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# Extraction processes of high added value molecules from wood waste: overview and comparison of processes for an eco-friendly recovery from paper industry by-product

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## ABSTRACT

The work aims to optimize an industrial greener extraction process of chemical components in wood waste from the paper industry. Extracts from a mixture of two species (*Picea abies* and *Abies alba*) were studied. Several parameters were found to play a key role in the extraction yield: to the conventional parameters such as the chemical composition of the extracting medium or the temperature, a key criterion was the choice of a process procedure in order to take into account the absorption of the solvent by the wood. The implementation of several operating modes, namely solid-liquid single-batch extraction, countercurrent extraction, percolation extraction, near supercritical, and supercritical fluid extraction is discussed in terms of yield and ease of implementation when scaling up.

## 1. Introduction

The paper industry annually produces considerable amounts of by-products, such as residues, bark, and knots, that are mostly used for the production of energy by combustion (Domingues et al., 2010). These by-products are a very rich source of high-value compounds such as phytosterols, oligolignans, lignans, stilbenes, flavonoids, and other phenolic compounds (Fernandes and Cabral, 2007; Willför et al., 2003a, 2005; Pietarinen et al., 2006; Holmbom et al., 2003). These substances can be extracted before combustion and valorized in the pharmaceutical and cosmetic industry (Patinha et al., 2013).

The wood's chemical composition depends on several parameters (Holmbom et al., 2003; Teacă, 2021; Wodzicki, 2001), such as sunlight, ground, altitude, age, ambient temperature, air pollution, tree species, location of wood in the tree, etc. The cell wall is generally constituted over 95% of its dry mass, of three main components: cellulose, hemicellulose, and lignin.

The complementary fraction (about 5% of the dry mass) includes low molecular weight compounds sometimes called "accidental" or "strange" wood materials. These components are classified into inorganic materials (ashes) ranging from 0.3% to 1% of the dry matter and organic (extractives), representing between 4% and 10% of the dry mass (Holmbom et al., 2003).

Extractives can have a significant influence on the wood properties (Holmbom et al., 2003; Kebbi-Benkeder et al., 2015a; Valette et al., 2017), such as growth, development, pigmentation, hygroscopic, odor, chemical defense against attacks by pathogens (bacteria, fungi, and termites), fixation of atmospheric dinitrogen by leguminous plants and plant reproduction (plant-insect interaction leading to pollination). Extractives can be classified into three categories: terpenes, the group represented by volatile substances responsible for the odor characteristic; aliphatic compounds and phenolic compounds with certain compounds of this group identified as lignin precursors.

In the 30 s, Häggglund and Larsson (1936) and Wegelius (1940) (Holmbom et al., 2003) found a large number of extractives present in the knots and, in 1966, Boutelje determined the amount present in the branches and the knots in several trees' species (Holmbom et al., 2003). However, most of the studies were interrupted for several years until 1998 when Willför et al (Willför et al., 2003a; Pietarinen et al., 2006; Holmbom et al., 2003; Willför et al., 2005), were again interested in this area. They studied the distribution of lignans in several species and discovered that the number of extractives in knots was higher than in the other parts of the tree, ranging from 10% to 20% of the dry weight of most trees. The knots of the *Picea*, *Abies*, and *Larix* families consist mainly of lignans and oligolignans. For example, Norway spruce (Family: Pinaceae, genus: *Picea*, species: *P. abies*, binomial name: *Picea abies* (L.) H.

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Karst) is extremely rich in lignans (6–24% dry weight) including  $\alpha$ -conidendrin, conidendric acid, lariciresinol (Lari), secoisolariciresinol (Seco), pinosresinol (Pino), matairesinol (MR), isolariciresinol, liovile, picearesinol, and especially hydroxymatairesinol (HMR), the most abundant lignan ranging from 65% to 85% of the dry weight.

HMR can be found in two isomeric forms differing in stereochemistry at C7. The major isomer is (7 S,8 R,8' R)-(-)-7-hydroxymatairesinol (HMR2) while the minor is (7 R,8 R,8' R)-(-)-allohydroxymatairesinol (HMR1), with an HMR1/HMR2 ratio of approximately 1/3 (Spilioti et al., 2014). It has also been found that HMR has anti-tumor activity (Heinonen et al., 2001; Saarinen et al., 2000, 2005; Miura et al., 2007; Katsuda et al., 2004; Oikarinen et al., 2000), a strong anti-inflammatory property in endothelial cells (Spilioti et al., 2014), antioxidant properties (Saarinen et al., 2000; Kangas et al., 2002), mild pro-estrogenic activity (Cosentino et al., 2007), and it is an excellent raw material for semi-synthesis of other important lignans (Holmbom et al., 2003). Furthermore, HMR is photosensitive and can be oxidated to allohydroxymatairesinol,  $\alpha$ -conidendrin, allo-7'-methoxymatairesinol, 7'-methoxymatairesinol, oxomatairesinol, vanillin in the presence of light (Kawamura et al., 1998).

The extraction of these molecules was proven to have an industrial importance. Faced with the need to use environmentally friendly extraction methods (non-toxic, bio-renewable), a particular interest has focused on extraction with supercritical fluids (SC). Carbon dioxide (CO<sub>2</sub>) is the most widely used solvent because it has a low critical point (P<sub>C</sub> = 73.8 bar; T<sub>C</sub> = 304.1 K) preventing degradation of thermosensitive compounds and reactions activated by temperature (Naik et al., 2010; Rozzi et al., 2002); it is neither toxic nor flammable (Kawamura et al., 1998; Rozzi et al., 2002); it has a low cost with good physical properties (high diffusivity, low viscosity, low surface tension) compared to conventional solvents (Ismadji et al., 2012; Jia et al., 2009); it can easily be separated from the extract due to its extreme volatility, which allows obtaining a final product completely free of solvent (Reverchon and De Marco, 2006; Costa et al., 2012). Furthermore, to promote the extraction of a target compound from a matrix containing many compounds, it is possible to control the physicochemical properties of the supercritical fluid (via pressure-temperature control), and thus solubilize one or even a family of molecules (Reverchon and De Marco, 2006). This can reduce or even avoid additional separation steps, which reduces the cost of the final product.

Carbon dioxide is widely used in the food, pharmaceutical, and cosmetic industries (Braga et al., 2008). It is ideal for the extraction of essential oils, lipophilic compounds, and slightly polar compounds. On the other side, CO<sub>2</sub> is less suitable in the case of compounds of high molecular weight or very polar molecules because of their low solubility in carbon dioxide. In this case, it is recommended to use modifiers (solvents) that are dissolved in the carbon dioxide, increasing its polarity and allowing the extraction of a greater range of compounds (Conde et al., 2013; Lang and Wai, 2001).

Patinha et al (Patinha et al., 2013). studied the lipophilic compounds extracted by supercritical extraction from the bark of *Eucalyptus grandis x globulus* (hybrid form). They found a significant amount of triterpenoids, being the most abundant compound the methyl morolate. Seabra et al (Seabra et al., 2012). studied the supercritical extraction from the bark of *Pinus pinaster Ait.* and observed that the most important outcome was the amount of ethanol relative to other operating parameters (temperature, pressure). Santos et al (Santos et al., 2012). were interested in phenolic compounds present in the bark of *Eucalyptus globulus*. Their study confirmed a better extraction of phenolic compounds using supercritical CO<sub>2</sub> with ethanol as a modifier compared to pure SC-CO<sub>2</sub> or in the presence of other modifiers (water or ethyl acetate). Furthermore, the amount of ethanol and the extraction temperature have a strong influence on the extraction yield and antioxidant activity of the extract.

The objective of this work is to validate, optimize, and analyze environmentally friendly (non-toxic and renewable) extraction methods of compounds present in wood waste to be implemented on an industrial

scale. The family of interesting compounds is lignans and particularly hydroxymatairesinol. Several operating parameters will be tested to study their impact on the profile of biomass extracts. For that, this work aims to compare:

- Processes that implement fluids in different physical states: liquid, near supercritical, supercritical.
- The implementation of several processes: single batch, a cascade of reactors, column, and near supercritical/supercritical extraction.

## 2. Materials and methods

### 2.1. Material and chemicals

A mixture of knots of 60% Norway spruce (*Picea abies*) and 40% silver fir (Family: *Pinaceae*, genus: *Abies*, species: *A. alba*, binomial name: *Abies alba* Mill.) was provided by the company Norske Skog Golbey (Golbey, France) (Patent EP, 2018) and stored in the refrigerator (5 °C). The initial water content in the material was 11% by weight of the wet mass.

Extractions were performed using carbon dioxide (CO<sub>2</sub>, 99.99 wt%, Messer, France) and ethanol absolute anhydrous (EtOH, 99.9 wt%, Carlo Erba, France). Liquid chromatography was performed using ethanol anhydrous HPLC grade (99.95 wt%, Carlo Erba, France).

