [13,19]. The advancement of this purification process has encountered challenges in equipment design due to the presence of emulsions and phase dispersion [20]. In recent years, the development of membrane contactors has emerged as a potential solution to overcome these issues [21].

In membrane contactors, the membrane acts as a barrier between two phases that prevents mixing but not controlling the rate of transport of different components between phases. It's typically composed of hydrophobic hollow fibers housed within a shell. In the context of LLE, this device facilitates the transfer

of a solute between two immiscible liquids [21]. This transfer occurs through the porous membrane without causing the liquids to mix. Specifically, one of the liquids acts as an organic solvent, filling the pores of the fibers, while the back pressure from an aqueous phase in the shell side stabilizes the interface located at these pores [22]. This limits the dispersion of the solvent in the aqueous phase and facilitates the separation of the two fluids, enabling the implementation of a continuous process.

LLE assisted by membrane contactors have been little studied for the recovery of phenolic acids, with the few existing studies using only model solutions [23–25]. To the best of our knowledge, this is the first application of a membrane contactor for the purification of a *p*-hydroxycinnamic acid from plant material.

The objective of this study is to investigate the utilization of a hollow fiber membrane contactor (HFMC) in LLE for the purification of sinapic acid from a hydrolysate derived from mustard bran, which is a by-product of mustard seed processing. To achieve this, an initial solvent screening was conducted to identify the most effective extraction solvents. Subsequently, the chosen solvents were employed in a pilot-scale (HFMC) to assess the feasibility of sinapic acid extraction from the hydrolysate of mustard bran. The operational parameters were then optimized to determine the maximum quantity of sinapic acid extractable per unit volume of solvent.

2. Materials and methods

2.1. Chemicals

Sinapic acid (purity > 98%) was produced in-house following the procedure of [26]. HCl (~ 37%), NaOH (purity ≥ 97%), acetic acid (purity of 99.7%) and sodium acetate (purity ≥ 99%) were from Fisher (UK). Acetonitrile (purity ≥ 99.9%) was from VWR. The selection of solvents was based on various criteria, encompassing their bio-based production, safety and sustainability according to the GSK's solvent sustainability guide updated in 2016 [27], as well as their potential affinity for hydroxycinnamic acids [18]. These solvents, characterized by differing chemical properties, were categorized based on their boiling points, with those boiling below 150 °C considered volatile and those boiling above 150 °C classified as non-volatile. A detailed description of these solvents is provided in Table 1.

Table 1: Solvents used to extract sinapic acid

Solvent	Chemical nature	Carbon chain length	Boiling point (° C)*	Purity	Supplier
Butanol	alcohol	C4	118	> 99%	Fischer
Octanol	alcohol	C8	196	99%	AlfaAesar
Dodecanol	alcohol	C12	258	> 99%	TCI
Ethyl acetate	ester	C2	77	99.9%	VWR
Octyl acetate	ester	C8	211	> 98%	TCI
Lauryl acetate	ester	C12	265	≥ 98%	Merck
2-MeTHF	cyclic ether	C5	80	> 98%	TCI
CPME	ether	C5	106	≥ 99%	Merck
MIBK	ketone	C6	117	100%	VWR

2-MeTHF: 2-methyl tetrahydrofuran, CPME: cyclopentyl methyl ether, MIBK: methyl isobutyl ketone.

*According to PubChem website datasheet [28]

2.2. Solvent screening on a model solution

Prior the use of a HFMC, a screening of solvents was first performed on a simple model solution using an adapted shake-flask method [29]. A model solution of sinapic acid (0.1 g/L) was prepared by dissolving sinapic acid in milli-Q water and adjusting the pH (Inlab Science Pro-ISM electrode, Mettler Toledo, Switzerland) from 3 to 7 with 0.1 M HCl or 0.1 M NaOH solutions. The shake flask method is described as follow: 5 mL of the model solution of sinapic acid was added in a 15 mL centrifuge tube. An equal volume of solvent was added (aqueous phase to solvent ratio of 1 v/v). The system was manually shaken for 3 x 1 min with equilibration times of 4 min and a final quiescent time of 15 min. The system was centrifuged (4000 g, 10 min) only when a persistent emulsion was observed. Finally, the aqueous feed was selectively taken and analyzed using HPLC. The HPLC method used for the quantification of sinapic acid was previously detailed [30].

The extraction efficiency of the solvents shown in Table 1 was first investigated by varying the pH (unbuffered) over a range of 3 to 7, at a temperature of 25 °C, while maintaining an initial sinapic acid concentration of 0.1 g/L in the feed phase. The extraction efficiency E (%) represents the disappearance of sinapic acid from the feed phase. It was calculated using Eq. 1.

$$E (\%) = \frac{m_{0(aq)} - m_{eq(aq)}}{m_{0(aq)}} \times 100 \quad \text{Eq. 1}$$

Where $m_{0(aq)}$ and $m_{eq(aq)}$ are the mass of sinapic acid (mg) in the aqueous phase at initial and equilibrium times respectively.

2.3. Effect of the initial concentration of sinapic acid in the aqueous phase

In order to assess the effect of the initial concentration of sinapic acid in the aqueous phase on the extraction efficiency, different concentrations of sinapic acid in the model solution (0.01, 0.05 and 0.1 g/L) were prepared at pH 4 and at a temperature of 25 °C. The efficiency of sinapic acid extraction from a model solution was compared to the one from a mustard bran hydrolysate using the shake-flask method.

The hydrolysate was obtained as follows: first, mustard bran was subjected to aqueous extraction (at 100 °C and pH 4, using 100 mL of solvent per gram of dry mustard bran). Subsequently, enzymatic hydrolysis was carried out using a cocktail of enzymes (Deltazym VR AC-100). The resulting hydrolysate underwent centrifugation (at 4000 g for 20 minutes) followed by filtration through a 2 µm cellulose filter. In this phase of the study, the sinapic acid concentration in the hydrolysate was measured to be 55 ± 4 mg/L a pH of 4.3 and a temperature of 25 °C.

2.4. Membrane-based solvent extraction

2.4.1. Description of the equipment

The extractions were carried out on a membrane contactor pilot developed by Seprosys (La Rochelle, France). The setup comprised a HFMC, specifically the 2.5x8 X50 Liqui-Cel™ module equipped with hydrophobic polypropylene fibers from 3M (Minnesota, United States). The module characteristics are detailed in Table 2 and the experimental setup of the pilot is presented *Figure 2*.

Table 2: Characteristics of the hollow fiber membrane contactor

Module material	Polypropylene	Fiber effective length	146 mm
Fiber material	Polypropylene	Wall thickness	40 µm
Pore size	0.03 µm	Shell internal diameter	58.4 mm
Porosity	40%	Shell internal length	20.3 mm
Inner fiber diameter	220 µm	Number of fibers	~ 9800
Outer fiber diameter	300 µm	Effective surface area	1.4 m ²

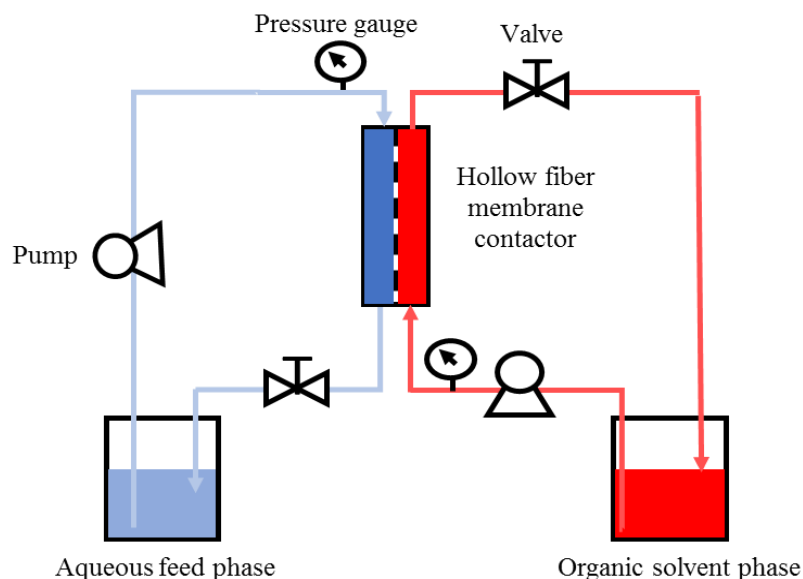


Figure 2: Experimental setup of the pilot for membrane based solvent extraction

The extractions were operated in crossflow countercurrent mode. Aqueous feed (0.5 L) was pumped through the fiber lumen side, while organic phase (0.5 L) was pumped through the module shell side at a flow rate of 50 ± 2 L/h for both phases. The aqueous phase pressure (P_{lumen}) was maintained higher than the organic phase pressure (P_{shell}) to prevent the organic phase (the wetting phase) from permeating [31]. A constant pressure difference (ΔP) between the two phases was kept at 0.5 bar.

2.4.2. Kinetic study of sinapic acid extraction with solvents in HFMC

The sinapic acid is initially contained in the feed phase (42 ± 4 mg/L) consisting of the mustard bran hydrolysate produced as described in part 2.3. The corresponding dry matter content is 0.5% (w/w) with a sinapic acid purity of 0.4 ± 0.1 %_{DM}. The purity of a compound on a dry matter (%_{DM}) of a hydrolysate or a solvent phase was calculated using Eq. 2.

$$P (\%_{DM}) = \frac{m_{\text{compound}}}{m_{DM}} \times 100 \quad \text{Eq. 2}$$

Where m_{compound} and m_{DM} are the mass of a compound and the mass of the dry matter in a hydrolysate or a solvent phase respectively.

Based on the screening step, four solvents were subjected to testing: two volatile solvents (MIBK and CPME) and two non-volatile solvents (octanol and octyl acetate). The extraction kinetics of sinapic acid were monitored by periodically collecting samples from the feed phase and subsequently analyzed using HPLC.

The extraction kinetics analysis enabled the determination of an apparent mass transfer coefficient, denoted as K_{app} (m/s). This coefficient quantifies the speed at which sinapic acid is extracted from the aqueous phase within the HFMC. To calculate K_{app} , an adapted equation from the work of Dahuron

and Cussler in 1988 [32] was employed. It's important to note that while Dahuron's relationship was initially established for a parallel f-current flow, the HFMC configuration used in our case involves a cross-current flow, thereby rendering K_{app} an apparent transfer coefficient. The detailed procedure for calculating K_{app} is presented in the supplementary material file.

2.4.3. Determination of mutual phase solubility

Certain solvents exhibit partial solubility in water, and conversely, water can also partially dissolve in some solvents. This occurrence becomes particularly notable when dealing with solvents characterized by shorter carbon chain lengths. Information about mutual phase solubilities proves valuable in understanding phase diffusion-induced losses, preventing phase emulsions through interfacial tension reduction, and facilitating the design of liquid-liquid extractors [33].

Consequently, after extraction within the HFMC, the mutual phase solubility was determined using diverse methodologies. To ascertain the solubility of water in the organic solvent, the Karl-Fischer method was employed with the 899 KF Titrino Plus and 899 coulometer instruments (Metrohm, Switzerland). The titrating reagents employed were HYDRANAL™ - Composite 5 (Honeywell, USA) and Aqualine complete 5K (Fisher Scientific, USA).

On the other hand, the solubility of solvents in the aqueous phase required distinct approaches. Specifically, the solubility of MIBK in the aqueous phase was quantified using HPLC-UV at 210 nm. Meanwhile, the solubility of CPME and octanol was assessed through Gas Chromatography-Mass Spectrometry (GCMS) coupled with Solid Phase Microextraction (SPME). In this procedure, a SPME fiber (Carboxen/PDMS) was immersed in 10 mL of water containing up to 10^{-3} % (v/v) of solvents for 60 seconds at 50 °C. Subsequently, solvent desorption occurred in a Gas Chromatograph (7890A, Agilent) at 300 °C for 600 seconds under a hydrogen flow rate of 2 mL/min. Separation took place in a ZB-5MSplus column (Phenomenex) (30 m x 250 μ m x 0.25 μ m) using a temperature gradient of 10 °C/min from 35 °C to 200 °C, followed by 20 °C/min to 300 °C for 5 minutes. Detection was performed by a mass spectrometer MS (5975C, Agilent). Calibration curves were established based on reference solvent standards diluted in osmosis water.

The final volume of each phase having been in contact depends on their mutual solubility. It was calculated using Eq. 3 for the aqueous phase and Eq. 4 for the solvent phase.

$$V_{eq(aq)} = V_0(aq) - \gamma V_0(orga) + \delta V_0(aq) \quad Eq. 3$$

$$V_{eq(orga)} = V_0(orga) + \gamma V_0(orga) - \delta V_0(aq) \quad Eq. 4$$

Where 0 and *eq* represent the initial and the equilibrium states, *aq* and *orga* represent the aqueous and solvent phases, *V* is the volume of a phase, γ is the solubility of water in solvent (% v/v), and δ is the

solubility of the solvent in water (% v/v). According to the final volumes determined, the volume loss can then be calculated using Eq. 5.

$$\Delta V = V_0 - V_{eq} \quad \text{Eq. 5}$$

2.4.4. Study of the effect of the initial concentration of sinapic acid in the feed phase

In order to intensify the extraction in the HFMC, the effect of the initial concentration of sinapic acid in the hydrolysate was studied. Higher dry matter content in the hydrolysate could have an impact both on the extraction efficiency and the mass transfer coefficient due to its possible accumulation in the aqueous boundary layer of the membrane of the fibers.

Therefore, the feed phase studied consists of different batches of hydrolysate having a sinapic acid concentrations of 43 ± 2 , 87 ± 1 and 274 ± 22 mg/L, with a dry matter content of 0.5%, 1% and 5% (w/w), respectively. The pH was around 4.2 for all batches. The sinapic acid purity in the hydrolysate remains constant at 0.4 ± 0.1 %_{DM}.

For this study, MIBK was selected as the solvent due to its notable extraction efficiency and high mass transfer coefficient. The extraction kinetics of sinapic acid were monitored through periodic sampling of the hydrolysate from the feed phase and subsequently quantified using HPLC.

2.4.5. Study of the effect of the feed-to-solvent ratio

To enhance the extraction process by achieving higher sinapic acid recovery with a reduced solvent quantity, the impact of the feed-to-solvent ratio was investigated using LLE assisted by HFMC. Various feed-to-solvent ratios were examined: 1:1, 2:1, 4:1, and 10:1 (v/v). While maintaining a constant initial volume of the solvent phase at 0.5 L, the volumes of the feed phase were set at 0.5, 1, 2, and 5 L, respectively.

The aqueous feed phase encompassed either the hydrolysate (characterized by a sinapic acid concentration of 230 ± 36 mg/L, purity of 0.4 ± 0.1 %_{DM}, and a dry matter content of 5% w/w) or a buffered model solution of sinapic acid (with a concentration of 250 mg/L, pH 4, and acetate buffer at 30 mM) for comparison. The extraction kinetics of sinapic acid was monitored through periodic sampling of the feed phase, and the measurements were performed using HPLC.

To assess the purity of the sinapic acid extracted within the solvent, our focus was solely on examining volatile solvents, specifically MIBK and CPME. The determination of purity requires the evaporation of the solvent, a process that was achieved utilizing a Rotavap (Büchi, France) with an overnight vacuum ramp.

The material balance was established through the procedure of obtaining a solvent phase sample upon completion of the extraction. This sample was diluted in acetonitrile at a tenfold ratio and subsequently

subjected to HPLC analysis. This analytical approach enabled the computation of the recovery yield of sinapic acid present in the solvent, expressed as a percentage. This computation was described by Eq. 6, wherein the mass of sinapic acid obtained within the organic phase was compared to the initial mass present in the aqueous feed phase.

The material balance was calculated by taking a sample of the solvent phase at the end of the extraction, diluting it in acetonitrile to the tenth, and analyzing it by HPLC. This allowed the calculation of a recovery yield of sinapic acid in the solvent (%), described by Eq. 6. It represents the mass of sinapic acid obtained in the organic phase compared to the initial mass in the aqueous feed phase. The difference with extraction efficiency could indicate a potential reduction in sinapic acid content throughout the extraction process, possibly attributable to degradation.

$$\text{Recovery yield (\%)} = \frac{m_{eq(orga)}}{m_{0(aq)}} \times 100 \quad \text{Eq. 6}$$

Where $m_{eq(orga)}$ is the mass of sinapic acid in the organic phase at equilibrium, $m_{0(aq)}$ is the initial mass of sinapic acid in the aqueous phase. The recovery yield in function of the feed-to-solvent ratio (F/S) can be predicted by a linear regression detailed Eq. 7.

$$\text{Recovery yield}^*(\%) = a \times F/S + b \quad \text{Eq. 7}$$

Where Recovery yield^* is the predicted recovery yield in function of the feed-to-solvent ratio (F/S), a and b are the slope and the intercept respectively. From the predicted recovery yield, a recovery capacity (g/L) can be predicted by Eq. 8. It represents the mass of sinapic acid that can be obtained in the solvent for a same initial volume of solvent used during the LLE.

$$\text{Recovery capacity}^*(g/L) = \frac{m_{0(aq)} \times (a \times F/S + b)}{V_{0(orga)}} \quad \text{Eq. 8}$$

Where $\text{Recovery capacity}^*(g/L)$ is the predicted recovery capacity in function of the feed-to-solvent ratio and depending on the parameters of the linear regression of the recovery yield, on $m_{0(aq)}$ the initial mass of sinapic acid in the feed phase, and on $V_{0(orga)}$ the initial volume of solvent. In this study, this prediction is obtained for an initial concentration of sinapic acid in the feed phase set at 230 mg/L, using a closed-loop extraction and one extraction cycle.

2.5. Statistical analysis

The data were analyzed statistically by one-way ANOVA. Differences between data were considered significant when $p < 0.05$. Error bars on the figures represent the standard deviation. Experiments were conducted at least in duplicate. The number of replicates performed is indicated by n.

3. Results

A screening approach for the selection of effective solvents and pH values for the extraction of sinapic acid by simple LLE is first described. Next, extraction kinetics using HFMC are presented with four selected solvents. Finally, the improvement of sinapic acid mass recovery by two volatile solvents by acting on the initial concentration in the feed phase and on the feed-solvent ratio is presented.

3.1. Solvent screening using simple LLE

3.1.1. Effect of solvent and pH on extraction efficiency

In this first part, the objective was to select the most efficient organic solvents to extract sinapic acid from a model solution (100 mg/L) at different pH. The extraction efficiencies are shown in Table 3.