Hydroxymatairesinol ( $\geq 95\%$ ), secoisolariciresinol ( $\geq 95\%$ ), and lariciresinol ( $\geq 95\%$ ) standards were purchased in Arbo Nova (Turku, Finland). Isolariciresinol ( $\geq 95.0\%$ ), coniferyl alcohol (98%), nortrachelogenin ( $\geq 95.0\%$ ), guaiaicol (98.0%),  $\alpha$ -conidendrin ( $\geq 95.0\%$ ), pinosresinol ( $\geq 95.0\%$ ), matairesinol ( $\geq 85.0\%$ ), enterodiol ( $\geq 95.0\%$ ) and enterolactone ( $\geq 95.0\%$ ) were purchased from Sigma Aldrich (Schnellendorf, Germany).

### 2.2. Solid-liquid extraction

This paper will study the following solid-liquid extractions:

- Soxhlet: to provide the composition of extractive compounds present in the solid material, its results were used as a reference for the following extractions.
- Single batch: extraction method focusing on the optimization of solvent composition and temperature.
- Simulated countercurrent extraction: series reactor with a countercurrent circulation of the solvent.
- Percolation (column extraction): optimized extraction method to avoid the downstream process.

#### 2.2.1. Soxhlet

Ethanol and acetone (Kebbi-Benkeder et al., 2015b; Brennan et al., 2021; Gérardin et al., 2023) are widely used solvents in the Soxhlet extraction process and, in the case of wood extraction, these solvents have the advantage that their boiling temperature is below the degradation temperature of most lignans. However, thermal degradation may occur depending on the interactions between the compounds present in the starting material (Gerstenmeyer et al., 2013), associated with high temperature and long extraction time (Zhang et al., 2018).

The Soxhlet extraction was performed using 600 mL of ethanol (T<sub>b</sub> = 78 °C) or acetone (T<sub>b</sub> = 56 °C) in contact with 50 g of wood using a porous cartridge (Whatman/GE Healthcare 603 Standard Cellulose, 48 × 200 mm, thickness 2.0 mm) for 8 h. This methodology was applied to limited extraction duration to avoid degradation of extractables.

#### 2.2.2. Single batch

Single batch extraction was carried out using a water-ethanol solvent mixture to have a greener process as it is considered an eco-friendly solvent mixture and is widely used as an extraction process in the

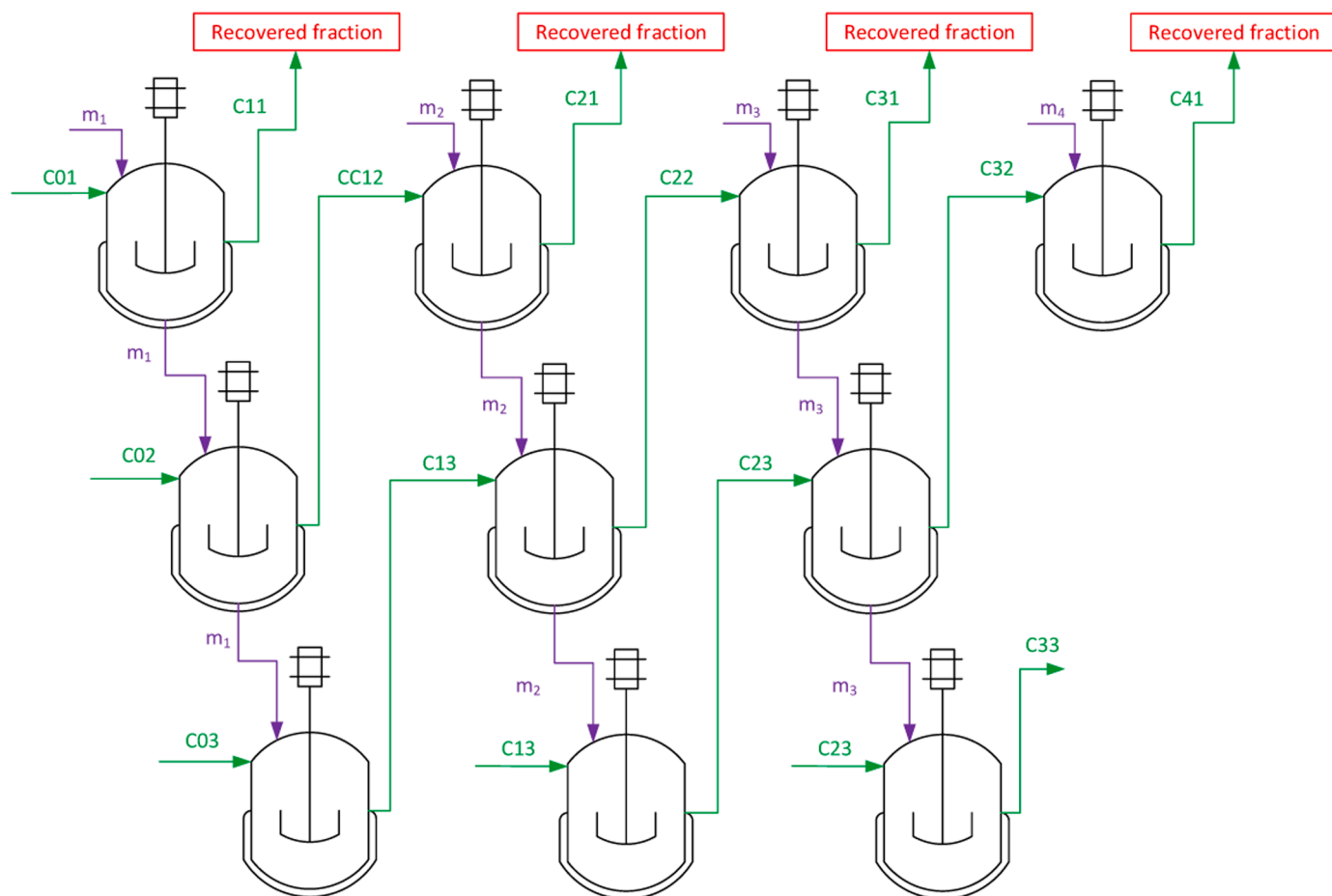


Fig. 1. Schematic illustration of the simulated countercurrent process.

pharmaceutical industry. Moreover, a water-ethanol mixture was selected as the extraction solvent due to its capability to extract a wide range of molecules from the wood material, encompassing both polar and less polar compounds. A preliminary study was conducted on another batch of nodes by altering the water: ethanol volumic ratio from 0:100–100:0, with an increment of 25. The best results for yield and lignans were observed in the 0:20 zone. The proportions of ethanol in this area have been varied due to this reason. Single batch extractions were carried out using Polytest 20 equipment (Bioblock Scientific), offering precise temperature control and gentle vortex agitation at 80 rpm. The volume of the bottles was adjusted to the equipment's 100 mL capacity. A solvent volume of 40 mL was selected to prevent any overflow. Initially, 1 g of sample was placed in 40 mL of solvent to ensure complete exhaustion of the material. Subsequently, the sample quantity was incrementally increased until a reduction in extraction yield was noted. To mitigate energy costs and prevent product degradation, the effect of temperature was investigated within the range of 30–60 °C, recognizing that temperature elevation could enhance specific physical phenomena such as solubility and viscosity.

### 2.2.3. Simulated countercurrent

The impact of successive extraction followed by a washing step using the same wood material was studied to recover a higher amount of extractables as discussed previously. Moreover, the effects of scaling up the process were investigated, whereas counter-current extraction was conducted using 50 g of wood material and 500 mL of solvent. To minimize solvent consumption during the washing steps, the developed methodology utilizes the recycling of rinsing solvent for subsequent extraction stages. As the experiment was conducted with a single

reactor, Fig. 1 illustrates a method for simulating countercurrent extraction using three reactors with a time-based sequence organization.

The first step corresponds to a solid-liquid extraction performed by mixing 50 g of wood ( $m_1$ ) with 500 mL of EtOH/Water (15:85, v/v) solvent at 40 °C for 24 h. The extract fraction (CC11) is recovered and stored.

The wood,  $m_1$ , from the first step, is washed with 500 mL of EtOH/Water (15:85, v/v) solvent at 40 °C for 1 h. The fraction CC12 is then recovered and stored. The washing step is repeated twice more and the CC13 and CC14 are recovered and stored.

The second step is therefore carried out by mixing 50 g of wood ( $m_2$ ) with the CC12 fraction, i.e., with the fraction obtained by the first washing of the wood  $m_1$ . After 24 h, the C21 fraction is recovered and stored. The final volume corresponded to the initial volume (500 mL). The wood,  $m_2$ , is then put in contact with the fraction C13 obtained by the third extraction/washing of the wood  $m_1$  of the first step. The next extractions and washes are carried out in the same way.

### 2.2.4. Percolation extraction

Percolation is a flow extraction process in which fresh solvent percolates through the material (here it is a wood sample) at a controlled rate with the continuous addition of fresh solvent. The diffusion is therefore improved by increasing the concentration gradient of the extractives from the plant material and the fresh solvent.

In conclusion, the purpose of this method is to reduce the total volume of solvent needed to perform the extraction, to reduce the extraction duration, and to simplify the unitary operations of downstream processing of the extract, e.g. centrifugation, filtration, and press.

Percolation was performed using a tubular column of dimension

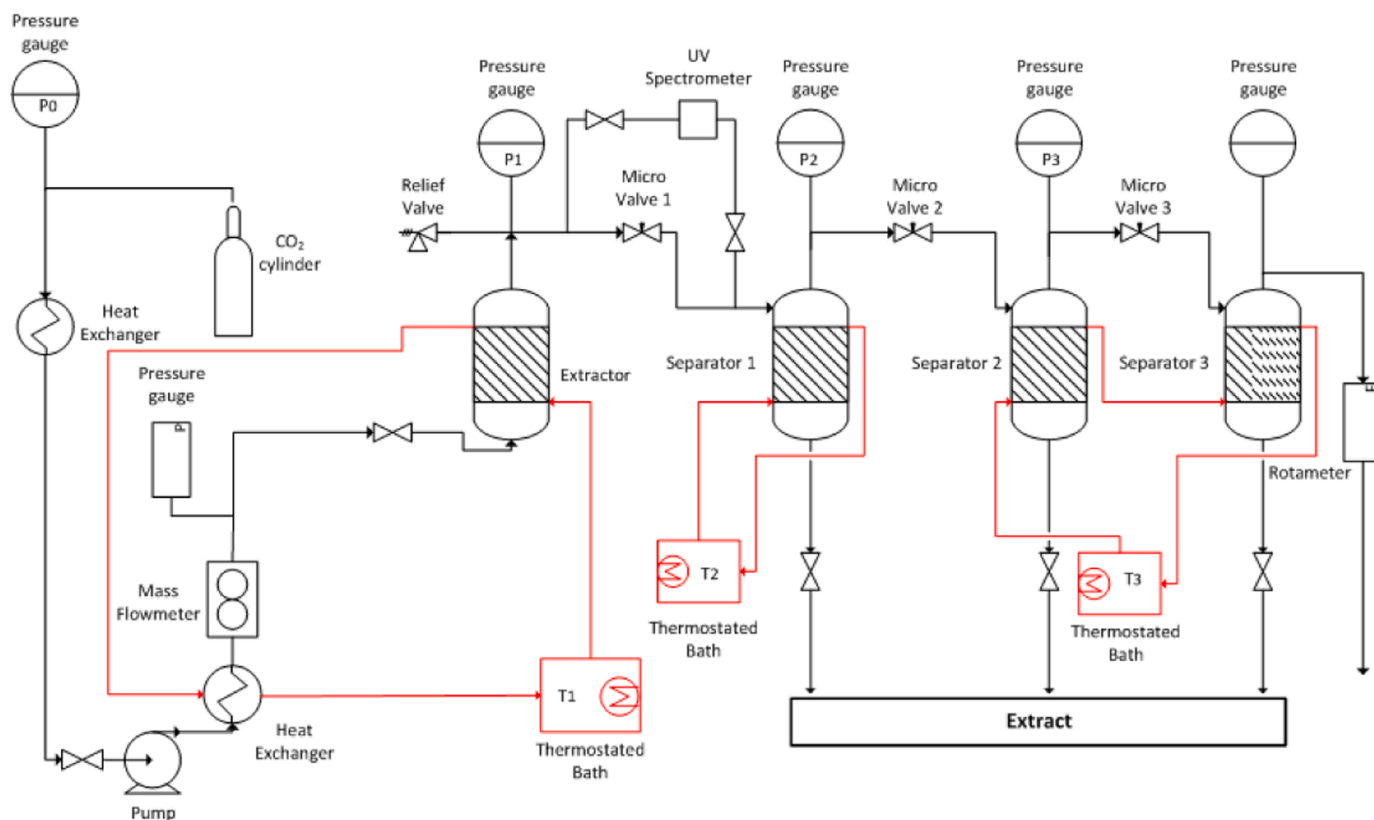


Fig. 2. Schematic drawing of the supercritical fluid extraction equipment.

20×120 mm ( $v=38$  mL) designed at our laboratory. This column is connected to a MasterFlex - Easyload 2 pump (Bioblock Scientific) using a Masterflex precision tube 96400–16. The column is then filled with the solid material (wood) and each end is closed with a sintered material allowing the passage of solvent (room temperature) and retaining the solid material.

Percolation extraction was performed at room ambient (25 °C), due to equipment limitation, with 6 g of wood material using EtOH/Water (15:85,  $v/v$ ) solvent. The flow rate was fixed at 10 mL/min and the extract was then divided into six fractions of 50 mL (5 minutes/fraction) for the study of the extraction kinetics.

### 2.3. Near supercritical and supercritical fluid extraction

Near and supercritical extraction methods have several advantages. These include (Ismadji et al., 2012; Jia et al., 2009; Reverchon and De Marco, 2006; Costa et al., 2012; Braga et al., 2008; Conde et al., 2013) the use of a fluid ( $\text{CO}_2$ ) with low viscosity and high diffusivity, low extraction temperature which reduces degradation reactions, the possibility of recycling the supercritical fluid, and increased antioxidant activity compared to traditional solid-liquid extraction methods.

Similar to the percolation process, near and supercritical extraction is a flow extraction method in which a fluid passes through the material containing the desired compounds and carries the extracted compounds to the separator(s). However, in contrast to percolation, the extract is completely separated from the fluid by decreasing its solvent power. The extract is precipitated and then recovered in a pure form at the separator outlet. In the case of wood extraction, the supercritical fluid is an alternative that can avoid the problems encountered in solid-liquid extraction, especially in terms of solvent recovery.

Supercritical extraction was performed using the experimental apparatus shown in Fig. 2.

Initially, the carbon dioxide (50 and 60 bar at room temperature) is

cooled using a heat exchanger to reach the temperature of 1 °C, which allows its liquefaction. A metal diaphragm pump (Dosparo Milton Roy - MILROYAL D) is then used to bring the fluid to the required pressure. At the exit of the pump, a Coriolis flowmeter (Micromotion  $\pm 1$  g) is used for measuring and recording the instantaneous and cumulative flow. The mass flowmeter is used to measure the amount of  $\text{CO}_2$  introduced into the extractor, which corresponds to the number of moles of  $\text{CO}_2$  at time zero,  $n_{\text{CO}_2}$ , and also the quantity of  $\text{CO}_2$  needed for an experiment  $m_{\text{CO}_2}$ .

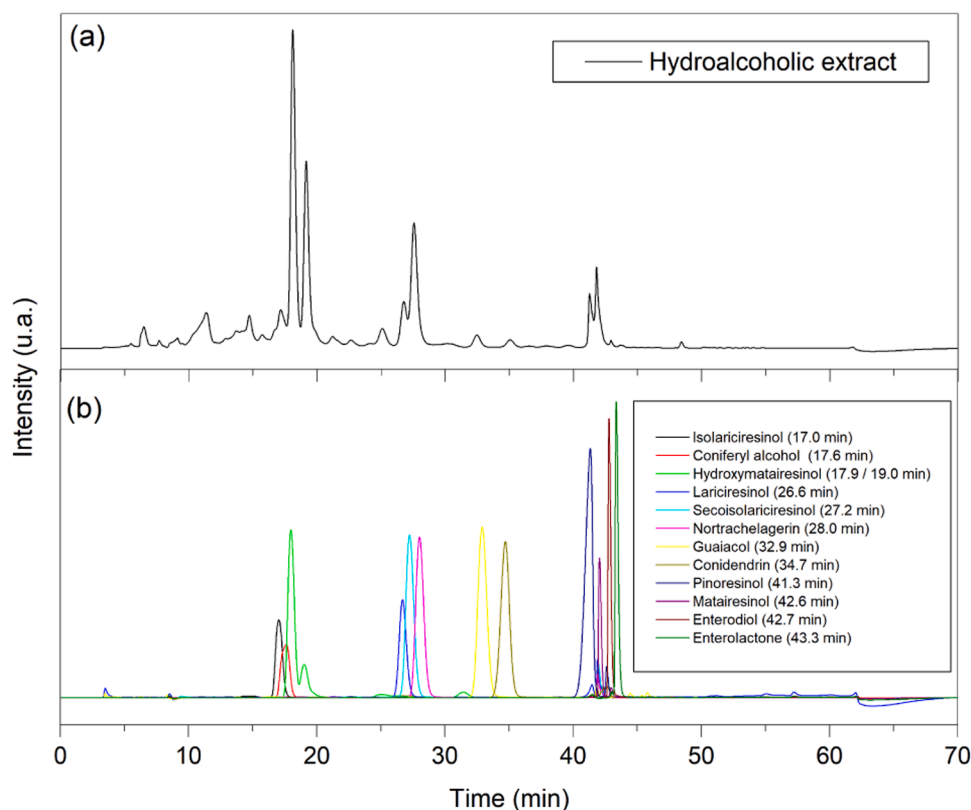
At the entrance of the extractor, a heat exchanger heats the fluid to pass to the supercritical state. Fluid – at the desired temperature and pressure – penetrates through the bottom of the extractor and is in contact with the material for a certain time called static contact time. Finally, it comes out at the top after the contact time has elapsed.