Table 3: Comparison of extraction efficiency (%) of sinapic acid for different solvents vs. pH

Solvents	pH 3	pH 4	pH 5	pH 6	pH 7
2-MeTHF	100.0 ± < 0.0 ^a	92.4 ± 0.5 ^b	39.6 ± 4.5 ^a	24.4 ± 0.9 ^b	19.3 ± 4.8 ^a
MIBK	-	98.5 ± 0.1 ^a	-	43.1 ± 4.8 ^a	-
CPME	94.7 ± 0.6 ^e	87.1 ± 1.9 ^{c,d}	18.9 ± 1.0 ^{c,d}	8.2 ± 0.5 ^c	3.7 ± 0.1 ^b
Ethyl acetate	96.0 ± 0.1 ^d	89.5 ± 0.1 ^{b,c}	26.3 ± 0.5 ^b	25.0 ± 4.2 ^b	15.8 ± 1.2 ^a
Octyl acetate	89.1 ± 0.5 ^f	86.2 ± 0.7 ^d	17.3 ± 0.5 ^d	10.9 ± 0.4 ^c	4.1 ± 1.4 ^b
Lauryl acetate	80.4 ± 0.4 ^g	79.0 ± 1.1 ^e	28.5 ± 0.3 ^b	13.9 ± 4.4 ^c	-
Butanol	97.0 ± 0.3 ^c	86.8 ± 0.2 ^d	16.1 ± 0.4 ^d	4.0 ± 2.5 ^c	1.2 ± 0.3 ^b
Octanol	98.0 ± 0.3 ^b	88.1 ± 0.1 ^{c,d}	20.2 ± 0.6 ^{c,d}	6.7 ± 1.3 ^c	7.3 ± 1.2 ^b
Dodecanol	93.8 ± 0.1 ^e	80.6 ± 0.3 ^e	23.2 ± 2.1 ^c	3.6 ± 2.3 ^c	3.7 ± 2.2 ^b

Different superscript letters (in column) indicate a significant difference between the extraction efficiency of solvents ($p < 0.05$) for each pH. Specifically, "a" designates the highest extraction efficiency at each pH, with "b," "c," and so forth, meaning significant decreasing levels of extraction efficiency. $C_{0(aq)} = 100$ mg/L, $n \geq 2$. The missing results for MIBK and lauryl acetate are due to the lower final pH observed which biased the results. This may be due to the undesired acidification of the un-buffered media by these solvents.

Table 3 shows that high extraction efficiencies (> 80%) are obtained for pH 3 and 4. Thus, solvents from the class of alcohols, esters, ether, cyclic ether and ketone can be considered efficient for the extraction of sinapic acid under acidic conditions. These results are in accordance with those obtained by [17,18] in their studies on similar *p*-hydroxycinnamic acids. Additionally, the solvents used in this study exhibit an intermediate level of polarity, which makes them well-suited for dissolving sinapic acid. This aligns with the predictive findings of Galanakis et al [34], which suggested that solvents with intermediate polarity, like alcohols, esters, and ketones, are particularly effective for dissolving hydroxycinnamic acids. Hydroxycinnamic acids have a low activity coefficient, and data predicted by the UNIFAC model

indicate that natural phenols including sinapic acid tend to favor solvents with intermediate polarity, such as alcohols and acetone, over more polar options like water, or less polar solvents like dichloromethane and diethyl ether. The current study supports this prediction, suggesting that the solvents used here should also be effective for dissolving other phenolic acids.

Increasing the pH results in a decrease in the extraction efficiency of sinapic acid, which is related to a lower proportion of its protonated (undissociated) form (Figure 3). Indeed, the proportion of the protonated form of sinapic acid is less than 1% (m/m) at two points above its first pKa (4.5) [35]. As a result, a notable decline in extraction efficiency becomes obvious at pH levels equal to or greater than 5. Extraction efficiency remains relatively modest (< 15%) up to a pH of 7 across the majority of tested solvents, with the exception of ethyl acetate, MIBK, and 2-MeTHF, which exhibit slightly higher yields (15% - 25%). However, it becomes clear that, to achieve an extraction efficiency surpassing 80%, the chosen pH must be maintained at values lower than 5. The significant extraction efficiency at pH 4 is noteworthy, given the natural pH of around 4.2 in the mustard bran hydrolysate. Therefore, in the rest of the study, the focus is on pH 4.

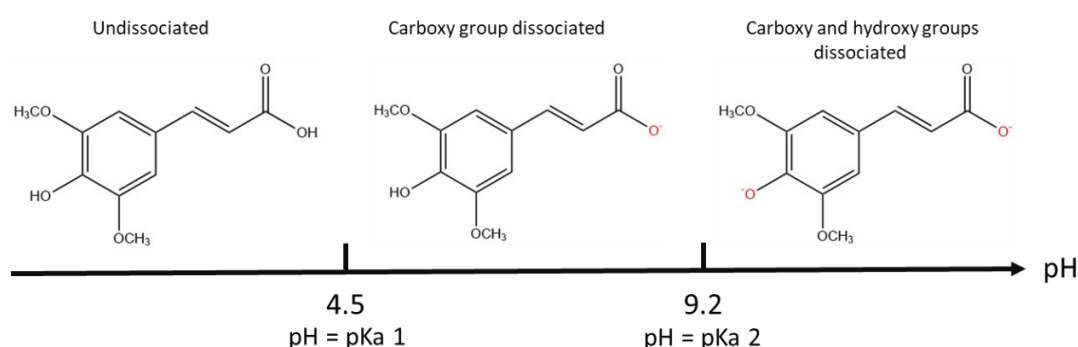


Figure 3: Main form of sinapic acid as a function of pH

3.1.2. Effect of the concentration of sinapic acid in the aqueous phase

This part reports on the study of two effects: (1) that of the initial concentration of sinapic acid, and (2) that of the nature of the aqueous phase (model solution solution or hydrolysate from a mustard bran). Their effects on the extraction efficiency of sinapic acid were studied at pH 4 with different solvents (Table 4).

Table 4: Comparison of extraction efficiency (%) of sinapic acid from a model solution or a hydrolysate vs. initial concentration at pH 4

Solvent	Concentration of sinapic acid mg/L			
	10 (model solution)	50 (model solution)	100 (model solution)	55 (hydrolysate)
2-MeTHF	98.2 ± 0.9 ^A	98.6 ± 0.2 ^A	92.4 ± 0.5 ^C	94.7 ± 0.1 ^B
MIBK	-	-	98.6 ± 0.2 ^A	96.2 ± 0.2 ^B

CPME	93.9 ± 1.9 ^A	90.1 ± 0.4 ^A	87.1 ± 1.9 ^B	85.5 ± 0.1 ^B
Butanol	97.0 ± 0.0 ^A	95.3 ± 0.4 ^B	86.8 ± 0.2 ^D	93.6 ± 0.0 ^C
Octanol	97.3 ± 0.4 ^A	95.8 ± 0.1 ^B	88.1 ± 0.1 ^D	92.1 ± 0.3 ^C
Dodecanol	92.3 ± 0.1 ^A	89.4 ± 0.0 ^B	80.6 ± 0.3 ^C	79.3 ± 0.6 ^D
Ethyl acetate	96.1 ± 0.1 ^A	94.3 ± 0.0 ^B	89.5 ± 0.1 ^C	94.6 ± 0.1 ^B
Octyl acetate	90.6 ± 1.5 ^A	86.8 ± 1.3 ^A	86.2 ± 0.7 ^A	77.8 ± 0.5 ^B
Lauryl acetate	98.8 ± 0.0 ^A	79.6 ± 0.0 ^B	79.0 ± 1.1 ^B	63.0 ± 1.1 ^C

Different superscript letters (in line) indicate a significant difference ($p < 0.05$) between concentrations. In the case of MIBK, the effect of the initial concentration was directly tested on HFMC (part 2.3.2.a).

Table 4 shows that the increase of the initial concentration of sinapic acid tends to lower the extraction efficiency. Indeed, the extraction efficiencies with CPME are 93.9 ± 1.9 %, 90.1 ± 0.4 % and 87.1 ± 1.9 % with concentration of sinapic acid of 10, 50 and 100 mg/L in the model solution, respectively. However, despite the slight loss of extraction efficiency when increasing the concentration of sinapic acid 10-fold, it remains above 80%. Therefore, it is considered in this study that the initial concentration of sinapic acid in a simple model solution has a low impact on the extraction efficiency in the range of concentration of sinapic acid from 10 to 100 mg/L. This result is coherent with the results of [36] on different *p*-hydroxycinnamic acids using CPME and 2-MeTHF as solvents.

According to Table 4, the extraction efficiencies of sinapic acid from the model solution (50 mg/L) and the hydrolysate (55 mg/L) are equivalent with ethyl acetate. However, when working with the hydrolysate, the extraction efficiency is comparatively lower by a margin of 5 points with 2-MeTHF, CPME, butanol, and octanol; by 10 points with dodecanol and octyl acetate; and even up to 17 points with lauryl acetate. Consequently, the hydrolysate appears to exert a diminishing effect on extraction efficiency in comparison to a binary solution containing sinapic acid and water. Considering practical implications, it is advisable to prioritize solvents exhibiting extraction efficiencies that closely approach or surpass the 80% threshold.

Given the demonstrated efficiency of all tested solvents for sinapic acid extraction from the hydrolysate, specific criteria were established to narrow down the solvent selection for employment with HFMC. These criteria encompass the following:

- 1- Short Carbon Chain Lengths: Solvents characterized by shortened carbon chains exhibit elevated water solubility, which could potentially impede extraction due to a reduction in interfacial tension between the aqueous and organic phases. Consequently, the exclusion of butanol and ethyl acetate was considered appropriate.
- 2- Long Carbon Chain Lengths: Solvents with protracted carbon chain lengths exhibit comparatively diminished extraction efficiency. Their heightened viscosity and melting points may also introduce

challenges during membrane contactor operation. Hence, the decision was made to exclude dodecanol and lauryl acetate.

- 3- Ether Safety: Within the ether category, safety concerns surrounding peroxide formation prompted the exclusion of 2-MeTHF, particularly in comparison to CPME, as previously highlighted by Watanabe et al., in 2007 [37].

As a result of these considerations, a selection of only four solvents was made for the extraction of sinapic acid from mustard bran hydrolysate utilizing HFMC. These solvents encompass two volatile options (MIBK, CPME) and two non-volatile choices (octyl acetate and octanol).