The stainless-steel cylindrical geometry extractor (300 × 23 mm – 125 mL) has a jacket connected to a thermostat (Huber). The solid material (wood) is disposed inside the extractor in a tube whose each end has a sintered material. The cosolvent is introduced directly into the extractor before starting the supercritical extraction. The tube is filled at its bottom with glass balls (diameter about 1.7 mm), which allows for increasing the contact surface between the fluid and the solid matrix and promotes a uniform distribution, avoiding the formation of preferential paths and agglomerations that can decrease the efficiency of the extraction.

At the outlet of the extractor, the fluid passes through three separators arranged in series, each comprising a jacket connected with a thermostat (Huber) – separators 2 and 3 are connected in the same bath – which allows individual temperature control. The extractor and separator are separated by micro-valves which allow control of the flow and evenly the local pressure in each system.

The cosolvent is introduced directly (mixed with the starting material) in the extractor before the start of the supercritical extraction to allow the extraction of compounds in a batch mode (static time),





**Fig. 3.** HPLC profile at  $\lambda = 280$  nm of (a) the extract after solid-liquid extraction. ( $V_{\text{solvent}} = 40$  mL,  $m_{\text{wood}} = 4$  g, 15:85, v/v, EtOH:water), (b) each of the compound standards.

followed by a flow to collect the extracted compounds diluted in ethanol.

Near supercritical and supercritical extraction was conducted at a temperature range of 20–60 °C, and 110–220 bar. This temperature and pressure range corresponds to a density ranging from 684.8 (110 bar, 40 °C) to 948.6 kg/m<sup>3</sup> (220 bar, 20 °C). The dynamic extraction duration ( $t_{\text{dynamic}}$ ) was 120 minutes, however, experimental data revealed that the extraction was finalized after 60 minutes. The minimum and maximum temperature was chosen to take into account the thermosensitive compounds and to keep the mixture in near supercritical conditions (liquid CO<sub>2</sub>). The maximum pressure was chosen due to equipment limitations ( $P_{\text{max}} = 250$  bar).

The extractive was recovered using three separators arranged in series at, respectively, 110 bar, 50 bar, and 10 bar if the extraction is conducted at 220 bar and 50 bar, 20 bar, and 10 bar if the extraction is conducted at 110 bar. The temperature of the separators is set at 20 °C to favor the precipitation. The extractive content was recovered only on the first two separators ( $m_{S1}$ ,  $m_{S2}$ ), and the yield presented below represents the overall yield considering the extract obtained in the two separators.

## 2.4. Analysis

### 2.4.1. Digital image analysis

Digital image analysis was performed using commercial software (Visilog 6). Several pictures (Sony XCD U100 CR) were taken using a microscope (Olympus, objective x0.5 and x1.5). The software treats the image and calculates the shape parameters of the wood material before extraction.

### 2.4.2. Sample preparation protocol

Following extraction and filtration, the solution undergoes dilution based on the extraction method employed to prevent detector

saturation:

- Soxhlet extraction samples: dilution of 1:4 in a 15% ethanol-water solution.
- SC-CO<sub>2</sub> extraction samples: dilution of 1:2 in a 15% ethanol-water solution.
- Liquid solid extraction samples: dilution of 1:1 in a 15% ethanol-water solution.

### 2.4.3. High-performance liquid chromatography

HPLC analysis (Fig. 3) was performed using a Shimadzu HPLC system (LC-20AD pump, SIL-20AC auto-injector, CTO-20A oven) equipped with a Photodiode Array detector (PDA) (SPD-M20A), a Kromasil® C18 HPLC column (5  $\mu\text{m}$  particle size, 100 Å pore size, length  $\times$  internal diameter 250  $\times$  4.6 mm) and a Kromasil® C18 guard column (5  $\mu\text{m}$  particle size, 100 Å pore size, length  $\times$  internal diameter 30  $\times$  4.6 mm). The columns were placed at 40 °C.

HPLC was performed with an injection volume (samples and standards) of 10  $\mu\text{L}$  and detection was carried out at 280 nm (wavelength with a satisfying sensitivity for the lignan group (Örså et al., 1997)). To minimize pressure loss, the pump flow rate was set at 0.3 mL/min. The mobile phase used was water (A) and ethanol (B). The gradient protocol was: 0–29 min, 25% B; 29–30 min, 25–60% B; 30–39 min, 60% B; 39–40 min, 60–90% B; 40–49 min, 90% B; 49–50 min, 90–25% B; 50–70 min, 25% B.

Fig. 3a shows a standard HPLC profile from an extract obtained by solid-liquid extraction ( $V_{\text{solvent}} = 40$  mL,  $m_{\text{wood}} = 4$  g, 15:85, v/v, EtOH:Water). The identification of compounds was obtained by comparing the retention time of the standards (Fig. 2b), such as isolariciresinol (17 minutes), coniferyl alcohol (17.6 minutes), hydroxymatairesinol epimers (17.9 and 19 minutes), lariciresinol (26.6 minutes), secoisolariciresinol (27.2 minutes), nortrachelogenin (28.0 minutes), guaiacol (32.9 minutes),  $\alpha$ -conidendrin (34.7 minutes), pinoresinol

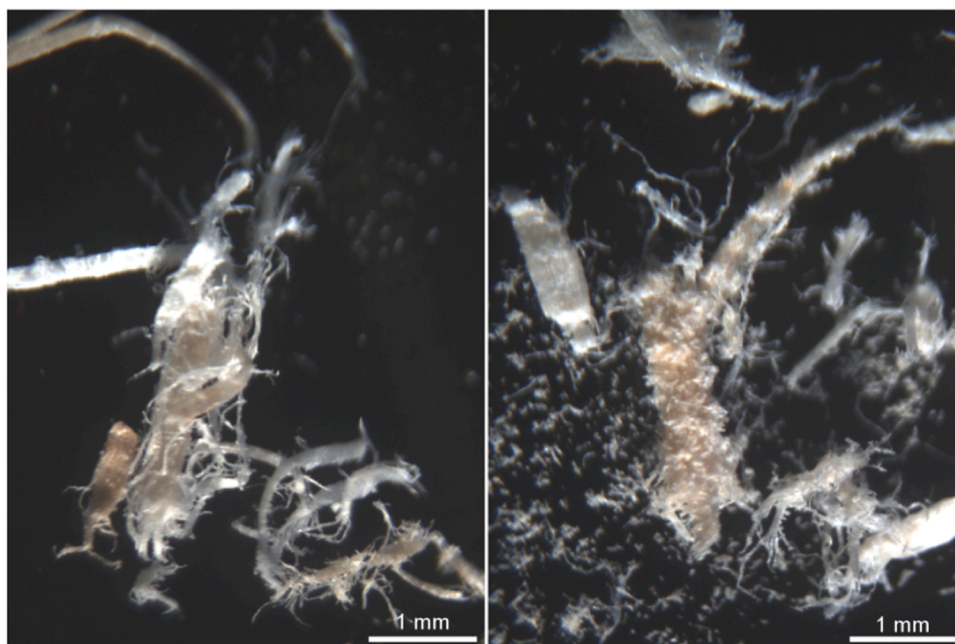


Fig. 4. Digital image of solid material (wood) before extraction (camera objective of x1.5).

(41.3 minutes), matairesinol (42.6 minutes), enterodiol (42.7 minutes) and enterolactone (43.3 minutes) (Supplementary Material, Table S1).

The content of target compounds (hydroxymatairesinol, secoisolariciresinol, and  $\alpha$ -conidendrin) was measured by HPLC-PDA and quantified by the method of external standards using calibration curves for each compound (Supplementary Material, Figures S1 to S4), expressed in g/100 g of dry wood. The standards were diluted in the solvent EtOH/H<sub>2</sub>O, 50/50 (v/v), and the concentrations of the sample extract were adapted according to the response of the detector. The % of lignans is derived from the areas under the peaks observed in the HPLC chromatogram, encompassing all compounds eluting after 17 minutes (which corresponds to the first identified lignan in the extract).

Some compounds such as coniferyl alcohol, pinoresinol, nortrachelogenin, matairesinol, and guaiacol were quantified by GC-MS because of their low concentration in the extract or co-elution with another compound.

#### 2.4.4. Gas chromatography

GC analysis was performed using a Shimadzu GC system (GC-2010) equipped with a mass spectrometer (GCMS-QP2010), a ZB-1 ms column (60 m x 0.25 mm, 0.25  $\mu$ m), at a helium flow rate of 1 mL/min and an injection volume of 1  $\mu$ L in split mode (1:15 and 1:100, T = 250 °C). The gradient temperature protocol was as follows: 70 °C to 300 °C at a rate of 15 °C/min, and hold for 20 min.

The content of target compounds (lariciresinol, coniferyl alcohol, and guaiacol diluted in ethanol, pinoresinol, nortrachelogenin, and matairesinol diluted in the solvent EtOH/H<sub>2</sub>O, 50/50 (v/v)) was measured by GC-FID and quantified by the method of external standards using calibration curves for each compound (Supplementary Material, Figures S9 to S14), expressed in g/100 g of dry wood. The concentrations of standards were adapted according to the response of the extracts.

An example of a GC-FID/MS chromatogram is shown in Figure S15, along with the mass spectra of 27 products.

Analysis of all extracts was carried out using HPLC and GC-MS.

#### 2.4.5. Extraction yield

The extraction yield was calculated from the dry mass of the residue obtained by drying 1 mL of the extract in an oven at 103 °C for 24 hours.

Subsequently, the dry residue was weighed to calculate the extraction yield.

### 3. Results and discussion

#### 3.1. Digital image analysis

Digital image analysis allows for observation of the wood fibers before extraction (Fig. 4). The initial water content in the material was 11% by weight of the wet mass.

Due to fiber orientation, the gridding allowed the obtaining of longitudinal particles and highly dispersed particle size. The average initial length is  $0.72 \pm 0.68$  mm, width is  $0.31 \pm 0.30$  mm and equivalent diameter is  $0.33 \pm 0.25$  mm.

#### 3.2. Liquid solid extraction

##### 3.2.1. Isolation of extracts from wood material

The final extract was separated from the wood by centrifugation. It was not efficient at removing all solid particles, so a porous medium was needed to filter out and hold particles larger than the surface pores. Filtration was conducted using filters with a pore size of 560  $\mu$ m, followed by a subsequent filtration step with filters having a pore size of 40  $\mu$ m. The resulting solution was opaque and had a dark yellow coloration.

To remove small particles still present in the liquid, three consecutive filtrations were performed under vacuum using Millipore hydrophilic nylon membrane filters (EMD Millipore, France). The filtration was performed sequentially at 5  $\mu$ m, 0.45  $\mu$ m, and 0.20  $\mu$ m to be able to remove all solid particles. The final solution was translucent and showed a light-yellow coloration.

The final amount of extractables was determined by accounting for the actual volume recovered after the extraction process.

An HPLC chromatogram for each extraction process is included in the Supplementary Material (Figures S5-S8, Figure S15).

##### 3.2.2. Soxhlet

After extraction and isolation, a recovery of 555 mL (ethanol) and 542 mL (acetone) of solvent was obtained, indicating that between 8%

**Table 1**

Content in target compounds (g/100 g of dry wood) for each solvent in Soxhlet.

| Solvent                              |                     | Ethanol | Acetone |
|--------------------------------------|---------------------|---------|---------|
| Total extractives                    | g/100 g of dry wood | 13.6    | 17.4    |
| Hydroxymatairesinol <sup>(1)</sup>   |                     | 4.7     | 6.3     |
| $\alpha$ -Conidendrin <sup>(1)</sup> |                     | 0.228   | 0.314   |
| Lariciresinol <sup>(2)</sup>         |                     | 0.334   | 0.321   |
| Matairesinol <sup>(2)</sup>          |                     | 0.320   | 0.270   |
| Pinoresinol <sup>(2)</sup>           |                     | 0.123   | 0.116   |
| Nortrachelogenin <sup>(2)</sup>      |                     | 0.055   | 0.073   |
| Guaiaicol <sup>(2)</sup>             |                     | 0.021   | 0.011   |
| Coniferyl alcohol <sup>(2)</sup>     |                     | 0.157   | 0.161   |
| Lignan (%) <sup>(3)</sup>            |                     | 75      | 75      |
| Solvent recovery rate (%)            |                     | 92      | 90      |

<sup>(1)</sup>HPLC, External quantification; <sup>(2)</sup> GC-MS/FID, external quantification; <sup>(3)</sup>HPLC, Peaks area %

and 10% of the initial solvent volume was retained in the wood material.

The total extraction yield was 13.6 g and 17.4 g/100 g of dry wood for ethanol and acetone, respectively. Hydroxymatairesinol is the major compound (in the form of two epimers) presented in the liquid extract with 34% for ethanol (Supplementary Material, Tables S3 to S5, Figures S16 and S17) and 36% for acetone extraction.

### 3.2.3. Single batch

Due to the numerous experimental parameters, we decided to maintain the volume of solvent at 40 mL for all experiments. The ethanol: water volume ratio was altered from 0:100–20:80 with a 5

**Table 2**Single batch extraction–  $V_{\text{solvent}} = 40$  mL.

| Ethanol (% v/v)                                | 0      | 5      | 10     | 15*    | 20     | 15     | 15     | 15     | 15     | 15     |
|--|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Temperature (°C)                               | 40     | 40     | 40     | 40     | 40     | 30     | 40     | 60     | 40     | 40     |
| Initial mass (g)                               | 1.0126 | 1.0298 | 0.9969 | 1.0393 | 1.0073 | 4.0197 | 4.0518 | 4.0257 | 2.0003 | 6.0076 |
| Liquid-solid ratio (mL/g)                      | 40     | 40     | 40     | 40     | 40     | 10     | 10     | 10     | 20     | 6.7    |
| Total extractives                              | 11.3   | 11.5   | 12.5   | 14.4   | 15.6   | 9.4    | 9.8    | 12.0   | 12.4   | 9.2    |
| Hydroxymatairesinol <sup>(1)</sup>             | 4.2    | 4.2    | 4.4    | 5.3    | 5.4    | 3.9    | 4.0    | 4.2    | 4.8    | 3.7    |
| Secoisolariciresinol <sup>(1)</sup>            | 0.91   | 0.82   | 0.84   | 0.94   | 1.10   | 0.80   | 0.75   | 0.96   | 0.98   | 0.92   |
| $\alpha$ -Conidendrin <sup>(1)</sup>           | 0.099  | 0.121  | 0.152  | 0.186  | 0.216  | 0.092  | 0.101  | 0.153  | 0.145  | 0.078  |
| Lariciresinol <sup>(2)</sup>                   | 0.203  | 0.201  | 0.223  | 0.240  | 0.307  | 0.184  | 0.152  | 0.204  | 0.256  | 0.197  |
| Matairesinol <sup>(2)</sup>                    | 0.113  | 0.116  | 0.211  | 0.176  | 0.221  | 0.798  | 0.755  | 0.960  | 0.980  | 0.925  |
| Pinoresinol <sup>(2)</sup>                     | ND     | 0.070  | 0.081  | 0.103  | 0.127  | 0.061  | 0.134  | 0.115  | 0.181  | 0.075  |
| Nortrachelogenin <sup>(2)</sup>                | ND     | ND     | ND     | ND     | ND     | 0.038  | 0.108  | 0.079  | 0.025  | ND     |
| o-Guaiaicol <sup>(2)</sup>                     | 0.022  | 0.022  | 0.036  | 0.026  | 0.062  | 0.035  | 0.053  | 0.047  | 0.053  | 0.030  |
| Coniferyl alcohol <sup>(2)</sup>               | 0.048  | 0.043  | 0.067  | 0.060  | 0.084  | 0.086  | 0.134  | 0.180  | 0.013  | 0.072  |
| Lignan (%) <sup>(3)</sup>                      | 91     | 89     | 87     | 84     | 82     | 88     | 88     | 85     | 86     | 90     |
| Average solvent recovery rate (%)              | 93     |        |        |        |        | 70     |        |        | 81     | 50     |
| Absorbed solvent / dry mass (mL/g of dry wood) | 3.1    |        |        |        |        | 3.4    |        |        | 4.3    | 3.7    |

ND: not detected

\*SM Figure S5

<sup>(1)</sup>HPLC, External quantification; <sup>(2)</sup> GC-MS/FID, external quantification; <sup>(3)</sup>HPLC, Peaks area %

**Table 3**Simulated countercurrent process results -  $V_{\text{solvent}} = 500$  mL,  $m_{\text{wood}} = 50$  g, 15:85 (v/v) EtOH:Water, T = 40 °C.

| Fraction | Total extractives | Hydroxymatairesinol <sup>(1)</sup><br>g/100 g of dry wood | Conidendrin <sup>(1)</sup> | Lignan <sup>(2)</sup> (%) | Solvent recovery rate (%) |
|----------|-------------------|---|----------------------------|---------------------------|---------------------------|
| CC11     | 5.9               | 2.4   | 0.040                      | 89                        | 54                        |
| CC12     | 4.7               | 1.8   | 0.049                      | 85                        | 100                       |
| CC13     | 2.6               | 0.9   | 0.033                      | 81                        | 100                       |
| CC14     | 1.0               | 0.3   | 0.020                      | 71                        | 100                       |
| CC21     | 7.3               | 2.9   | 0.060                      | 90                        | 54                        |
| CC22     | 7.2               | 2.9   | 0.080                      | 86                        | 100                       |
| CC23     | 4.2               | 1.5   | 0.055                      | 81                        | 100                       |
| CC31     | 8.2               | 3.3   | 0.065                      | 91                        | 54                        |
| CC32     | 8.7               | 3.5   | 0.081                      | 87                        | 100                       |
| CC41     | 8.3               | 3.4   | 0.055                      | 91                        | 54                        |

<sup>(1)</sup>HPLC, External quantification; <sup>(2)</sup>HPLC, Peaks area %

increment. As the increase in sample mass caused a decrease in yield, we varied the temperature in the hope of improving it. We decided to examine the impact of sample mass more closely because this is not the case. Table 2 shows the results obtained for single batch extraction considering the influence of the 3 chosen parameters: ethanol concentration, temperature, and feed mass.