3.2. Study of sinapic acid extraction in HFMC

In order to understand the LLE of sinapic acid assisted by HFMC, the kinetics of extraction were established for the four solvents selected, and the extraction efficiency obtained were compared to those of the simple LLE. Despite the non-dispersion of the phases into each other with the membrane contactor, some diffusion may occur due to their mutual solubility. Therefore, the solubility of the mutual phases was also measured.

3.2.1. Extraction kinetics

The four solvents selected are MIBK, CPME, octanol and octyl acetate. The extraction kinetics of sinapic acid using the HFMC from a feed phase consisting of the hydrolysate are presented in Figure 44.

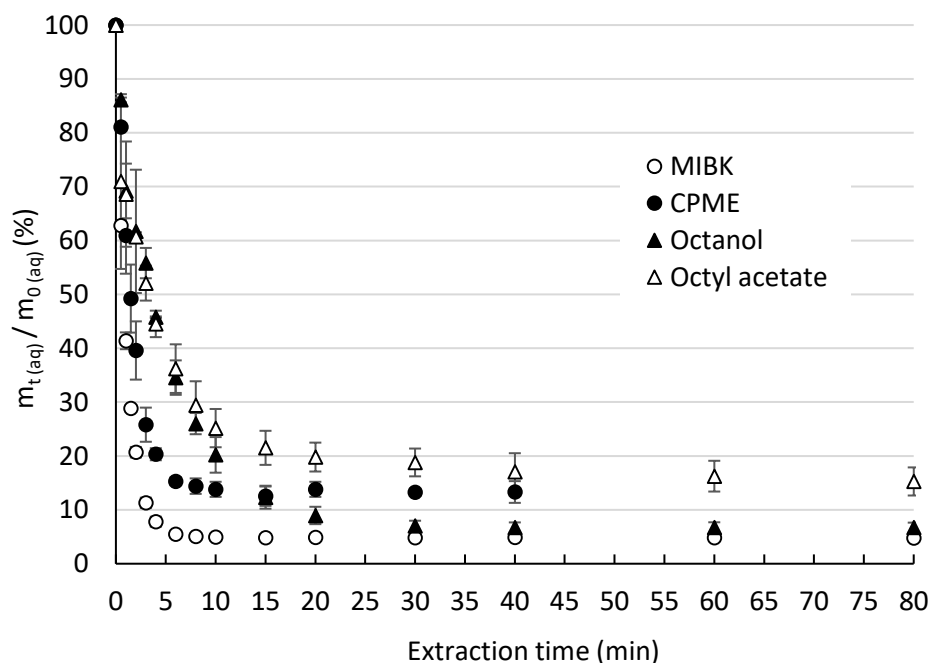


Figure 4: Extraction kinetic of sinapic acid in the HFMC with different solvents

Feed phase in the lumen side of the fibers, feed-to-solvent ratio: 1:1 (v/v), flow rates 50 ± 2 L/h, pH 4.3, $C_0(aq) = 42 \pm 4$ mg/L, $n = 2$.

Figure 4 shows that the concentration of sinapic acid drop sharply in function of the extraction time with all the solvent tested, meaning that the extraction of sinapic acid in the HFMC from a mustard bran hydrolysate is effective. The extraction equilibrium is reached after 8 min with MIBK and CPME, whereas it takes 30 min with octanol. However, in the case of octyl acetate, the kinetic was followed up to 160 min, although equilibrium was challenging to achieve (Figure S2 in supplementary material). This means that the extraction is carried out at a low speed. This phenomenon could be attributed to the higher viscosity of octyl acetate (11 mPa·s at 25 °C) compared to other solvents, such as octanol (7.3 mPa·s at 25 °C). Therefore, octyl acetate appears to be a solvent of lower interest as regards its reliability for comparing extraction yields.

3.2.2. Comparison of the extraction efficiency of the solvents between HFMC and simple LLE

After obtaining the extraction kinetics of sinapic acid by the four solvents selected, the extraction efficiencies obtained with the implementation in the HFMC is compared to the one obtained with a simple liquid-liquid extraction. Extraction kinetics can also be used to determine the mass transfer coefficients, which indicate the speed of extraction in the membrane contactor. These results are presented Table 5.

Table 5: Comparison of the a simple LLE and LLE assisted by HFMC

Solvents	Simple LLE	LLE assisted by HFMC	
	Extraction efficiency (%)	Extraction efficiency (%)	Mass transfer coefficient (10^{-6} m/s)
MIBK	96.2 ± 0.2 ^{a A}	95.1 ± 0.1 ^{a B}	25 ± 1 ^a
CPME	85.5 ± 0.1 ^{c A}	87 ± 2 ^{b,c A}	15.3 ± 0.5 ^b
Octanol	92.1 ± 0.3 ^{b A}	93.1 ± 0.7 ^{a,b A}	4.1 ± 0.2 ^c
Octyl acetate	77.8 ± 0.5 ^{d A}	80.2 ± 2.7 ^{c A} (at 20 min)	4.5 ± 0.5 ^c

Different superscript letters indicate a significant difference between groups ($p < 0.05$) (lowercase letter in column and capital letter in line).

Table 5 shows that there are no significant differences between the simple LLE and the LLE assisted by HFMC with regards to the extraction efficiency, except for MIBK. However, the difference being low for an extraction efficiency, it will be neglected. Thus, the use of membrane contactor has no effect on the extraction efficiency of sinapic acid compared to a simple LLE. However, the use of a HFMC avoids mixing of the phases.

According to Table 5, the highest mass transfer coefficients were obtained with the volatile solvents, MIBK and CPME (25 ± 1 and $15,3 \pm 0,5 \cdot 10^{-6}$ m/s, respectively), whereas the lowest were given with the non-volatile solvents, octanol and octyl acetate ($4.1 \pm 0,2 \times 10^{-6}$ and $4.5 \pm 0,5 \times 10^{-6}$ m/s, respectively). Therefore, the extraction with volatile solvents is 4 to 6 times faster compared to non-volatile ones. It seems that the diffusion of sinapic acid in the stationary phase of the solvent located in the pore of the membrane appears more limited with non-volatile solvents. The lower diffusion could be due to steric hindrance linked to the long chain length of the non-volatile solvents. Concerning the mass transfer coefficients obtained with MIBK and CPME, they are consistent with the same orders of magnitude as those found by Sciubba et al. in 2009 [25] in a HFMC, dealing with the extraction of vanillin by butyl acetate.

Besides, the extraction efficiency with octanol (93.1 ± 0.7 %) is higher than with CPME (87.0 ± 2.0 %), whereas its mass transfer coefficient is lower. Therefore, the speed of extraction does not seem to be related to the extraction efficiency. It means that an increase of the feed flow rate to reduce the extraction time [23] can be expected to not reduce the extraction efficiency.

3.2.3. Mutual phase solubilities

The mutual solubilities of the phases were measured after HFMC-assisted LLE using mustard bran hydrolysate and the four selected solvents. Indeed, such solubilities are useful information to determine the loss of each phase in the other phase by diffusion, to prevent phase emulsion by the drop of interfacial tension, or to design liquid-liquid extractors [33]. The results are presented in Table 6 **Erreur ! Source du renvoi introuvable.**

Table 6: Mutual phase solubility of water and solvent

	Measured solubility of water (% v/v)	Literature data “solubility of water” (% v/v)	Measured solubility in water (% v/v)	Literature data “solubility in water” (% v/v)
Octanol	3.62 ± 0.12	3.72	0.16 ± 0.03	0.06
Octyl acetate	0.22 ± 0.08	0.37	$< 10^{-3}$	0.04
CPME	0.58 ± 0.03	0.26	1.07 ± 0.05	1.28
MIBK	1.41 ± 0.07	2.01	2.6 ± 0.5	1.5 – 2.4

Aqueous phase: mustard bran hydrolysate, pH 4.3, 25 °C. References for literature data : [33,37–40]

Table 6 shows that the solubility of water is important in octanol (3.62 ± 0.12 % v/v) and in a lesser extend in MIBK (1.41 ± 0.07 % v/v), whereas its solubility is limited in CPME and octyl acetate (0.58 ± 0.03 % and 0.22 ± 0.08 % v/v, respectively).

In addition, the solubilities of the non-volatile solvents (octanol and octyl acetate) in water are negligible (0.16 ± 0.03 % and $< 10^{-3}$ % v/v, respectively). However, the solubilities of the volatile solvent (MIBK

and CPME) in water are significant and thus must be considered (2.6 ± 0.5 % and 1.07 ± 0.05 % v/v, respectively). All of the measured solubilities are in accordance with the literature.

According to these results, it appears that the mutual solubility of MIBK and water is not negligible. It should be considered when high volumes are processed in terms of solvent loss and contamination of the solvent and aqueous phases, having cost and environmental consequences.

After stating the basis of the extraction of sinapic acid with the HFMC, and showing the proof of concept with different solvents, some operating conditions were studied to find the optimal mass of sinapic acid that can be recovered in the solvents.

3.3. Improvement of the quantity of sinapic acid extracted by LLE assisted by HFMC

The improvement of quantity of sinapic acid extracted in the solvent was carried out using HFMC by studying the effect of the initial concentration of sinapic acid in the feed phase and the effect of the feed-to-solvent ratio. In addition to the calculation of the extraction efficiency, the speed of extraction with the mass transfer coefficient, and the loss of solvent in the aqueous feed phase due to the solubility of solvent in water, the purity of sinapic acid was also investigated. The purity can be easily calculated after evaporation of the volatile solvents. However, for non-volatile solvents, this implies a more complex desextraction step such as alkaline stripping or diluent-swing [41]. Therefore, only the two volatile solvents (MIBK and CPME) were selected for this study.