**3.2.3.1. Ethanol concentration.** Extraction by single batch was studied in a range between 0 (pure water) and 20% (v/v) ethanol in water. This range of composition is close to the ideal one to purify the extract, as published before (Ravetti Duran et al., 2023). Extraction was carried out with 1 g of solid material (wood) with 40 mL of EtOH/Water solvent at 40 °C for 24 h to achieve thermodynamic equilibrium.

Ethanol concentration has a major influence on the extraction process (Fig. 5) - as the polarity of the mixture decreases, the total extraction yield goes from 11.3 with pure water to 15.6 g/100 g of dry wood with a 20:80 (v/v) solvent mixture. At the same time, the extract composition evolves with the ethanol concentration by enhancing from 9% to 18% in the presence of low-polar compounds (i.e. non-lignan compounds). HMR epimers extraction follows the same trend as the total extraction yield and stabilizes at a maximum of 5.4 g/100 g dry wood for a 15:85 (v/v) water-ethanol mixture.

The results indicate that on average 93% of the initial solvent volume was recovered after extraction and isolation.

The optimal ethanol concentration of 15% (v/v) was chosen for the following experiments because it favors the extraction of HMR at the same time as it increases the number of total extractable.

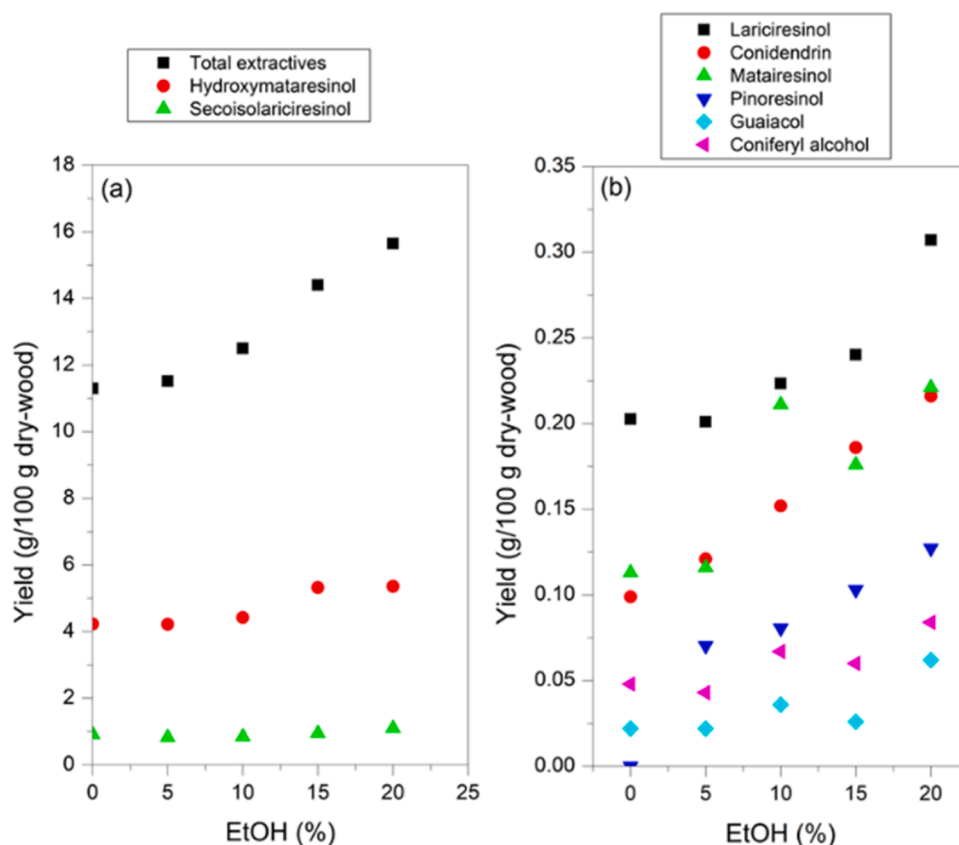


**Table 4**Percolation extraction results -  $m_{\text{wood}} = 6 \text{ g}$ , 15:85 (v/v) EtOH:Water,  $T = 25 \text{ }^{\circ}\text{C}$ .

| Volume Fraction (mL) | Total extractives | Cumulated total extractive<br>g/100 g of dry wood | Hydroxymatairesinol <sup>(1)</sup> | Conidendrin <sup>(1)</sup> | Lignan (%) <sup>(2)</sup> |
|----------------------|-------------------|---|------------------------------------|----------------------------|---------------------------|
| 0–50*                | 7.3               | 7.3   | 3.1                                | 0.031                      | 91                        |
| 50–100               | 2.0               | 9.3   | 0.9                                | 0.020                      | 86                        |
| 100–150              | 1.1               | 10.4  | 0.4                                | 0.016                      | 81                        |
| 150–200              | 0.6               | 11.0  | 0.2                                | 0.012                      | 76                        |
| 200–250              | 0.4               | 11.4  | 0.1                                | 0.009                      | 74                        |
| 250–300              | 0.3               | 11.7  | 0.1                                | 0.007                      | 72                        |

<sup>(1)</sup>HPLC, External quantification; <sup>(2)</sup>HPLC, Peaks area %

\* SM Figure S7

**Fig. 5.** Effect of ethanol concentration on the extraction yield of (a) total extractives, HMR (isomers), secoisolariciresinol, and (b) conidendrin, lariciresinol, matairesinol, pinoresinol, guaiaicol, coniferyl alcohol – Single batch extraction –  $V_{\text{solvent}} = 40 \text{ mL}$ ,  $T = 40 \text{ }^{\circ}\text{C}$ ,  $m_{\text{wood}} = 1 \text{ g}$ .

**3.2.3.2. Temperature.** The temperature is an important operational factor because it may increase the solubility of compounds in the solvent. Extraction by single batch was studied in a range between 30 and 60 °C. For this, 4 g of starting material was contacted with 40 mL of EtOH/Water (15:85, v/v) solvent for 24 h.

The temperature has a positive impact on extraction yield, increasing it from 9.4 at 30 °C to 12.0 g/100 g of dry wood at 60 °C (Fig. 6). However, HMR remains stable with the increase in temperature. Furthermore, there was a slight decrease in lignan content in the liquid extract as the temperature increased, dropping from 88% at 30 °C to 85% at 60 °C.

The optimal temperature of 40 °C was chosen for the following experiments because it favors the extraction of total extractable and, at the same time, avoids the degradation of thermosensitive compounds.

**3.2.3.3. Starting material mass.** Solid-liquid extraction was performed with 40 mL of EtOH/Water (15:85, v/v) solvent at 40 °C for 24 h. Extraction was performed with 1, 2, 4, and 6 g of wood material.

The results of this experiment demonstrate a correlation between the mass of the wood material and the solvent recovery rate, highlighting the extent to which the wood material absorbs the solvent which leads to a decrease in overall extraction yield. The results reveal a decline in solvent recovery with an increase in the mass of the wood material.

A solvent recovery of 93% was obtained using 1 g of wood, diminishing to 81% with 2 g, 70% with 4 g, and 50% with 6 g. Analyzing the absorbed solvent volume per unit mass of dry wood (Table 2) demonstrates variability, with an average value approximating 3.6 mL/g of dry wood.

The absorbed solvent phenomena directly affect the overall extraction yield, resulting in a decrease from 15.6 to 9.2 g/100 g of dry wood for initial wood material quantities of 1 g and 6 g, respectively.