3.3.1. Effect of the initial concentration in the feed phase

According to part 3.1.2. , there is little impact of the initial concentration of sinapic acid in a model solution on the extraction efficiency. In this part, the study deals with the effect of the initial concentration of sinapic acid contained in different batches of mustard bran hydrolysate. The initial concentrations of sinapic acid in the hydrolysates are 43 ± 2 , 87 ± 1 and 274 ± 22 mg/L, with corresponding dry matter contents of 0.5%, 1% and 5% (w/w), respectively. The extraction kinetics of sinapic acid in the HFMC by MIBK are presented in Figure 5.

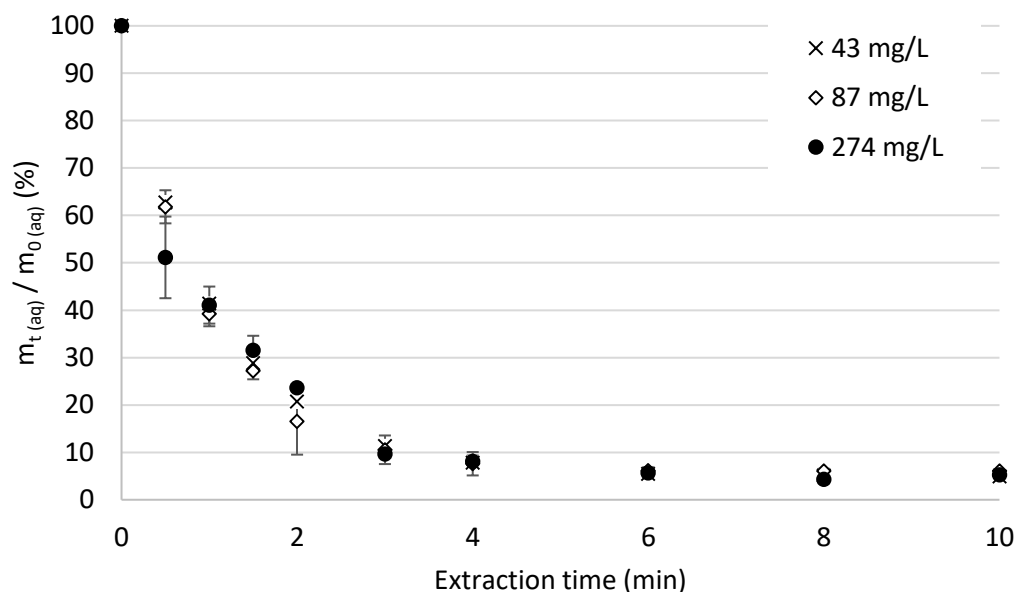


Figure 5: Evolution of sinapic acid extraction with MIBK vs the initial concentration of sinapic acid in the feed phase

pH 4.1 – 4.3, 25 °C, feed in the lumen side of the fibers, feed-to-solvent ratio: 1:1 (v/v).

Figure 4 shows equivalent extraction kinetics whatever the initial concentration of sinapic acid in the hydrolysates. The extraction efficiency at equilibrium is $95.2 \pm 1.0 \%$, and is not significantly different from that obtained with the simple LLE (part 3.1.2.) ($p > 0.05$). This shows that the initial concentration of sinapic acid in the hydrolysate does not impact the extraction efficiency over the concentration range studied (43 – 274 mg/L).

In addition, the mass transfer coefficients obtained with the different initial concentrations are equivalent ($p > 0.05$) with a value of $27 \pm 3 \times 10^{-6}$ m/s. Therefore, the speed of extraction is not dependent of the initial concentration of sinapic acid in the hydrolysate. As a consequence, its initial concentration should be as high as possible to recover the highest concentration in the solvent. In the present study, the maximal initial concentration in the hydrolysate was limited to 274 ± 22 mg/L due to the process used to produce the hydrolysate.

3.3.2. Effect of the feed-to-solvent ratio

The interest to increase the feed-to-solvent ratio lies in the potential increase of the mass of sinapic acid recovered par volume of solvent used, leading to the reduction of the solvent consumption. The feed phase used was either the hydrolysate or a buffered model solution of sinapic acid for comparison (concentration of sinapic acid of 230 ± 36 mg/L, pH 4.1, 30 mM acetate buffer). The feed-to-solvent ratios tested were 1:1, 2:1, 4:1 and 10:1 (v/v). The extraction kinetics with MIBK and CPME as solvents in function of the feed-to-solvent ratio are presented in Figure 6.

(a)

(b)

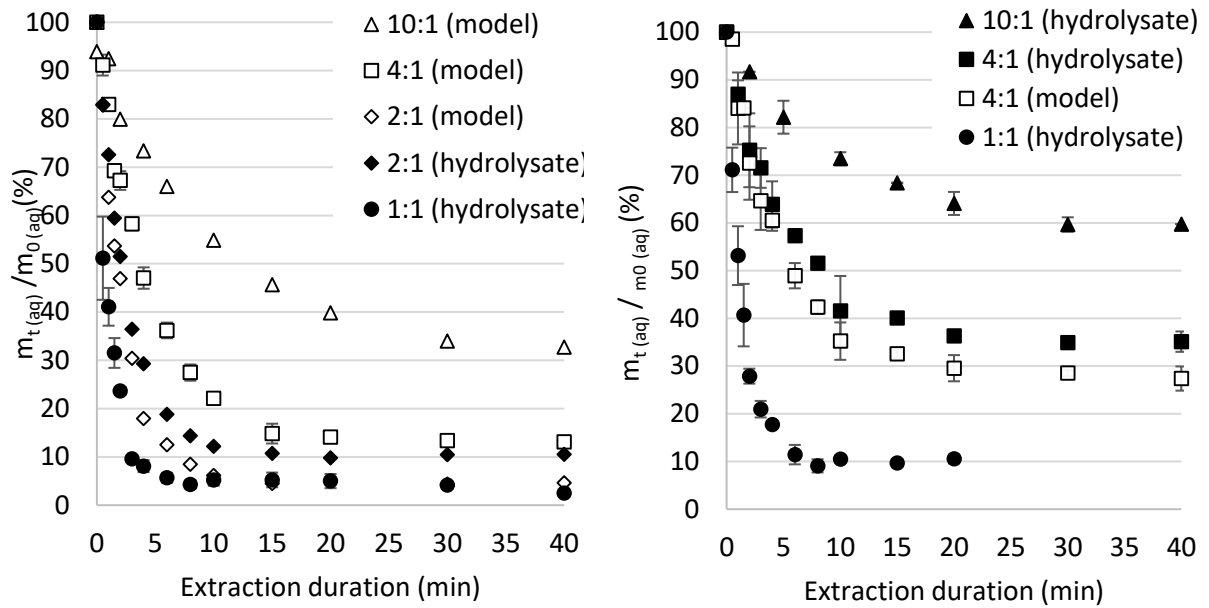


Figure 6: Extraction kinetics of sinapic acid with MIBK (a) or CPME (b) vs the feed-to-solvent ratio pH 4.1, 25 °C, $C_{0(aq)} = 230 \pm 36$ mg/L, feed in the lumen side of the fibers, using a mustard bran hydrolysate or a buffered model solution of sinapic acid.

Figure 5Figure 6 shows that the extraction equilibrium is delayed as the feed-to-solvent ratio increases. Indeed, for the MIBK, the extraction equilibrium is reached at 5, 15, 15-20 and > 40 min for the feed-to-solvent ratios of 1:1, 2:1, 4:1, 10:1 (v/v) respectively. The same phenomenon is observed for the CPME; the extraction equilibrium is reached at 6, 20-30 and 30 min for the feed-to-solvent ratios of 1:1, 4:1, 10:1 (v/v) respectively. Thus, the extraction durations appear longer with higher feed-to-solvent ratios. To have a more precise comparison, the mass transfer coefficients were calculated according to the kinetics. It was found that the highest mass transfer coefficients with MIBK and CPME are at a feed-to-solvent ratio of 1:1 (v/v) ($27.3 \pm 0.4 \times 10^{-6}$ and $19 \pm 3 \times 10^{-6}$ m/s, respectively), whereas it is low at 10:1 (v/v) (19×10^{-6} and $11 \pm 2 \times 10^{-6}$ m/s, respectively). Therefore, the increase of the feed-to-solvent ratio tends to decrease the mass transfer coefficient, either using the hydrolysate or the model buffered solution.

In addition, in the case of MIBK for a feed-to-solvent ratio of 2:1 (v/v), the mass transfer coefficient is found higher with the model buffered solution (26×10^{-6} m/s) than with the hydrolysate (21×10^{-6} m/s). This is also observed with CPME at 4:1 (v/v) ($16 \pm 1 \times 10^{-6}$ and $12 \pm 1 \times 10^{-6}$ m/s respectively). Therefore, the lower mass transfer coefficient observed with the hydrolysate might be explained by a higher resistance from the aqueous boundary layer compared to the model buffered solution, represented in Figure 7.

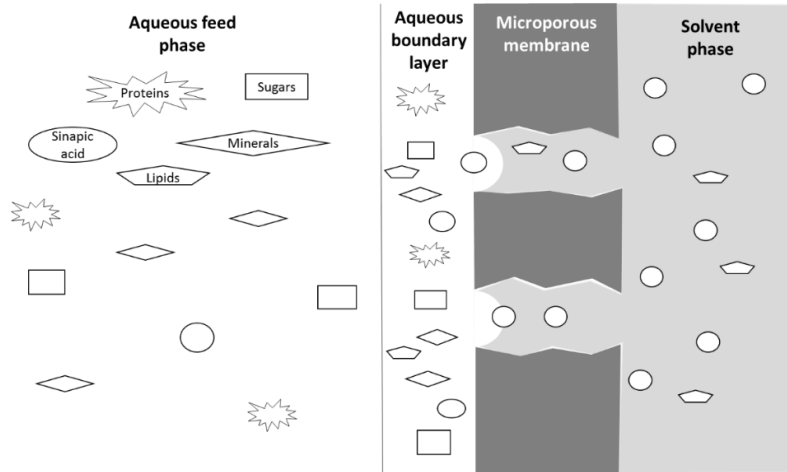


Figure 7: Suggestion of schematic representation of the accumulation the dry matter in the aqueous boundary layer of the HFMC

Indeed, the complex solution of the hydrolysate (dry matter content of 5% w/w) compared to the model buffered solution (< 0.1% w/w) might slow the speed of extraction of sinapic acid by accumulation of some dry matter (sugars, proteins, minerals) in the aqueous boundary layer.

According to the extraction kinetics, the extraction efficiencies at equilibrium, representing the disappearance of sinapic acid from the aqueous feed phase, are determined in function of the feed-to-solvent ratios. It is presented in Figure 8 with a comparison to the recovery yields, representing the effective mass of sinapic acid obtained in the solvent.

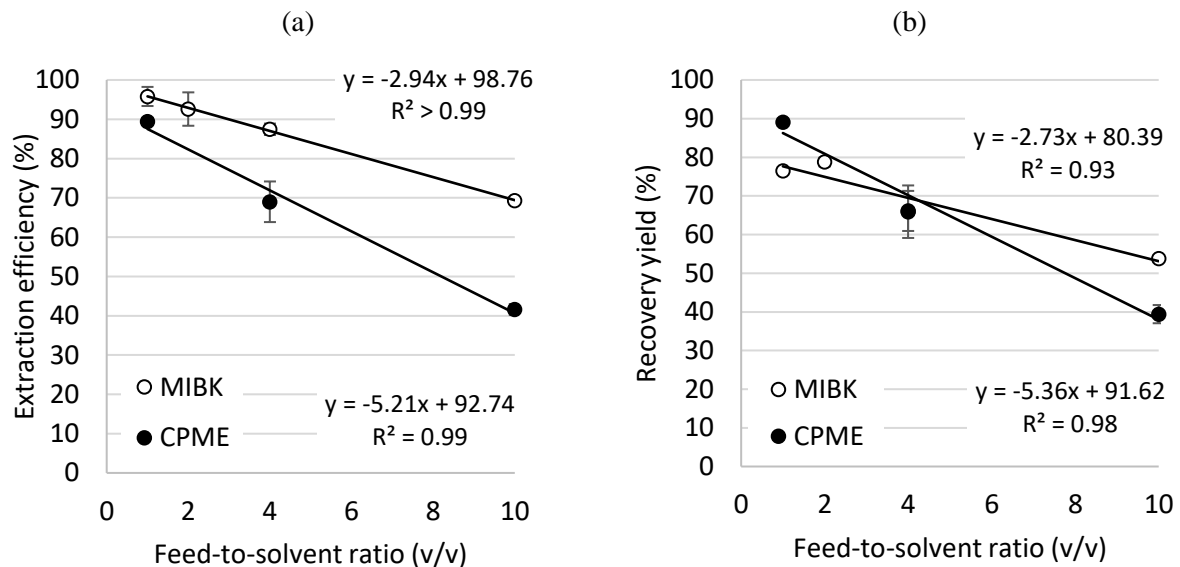
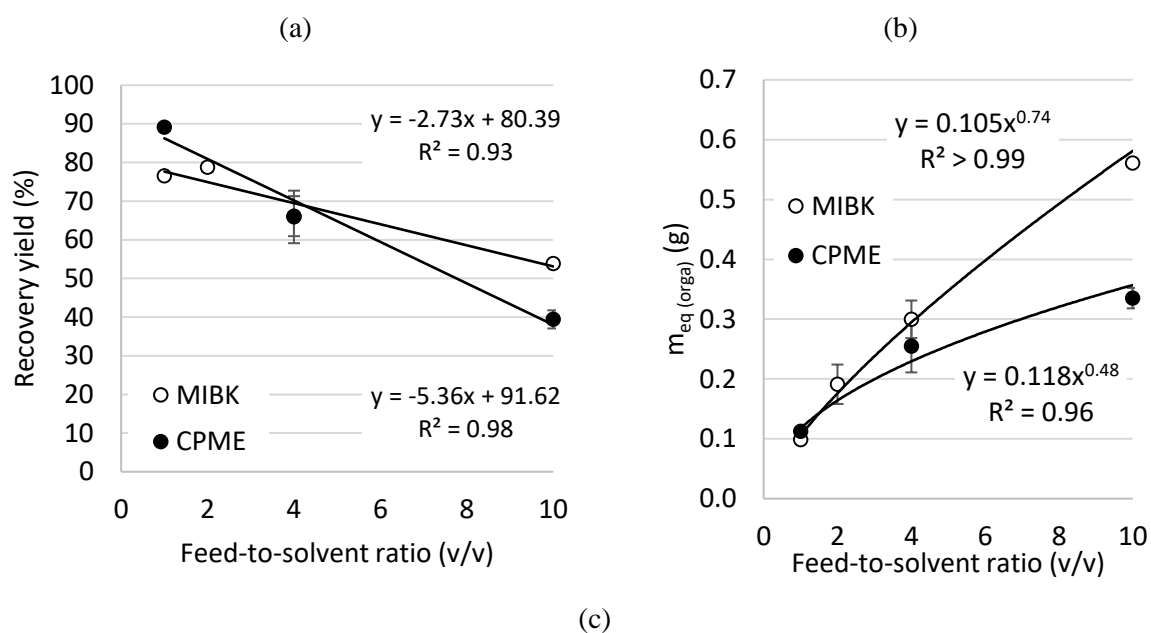


Figure 8 : Effect of the feed-to-solvent ratio on (a) the extraction efficiency and (b) the recovery yield when extracting sinapic acid from a hydrolysate with the membrane contactor pH 4, 25 °C, $C_{0(aq)} = 230 \pm 36$ mg/L, feed in the lumen side of the fibers.

The Figure 88 (a) shows that the extraction efficiencies with MIBK and CPME decrease linearly with the increase of the feed-to-solvent ratio between 1:1 to 10:1 (v/v) (slopes of - 2.94 and - 5.21, respectively). Thus, the decrease in extraction efficiency with CPME is twice as sensitive as MIBK. However, some degradation of sinapic acid can occur during the extraction. Therefore, the recovery yield (Figure8(b)) is also a relevant parameter to consider as it represents the effective mass of sinapic acid obtained in the solvent with respect to the initial mass in the aqueous feed phase. While the extraction efficiency is equal to the recovery yield in the case of CPME, this is not the case for MIBK. Indeed, the recovery yield is lower for all the ratios, showing a loss up to 20 points between the extraction efficiency and the recovery yield, represented by ordinates at the origin. However, the slope remains equivalent (- 2.73), leading to an equivalent effect of the feed-to-solvent ratio. Further investigation showed that sinapic acid is oxidized with MIBK into thomasidioic acid (Figure S1 in supplementary material), accounting for the loss of sinapic acid.

The antagonistic effect of the feed-to-solvent ratio on the recovery yield and on the mass of sinapic acid recovered in the solvent is presented in Figure 9. The integration of these two effects is represented by the recovery capacity, also presented.



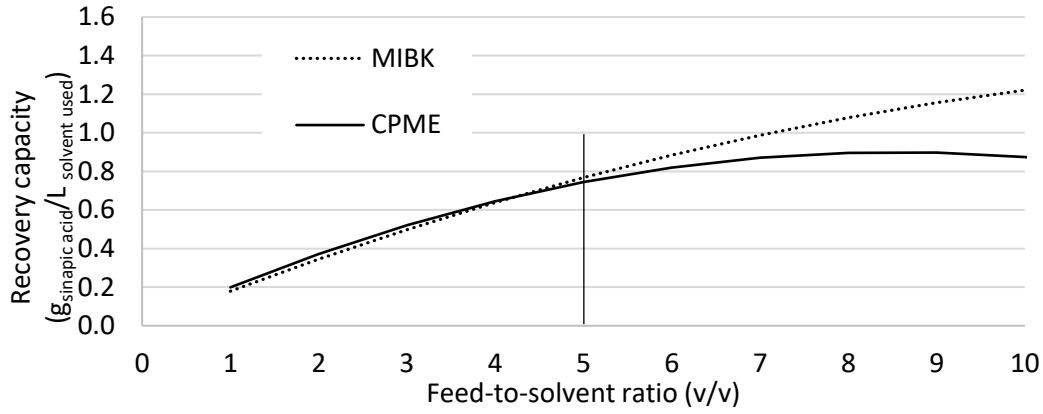


Figure 9 : Effect of the feed-to-solvent ratio on (a) the recovery yield, (b) the mass of sinapic acid recovered in the solvent, (c) the recovery capacity, when extracting sinapic acid from a hydrolysate with the membrane contactor

$pH\ 4$, $25\ ^\circ C$, $C_{0(aq)} = 230 \pm 36\ mg/L$, feed in the lumen side of the fibers, $V_{0(orga)} = 0.5\ L$.

Figure 99 shows that an increase of the feed-to-solvent ratio implies a linear reduction of the recovery yield (Figure 99(a)), and a power law increase of the mass of sinapic acid recovered in the solvent accordingly (Figure 99(b)). This antagonistic effect can be integrated and represented by the recovery capacity. It corresponds to the mass of sinapic acid that could be obtained for a same initial volume of solvent used for the LLE assisted by HFMC. This could be calculated by Eq. 8 using the parameters of the linear regression of the predicted recovery yield in function of the feed-to-solvent ratio (Table 7) and the initial mass of sinapic acid in the aqueous feed phase.