### 3.2.4. Simulated countercurrent

This experiment effectively illustrates the advantage of the washing steps based on recovering molecules that were absorbed with the solvent into the wood material. Using  $m_1$  wood as an example (Fig. 1), the initial

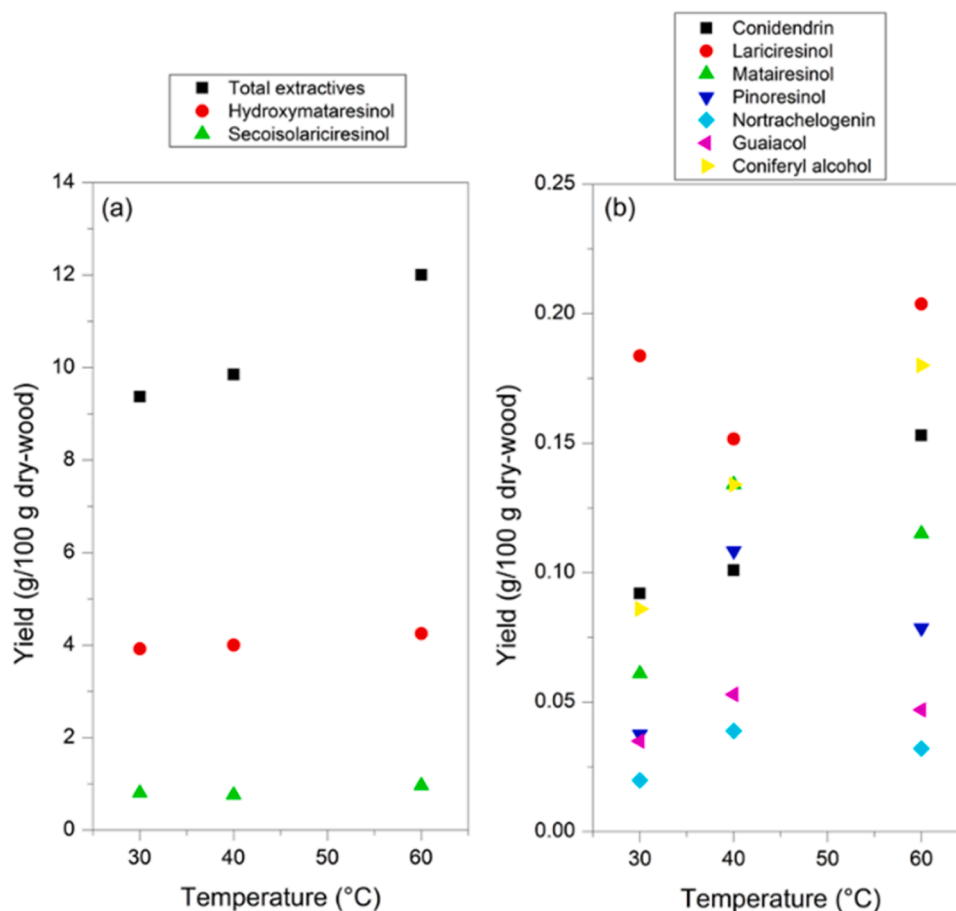


Fig. 6. Effect of the temperature on the extraction yield of (a) total extractives, HMR (isomers), secoisolariciresinol, and (b) conidendrin, lariciresinol, matairesinol, pinoresinol, nortrachelogenin, guaiacol, coniferyl alcohol – Single batch extraction –  $V_{\text{solvent}} = 40$  mL,  $m_{\text{wood}} = 4$  g, 15:85 (v/v) EtOH:Water.

extraction yielded 5.9 g/100 g of dry wood. Subsequent washing steps recovered 4.7 g/100 g of dry wood after the first wash, 2.6 g/100 g of dry wood after the second wash, and 1.0 g/100 g of dry wood after the third wash. In essence, the cumulative effect of the successive washing steps enhanced the overall extraction yield by an additional.

Furthermore, the solvent recovery rate exhibited variation depending on the process step. During the initial extraction step, wherein the wood material had not yet absorbed the solvent, only 54% of the initial

volume was recovered. Conversely, during the washing steps, as the wood had already reached saturation by absorbing the solvent, a 100% solvent recovery rate was achieved.

The final recovered fraction (CC11, CC21, CC31, CC41) exemplifies the enhancement in total extractable yield while employing the same quantity of solvent (500 mL) with a recovery of 270 mL. This enhancement is evidenced by an increase in the total extractable yield from 5.9 g to 8.3 g/100 g of dry wood for C11 and C41 (Supplementary

Table 5

Supercritical extraction – P = 110 bar,  $t_{\text{dynamic}} = 120$  minutes.

| Temperature (°C)                     | 20                  | 40    | 60    | 40    | 40    | 40    |
|--------------------------------------|---------------------|-------|-------|-------|-------|-------|
| $t_{\text{static}}$ (min)            | 30                  | 30    | 30    | 30    | 30    | 30    |
| $V_{\text{EtOH}}$ (mL)               | 30                  | 30    | 30    | 5     | 10    | 20    |
| $n_{\text{CO}_2}$ (mol)              | 1.48                | 1.45  | 1.10  | 1.68  | 1.65  | 1.43  |
| $x_{\text{EtOH}}$                    | 0.256               | 0.260 | 0.316 | 0.051 | 0.094 | 0.192 |
| $Q_{\text{CO}_2}$ (g/min)            | 10.5                | 11.4  | 11.1  | 9.6   | 12.1  | 11.0  |
| $m_{\text{dry wood}}$ (g)            | 7.9                 | 7.7   | 7.8   | 7.5   | 11.0  | 7.9   |
| Total extractives                    | g/100 g of dry wood |       |       |       |       |       |
| Hydroxymatairesinol <sup>(1)</sup>   | 3.9                 | 4.1   | 4.1   | 1.0   | 1.4   | 2.9   |
| $\alpha$ -Conidendrin <sup>(1)</sup> | 1.7                 | 1.4   | 1.7   | 0.1   | 0.1   | 0.9   |
| Lariciresinol <sup>(2)</sup>         | 0.075               | 0.075 | 0.070 | 0.002 | 0.008 | 0.044 |
| Matairesinol <sup>(2)</sup>          | 0.099               | 0.112 | 0.038 | ND    | ND    | ND    |
| Pinoresinol <sup>(2)</sup>           | 0.053               | 0.077 | 0.038 | ND    | ND    | ND    |
| Nortrachelogenin <sup>(2)</sup>      | 0.031               | 0.047 | 0.018 | ND    | ND    | ND    |
| o-Guaiacol <sup>(2)</sup>            | 0.001               | 0.006 | 0.002 | ND    | ND    | ND    |
| Coniferyl alcohol <sup>(2)</sup>     | 0.003               | 0.007 | 0.001 | ND    | ND    | ND    |
| % Lignan <sup>(3)</sup>              | 0.007               | 0.009 | 0.002 | ND    | ND    | ND    |
|                                      | 74                  | 78    | 77    | 19    | 36    | 72    |

ND: not detected

<sup>(1)</sup>HPLC, External quantification; <sup>(2)</sup>GC-MS/FID, external quantification; <sup>(3)</sup>HPLC, Peaks area %