Table 7: Parameters of the linear regression of the recovery yield in function of the feed-to-solvent ratio with MIBK and CPME

	slope	y-intercept	R ²
MIBK	-2.73	80.39	0.83
CPME	-5.36	91.62	0.98

The models obtained showed Figure 8(c) seem parabolic. In the case of CPME, a maximum recovery capacity is reached with a feed-to-solvent ratio of 8:1 (v/v), corresponding to 0.9 gram of sinapic acid recovered per liter of CPME implemented. This result is obtained in the following conditions: for an initial concentration of sinapic acid in the feed phase set at 230 mg/L and a closed-loop extraction with one extraction cycle. In the case of MIBK though, the maximum recovery capacity is not reached with the maximum feed-to-solvent ratio tested (10:1 v/v). The corresponding recovery capacity (1.2 g/L) is still higher than the one obtained with CPME, and as from the feed-to-solvent ratio of 5:1 (v/v). Therefore, MIBK seems to be a better solvent than CPME regarding the recovery capacity. In order to

improve the recovery capacity of the solvents, further study should focus on the intensification of the enrichment of sinapic acid in the initial hydrolysate, and on the development of technics for solvent recycling.

In addition to the recovery capacity, the loss of solvent by diffusion in the aqueous feed phase should also be taken into account. After measuring the mutual phase solubilities, the loss of solvents in the hydrolysate can be calculated in function of the feed-to-solvent ratio. This is presented in Figure 10.

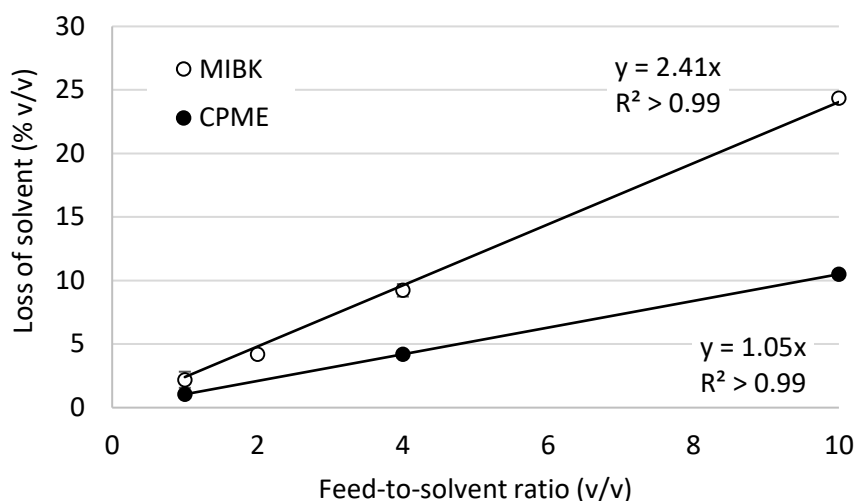


Figure 10 : Effect of the feed-to-solvent ratio on the loss of solvent when extracting sinapic acid from a hydrolysate with the membrane contactor

$pH\ 4$, $25\ ^\circ C$, $C_{0(aq)} = 230 \pm 36\ mg/L$, feed in the lumen side of the fibers.

Figure 10 shows that the loss of the solvents in the hydrolysate increases linearly between the feed-to-solvent ratio 1:1 to 10:1 (v/v) with MIBK and CPME (slopes of 2.41 and 1.05 respectively). Thus, the loss of solvent is 2.5 times higher with MIBK than with CPME. This is due to the higher solubility of MIBK in water ($2.6 \pm 0.5\ \%\ v/v$) than CPME in water ($1.07 \pm 0.05\ \%$). Therefore, an increase of the feed-to-solvent ratio increases the volume of hydrolysate in which the solvent can solubilize, leading to higher losses of solvent. The losses of solvents represent 11% and 24% (v/v) at a phase ratio of 10:1 (v/v) for CPME and MIBK, respectively. The difficulties to recover the solvents lost in the hydrolysate may have a significant impact on the costs of the process. Therefore, the choice in selecting the best solvent between MIBK and CPME would require a techno-economic evaluation and a life cycle analysis.

The purity of sinapic acid could be calculated after evaporation of the solvents. The purities obtained were $29.4 \pm 0.4\ \%\ (w/w)$ with CPME and $33 \pm 8\ \%\ (w/w)$ with MIBK. Therefore, the use of CPME or MIBK resulted in equivalent purities ($p > 0.05$). It is important to mention that further investigations in the case of CPME showed that benzoic acid and sorbic acid, coming from the enzymatic cocktail implemented during the production of the mustard bran hydrolysate, were the main impurities ($36.1 \pm 0.6\ \%$ and $12.8 \pm 0.4\ \%\ w/w$, respectively). Finally, the sinapic acid purity in the initial hydrolysate dry

matter was 0.4 ± 0.1 % (w/w). Therefore, the use of the HFMC allowed the concentration of the sinapic acid by 70 in the solvent phase.

4. Conclusion

In this study, different solvents from the class of alcohols, esters, ethers and ketone were found efficient to extracted more than 80% (w/w) of sinapic acid from an aqueous phase for $\text{pH} < 5$. They were efficient for model aqueous solution of sinapic acid and mustard bran hydrolysate.

The implementation of a HFMC was found operational for the extraction of sinapic acid from the hydrolysate with either volatile and non-volatile solvents. The initial concentration of sinapic acid in the feed phase was found not limiting the extraction efficiency or speed, thus allowing its concentration in the solvent.

The volatile solvents tested with HFMC were proportionally lost in the aqueous feed phase by the increase of the feed-to-solvent ratio, leading to increasing cost of the process. Also, the recovery yield of sinapic acid decreases with the increase of the feed-to-solvent ratio. Therefore, there is an optimal condition in order to obtain the highest recovery capacity of sinapic acid in the solvent. It was found at 8:1 (v/v) with CPME and above 10:1 (v/v) with MIBK.

The use of the HFMC effectively circumvented phase emulsions and facilitated a 70-fold increase in sinapic acid concentration within the solvent in contrast to its initial content within the hydrolysate. Consequently, LLE assisted by HFMC emerges as a straightforward and scalable technological approach for generating commercially viable bio-based sinapic acid from *Brassicaceae* by-products.

The purity of the obtained extracts is influenced to a greater extent by the impurities inherent in the commercial enzymatic cocktails and additional substances found in mustard bran. These substances arise from the mustard manufacturing process. In order to improve the extract's purity, further efforts are required to address these factors.

5. Acknowledgements

This work was supported by Extractis (Amiens, France). The Region Grand Est, the Conseil Départemental de la Marne and the Grand Reims are thanked for their financial support, as well as Charbonneaux-Brabant for providing the mustard bran. We express our gratitude to Dr. Marwen Moussa and Prof. Violaine Athès from Université Paris-Saclay for their valuable assistance during this work and in the implementation of the membrane contactor set-up.

6. References

- [1] M.D. Horbury, E.L. Holt, L.M.M. Mouterde, P. Balaguer, J. Cebrián, L. Blasco, F. Allais, V.G. Stavros, Towards symmetry driven and nature inspired UV filter design, *Nature Communications*. 10 (2019). <https://doi.org/10.1038/s41467-019-12719-z>.
- [2] F. Diot-Néant, L. Migeot, L. Hollande, F.A. Reano, S. Domenek, F. Allais, Biocatalytic Synthesis and Polymerization via ROMP of New Biobased Phenolic Monomers: A Greener Process toward Sustainable Antioxidant Polymers, *Front. Chem.* 5 (2017) 126. <https://doi.org/10.3389/fchem.2017.00126>.
- [3] N. Nićiforović, H. Abramović, Sinapic Acid and Its Derivatives: Natural Sources and Bioactivity, *Comprehensive Reviews in Food Science and Food Safety*. 13 (2014) 34–51. <https://doi.org/10.1111/1541-4337.12041>.
- [4] L.M.M. Mouterde, F. Allais, Microwave-Assisted Knoevenagel-Doebner Reaction: An Efficient Method for Naturally Occurring Phenolic Acids Synthesis, *Frontiers in Chemistry*. 6 (2018). <https://doi.org/10.3389/fchem.2018.00426>.
- [5] C. Peyrot, A.A.M. Peru, L.M.M. Mouterde, F. Allais, Proline-Mediated Knoevenagel–Doebner Condensation in Ethanol: A Sustainable Access to p-Hydroxycinnamic Acids, *ACS Sustainable Chemistry & Engineering*. 7 (2019) 9422–9427. <https://doi.org/10.1021/acssuschemeng.9b00624>.
- [6] J. van Schijndel, L.A. Canalle, J. Smid, J. Meuldijk, Conversion of Syringaldehyde to Sinapinic Acid through Knoevenagel-Doebner Condensation, *Open Journal of Physical Chemistry*. 06 (2016) 101–108. <https://doi.org/10.4236/ojpc.2016.64010>.
- [7] S. Sinichi, A.V.L. Siañez, L.L. Diosady, Recovery of phenolic compounds from the by-products of yellow mustard protein isolation, *Food Research International*. 115 (2019) 460–466. <https://doi.org/10.1016/j.foodres.2018.10.047>.
- [8] E. Odinot, F. Fine, J.-C. Sigoillot, D. Navarro, O. Laguna, A. Bisotto, C. Peyronnet, C. Ginies, J. Lecomte, C.B. Faulds, A. Lomascolo, A Two-Step Bioconversion Process for Canolol Production from Rapeseed Meal Combining an *Aspergillus niger* Feruloyl Esterase and the Fungus *Neolentinus lepideus*, *Microorganisms*. 5 (2017) 67. <https://doi.org/10.3390/microorganisms5040067>.
- [9] M. Chadni, N. Boussetta, C. Guerin, F. Lagalle, A. Zoghalmi, P. Perré, F. Allais, N. Grimi, I. Ioannou, Improvement of Sinapine Extraction from Mustard Seed Meal by Application of Emerging Technologies, *Foods*. 12 (2023) 520. <https://doi.org/10.3390/foods12030520>.
- [10] M. Chadni, A.L. Flourat, V. Reungoat, L.M.M. Mouterde, F. Allais, I. Ioannou, Selective Extraction of Sinapic Acid Derivatives from Mustard Seed Meal by Acting on pH: Toward a High Antioxidant Activity Rich Extract, *Molecules*. 26 (2021) 212. <https://doi.org/10.3390/molecules26010212>.
- [11] V. Reungoat, L.M.M. Mouterde, M. Chadni, J. Couvreur, E. Isidore, F. Allais, H. Ducatel, I. Ioannou, Simultaneous extraction and enzymatic hydrolysis of mustard bran for the recovery of sinapic acid, *Food and Bioproducts Processing*. 130 (2021) 68–78. <https://doi.org/10.1016/j.fbp.2021.09.003>.
- [12] V. Reungoat, F. Allais, H. Ducatel, I. Ioannou, Extraction and Purification Processes of Sinapic Acid Derivatives from Rapeseed and Mustard Seed By-Products, *Separation & Purification Reviews*. (2021). <https://doi.org/10.1080/15422119.2021.2004550>.
- [13] N. Prapakornwiriya, L.L. Diosady, Recovery of sinapic acid from a waste stream in the processing of yellow mustard protein isolate, *Journal of Food Process Engineering*. 31 (2008) 173–185. <https://doi.org/10.1111/j.1745-4530.2007.00146.x>.
- [14] S. Sinichi, A.V.L. Siañez, L.L. Diosady, Recovery of phenolic compounds from the by-products of yellow mustard protein isolation, *Food Research International*. 115 (2019) 460–466. <https://doi.org/10.1016/j.foodres.2018.10.047>.
- [15] E. Odinot, F. Fine, J.-C. Sigoillot, D. Navarro, O. Laguna, A. Bisotto, C. Peyronnet, C. Ginies, J. Lecomte, C. Faulds, A. Lomascolo, A Two-Step Bioconversion Process for Canolol Production from Rapeseed Meal Combining an *Aspergillus niger* Feruloyl Esterase and the Fungus *Neolentinus lepideus*, *Microorganisms*. 5 (2017) 67. <https://doi.org/10.3390/microorganisms5040067>.

- [16] A. Thiel, K. Muffler, N. Tippkötter, K. Suck, U. Sohling, S.M. Hruschka, R. Ulber, A novel integrated downstream processing approach to recover sinapic acid, phytic acid and proteins from rapeseed meal: A novel downstream processing approach for rapeseed meal, *Journal of Chemical Technology & Biotechnology*. 90 (2015) 1999–2006. <https://doi.org/10.1002/jctb.4664>.
- [17] R. Cañadas, M. González-Miquel, E.J. González, I. Díaz, M. Rodríguez, Evaluation of bio-based solvents for phenolic acids extraction from aqueous matrices, *Journal of Molecular Liquids*. 338 (2021) 116930. <https://doi.org/10.1016/j.molliq.2021.116930>.
- [18] J. Combes, E. Clavijo Rivera, T. Clément, C. Fojcik, V. Athès, M. Moussa, F. Allais, Solvent selection strategy for an ISPR (In Situ/In stream product recovery) process: The case of microbial production of p-coumaric acid coupled with a liquid-liquid extraction, *Separation and Purification Technology*. 259 (2021) 118170. <https://doi.org/10.1016/j.seppur.2020.118170>.
- [19] K.L. Morley, S. Grosse, H. Leisch, P.C.K. Lau, Antioxidant canolol production from a renewable feedstock via an engineered decarboxylase, *Green Chem.* 15 (2013) 3312. <https://doi.org/10.1039/c3gc40748a>.
- [20] S. Mohanty, Modeling of liquid-liquid extraction column: a review, *Reviews in Chemical Engineering*. 16 (2000). <https://doi.org/10.1515/REVCE.2000.16.3.199>.
- [21] M. Chadni, M. Moussa, V. Athès, F. Allais, I. Ioannou, Membrane contactors-assisted liquid-liquid extraction of biomolecules from biorefinery liquid streams: A case study on organic acids, *Separation and Purification Technology*. 317 (2023) 123927. <https://doi.org/10.1016/j.seppur.2023.123927>.
- [22] A. Gabelman, S.-T. Hwang, Hollow fiber membrane contactors, *Journal of Membrane Science*. 159 (1999) 61–106. [https://doi.org/10.1016/S0376-7388\(99\)00040-X](https://doi.org/10.1016/S0376-7388(99)00040-X).
- [23] G.K. Agrahari, N. Verma, P.K. Bhattacharya, Removal of Benzoic Acid from Water by Reactive Extraction Using Hollow Fiber Membrane Contactor: Experiment and Modeling, *CLEAN – Soil, Air, Water*. 42 (2014) 901–908. <https://doi.org/10.1002/clen.201300769>.
- [24] M.T.A. Reis, O.M.F. Freitas, S. Agarwal, L.M. Ferreira, M.R.C. Ismael, R. Machado, J.M.R. Carvalho, Removal of phenols from aqueous solutions by emulsion liquid membranes, *Journal of Hazardous Materials*. 192 (2011) 986–994. <https://doi.org/10.1016/j.jhazmat.2011.05.092>.
- [25] L. Sciubba, D. Di Gioia, F. Fava, C. Gostoli, Membrane-based solvent extraction of vanillin in hollow fiber contactors, *Desalination*. 241 (2009) 357–364. <https://doi.org/10.1016/j.desal.2007.10.104>.
- [26] M. Janvier, L. Hollande, A.S. Jaufurally, M. Pernes, R. Ménard, M. Grimaldi, J. Beaugrand, P. Balaguer, P.-H. Ducrot, F. Allais, Syringaresinol: A Renewable and Safer Alternative to Bisphenol A for Epoxy-Amine Resins, *ChemSusChem*. 10 (2017) 738–746. <https://doi.org/10.1002/cssc.201601595>.
- [27] C.M. Alder, J.D. Hayler, R.K. Henderson, A.M. Redman, L. Shukla, L.E. Shuster, H.F. Sneddon, Updating and further expanding GSK's solvent sustainability guide, *Green Chemistry*. 18 (2016) 3879–3890. <https://doi.org/10.1039/C6GC00611F>.
- [28] PubChem, PubChem, PubChem. (2021). <https://pubchem.ncbi.nlm.nih.gov/> (accessed September 12, 2021).
- [29] A. Berthod, S. Carda-Broch, Determination of liquid–liquid partition coefficients by separation methods, *Journal of Chromatography A*. 1037 (2004) 3–14. <https://doi.org/10.1016/j.chroma.2004.01.001>.
- [30] V. Reungoat, M. Gaudin, A.L. Flourat, E. Isidore, L.M.M. Mouterde, F. Allais, H. Ducatel, I. Ioannou, Optimization of an ethanol/water-based sinapine extraction from mustard bran using Response Surface Methodology, *Food and Bioproducts Processing*. 122 (2020) 322–331. <https://doi.org/10.1016/j.fbp.2020.06.001>.
- [31] A. Dupuy, Stabilisation de l'interface liquide-liquide dans un contacteur membranaire: Application à l'extraction sélective de terpènes oxygénés d'huile essentielle d'agrumes, 2010.
- [32] L. Dahuron, E.L. Cussler, Protein extractions with hollow fibers, *AIChE Journal*. 34 (1988) 130–136. <https://doi.org/10.1002/aic.690340115>.
- [33] R. Stephenson, J. Stuart, M. Tabak, Mutual solubility of water and aliphatic alcohols, *J. Chem. Eng. Data*. 29 (1984) 287–290. <https://doi.org/10.1021/je00037a019>.

- [34] C.M. Galanakis, V. Goulas, S. Tsakona, G.A. Manganaris, V. Gekas, A Knowledge Base for The Recovery of Natural Phenols with Different Solvents, *International Journal of Food Properties*. 16 (2013) 382–396. <https://doi.org/10.1080/10942912.2010.522750>.
- [35] B. Smyk, R. Drabent, Spectroscopic investigation of the equilibria of the ionic forms of sinapic acid, *The Analyst*. 114 (1989) 723. <https://doi.org/10.1039/an9891400723>.
- [36] R. Cañadas, M. González-Miquel, E.J. González, A. Núñez de Prado, I. Díaz, M. Rodríguez, Sustainable Recovery of High Added-Value Vanilla Compounds from Wastewater Using Green Solvents, *ACS Sustainable Chem. Eng.* 9 (2021) 4850–4862. <https://doi.org/10.1021/acssuschemeng.1c00168>.
- [37] K. Watanabe, N. Yamagiwa, Y. Torisawa, Cyclopentyl Methyl Ether as a New and Alternative Process Solvent, *Org. Process Res. Dev.* 11 (2007) 251–258. <https://doi.org/10.1021/op0680136>.
- [38] Hiromitsu. Kanai, Veronica. Inouye, Reginald. Goo, Helen. Wakatsuki, Solubility of 4-methyl-2-pentanone in aqueous phase of various salt concentrations, *Anal. Chem.* 51 (1979) 1019–1021. <https://doi.org/10.1021/ac50043a055>.
- [39] R. Stephenson, J. Stuart, Mutual binary solubilities: water-alcohols and water-esters, *J. Chem. Eng. Data*. 31 (1986) 56–70. <https://doi.org/10.1021/je00043a019>.
- [40] R.M. Stephenson, Mutual solubilities: water-ketones, water-ethers, and water-gasoline-alcohols, *J. Chem. Eng. Data*. 37 (1992) 80–95. <https://doi.org/10.1021/je00005a024>.
- [41] F. Chemarin, M. Moussa, F. Allais, I.C. Trelea, V. Athès, Recovery of 3-hydroxypropionic acid from organic phases after reactive extraction with amines in an alcohol-type solvent, *Separation and Purification Technology*. 219 (2019) 260–267. <https://doi.org/10.1016/j.seppur.2019.02.026>.