**Table 6**Supercritical extraction – P = 220 bar,  $t_{\text{dynamic}} = 120$  minutes.

| Temperature (°C)                     | 20    | 40    | 60    | 40   | 40    | 40    | 40    | 40    | 40    | 40    | 40    | 40    |     |
|--------------------------------------|-------|-------|-------|------|-------|-------|-------|-------|-------|-------|-------|-------|-----|
| $t_{\text{static}}$ (min)            | 30    | 30    | 30    | 30   | 30    | 30    | 30    | 30    | 30    | 30    | 0     | 120   | 240 |
| $V_{\text{EtOH}}$ (mL)               | 30    | 30    | 30    | 0    | 5     | 10    | 20    | 25    | 40    | 30    | 30    | 30    |     |
| $n_{\text{CO}_2}$ (mol)              | 1.42  | 1.70  | 1.30  | 2.00 | 2.03  | 1.94  | 1.54  | 1.58  | 1.34  | -     | 1.79  | 1.89  |     |
| $x_{\text{EtOH}}$                    | 0.266 | 0.232 | 0.283 | 0    | 0.041 | 0.081 | 0.182 | 0.213 | 0.338 | -     | 0.223 | 0.214 |     |
| $Q_{\text{CO}_2}$ (g/min)            | 11.5  | 10.6  | 11.2  | 10.8 | 10.8  | 11.3  | 11.0  | 11.0  | 10.5  | 10.5  | 10.2  | 10.9  |     |
| $m_{\text{dry wood}}$ (g)            | 7.8   | 8.0   | 7.8   | 7.8  | 7.4   | 7.9   | 7.9   | 7.8   | 7.9   | 7.6   | 7.8   | 7.8   |     |
| Total extractives                    | 4.7   | 4.4   | 4.3   | 0.1  | 1.4   | 1.8   | 3.2   | 3.9   | 5.7   | 4.7   | 4.2   | 4.8   |     |
| Hydroxymatairesinol <sup>(1)</sup>   | 1.7   | 1.6   | 1.7   | 0.1  | 0.1   | 0.2   | 1.0   | 1.4   | 2.0   | 1.7   | 1.7   | 1.9   |     |
| $\alpha$ -Conidendrin <sup>(1)</sup> | 0.080 | 0.095 | 0.083 | -    | 0.013 | 0.023 | 0.052 | 0.070 | 0.115 | 0.127 | 0.077 | 0.096 |     |
| Lariciresinol <sup>(2)</sup>         | 0.124 | 0.152 | 0.105 | ND   | ND    | ND    | ND    | ND    | ND    | 0.129 | ND    | 0.123 |     |
| Matairesinol <sup>(2)</sup>          | 0.070 | 0.095 | 0.079 | ND   | ND    | ND    | ND    | ND    | ND    | 0.092 | ND    | 0.087 |     |
| Pinoresinol <sup>(2)</sup>           | 0.025 | 0.042 | 0.034 | ND   | ND    | ND    | ND    | ND    | ND    | 0.052 | ND    | 0.028 |     |
| Nortrachelogenin <sup>(2)</sup>      | 0.017 | 0.018 | 0.025 | ND   | ND    | ND    | ND    | ND    | ND    | 0.014 | ND    | 0.016 |     |
| o-Guaiacol <sup>(2)</sup>            | 0.006 | 0.006 | 0.005 | ND   | ND    | ND    | ND    | ND    | ND    | 0.007 | ND    | 0.007 |     |
| Coniferyl alcohol <sup>(2)</sup>     | 0.008 | 0.012 | 0.009 | ND   | ND    | ND    | ND    | ND    | ND    | 0.010 | ND    | 0.012 |     |
| % Lignan <sup>(3)</sup>              | 76    | 73    | 77    | 41   | 45    | 52    | 70    | 70    | 75    | 69    | 76    | 76    |     |

ND: not detected

<sup>(1)</sup>HPLC, External quantification; <sup>(2)</sup>GC-MS/FID, external quantification; <sup>(3)</sup>HPLC, Peaks area %

Material, Figure S6), respectively.

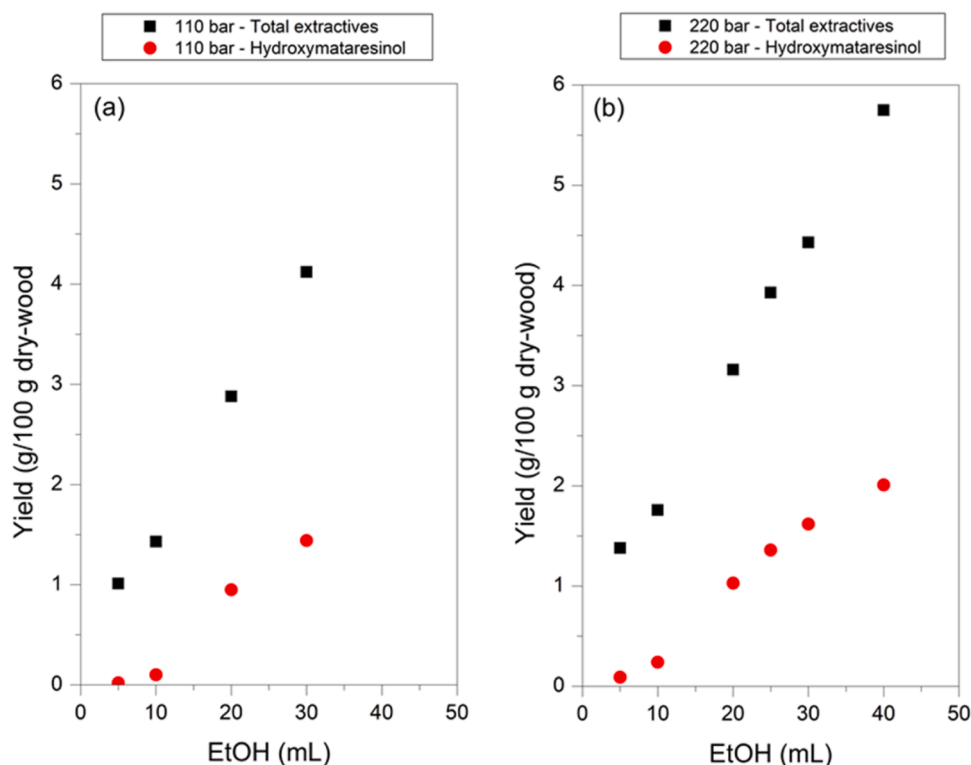
### 3.2.5. Percolation extraction

The polar compounds, as lignans, exhibit a preferential extraction pattern, with a reduction in their extraction rate observed from 91% for the first fraction to 72% for the sixth fraction. Correspondingly, the content of extractable compounds diminished notably, declining from 7.3 g to 0.3 g/100 g of dry wood for the first and sixth fractions, respectively. The cumulative extraction yield for these fractions amounted to 11.6 g/100 g of dry wood, employing 300 mL of solvent throughout the percolation extraction process.

### 3.3. Near supercritical and supercritical fluid extraction

Experimental conditions and data are summarized in [Supplementary Material, Tables S6 and S7](#). Supercritical extraction was first performed with CO<sub>2</sub> (40 °C, 220 bar, 30 minutes of static time, 8.75 g of wood), however, the extraction yield was much lower than the previous extraction methods (0.06 g/100 g dry wood). Furthermore, it enables to extract preferentially lipophilic compounds (69% HPLC area) instead of lignans. This may be explained by the fact that major components of wood are hydrophilic ([Willför et al., 2003b](#)), and hence cannot be extracted only with carbon dioxide.

Thus, we have chosen to use a cosolvent to increase the polarity of carbon dioxide and facilitate the extraction. Ethanol was chosen because it is considered an eco-friendly solvent and it is widely used as a modifier

Fig. 7. Effect of ethanol volume at (a) 110 bar and (b) 220 bar in SC-CO<sub>2</sub> extracts (40 °C).

for supercritical extraction.

The near supercritical/supercritical extraction was studied taking into account the following parameters (Tables 5 and 6):

- Static time: 0–240 minutes
- Pressure: 110 and 220 bar
- Temperature: from 20 °C to 60 °C
- Cosolvent (ethanol) volume: 0–40 mL.

The amount of cosolvent is an important operational factor for the extraction of lignans due to their high polarity. Pure CO<sub>2</sub> is less suitable for the extraction of polar molecules, so the use of modifiers (cosolvents) dissolved in the carbon dioxide is fundamental to increase solvent polarity and allow the extraction of a greater range of compounds, especially lignans. It is important to highlight that the addition of ethanol modifies the supercritical point (Ravetti Duran et al., 2018), and consequently the density values. Zhang et al (Zhang et al., 2003). studied the dependence of ethanol concentration on the density and critical properties (pressure and temperature), and Cunico et al (Cunico and Turner, 2017). observed that in the range of mole fraction of ethanol from 0.1% to 0.5% (308 K, 220 bar), the density increases from 807.0 to 858 kg/m<sup>3</sup>, respectively.

The addition of ethanol as cosolvent increased the lignan, HMR, and total extraction yield in both cases (Fig. 7), at 110 bar and 220 bar (Supplementary Material, Figures S8 and S15). The use of 5–40 mL of ethanol as cosolvent resulted in a total extract yield in the range of 1.38 g to 5.75 g/100 g of the dry wood depending on the chosen pressure. The major compound is HMR resulting in an extract yield in the range of 0.09–2.01 g HMR/100 g of dry wood. The optimal operating condition for maximizing total extract yield, HMR extract yield, and highest phenolic concentration was obtained operating at 40 °C, 220 bar, and 40 mL of ethanol (SM Figure S8).

Seabra et al (Gérardin et al., 2023). observed that the variation of ethanol concentration in supercritical CO<sub>2</sub> changes the extraction yield from 3% to 6%, for 10 wt% and 90 wt% of ethanol, respectively. Furthermore, a higher phenolic concentration in the obtained extract was observed. The difference in species and the portion of the tree used for extraction could explain our higher extraction yield. Beyond that, the cosolvent was continuously injected with the CO<sub>2</sub> flow before contact with the wood.

The results of this study indicate that under the proposed conditions, optimization of static time, pressure, and temperature does not have a significant impact on the extract yield. This finding suggests that ethanol effectively extracts the desired compounds before the supercritical extraction. As a result, the use of a flow of SC-CO<sub>2</sub> leads to the solubilization of ethanol containing the target compounds, which is subsequently recovered at the separators. The setup is similar to solid-liquid extraction, considering only ethanol. However, this method has three main advantages:

- Reduces the total amount of solvent required, resulting in a concentrated extract. This also eliminates or reduces the need for a concentration step.
- Facilitates the separation of extractables from the raw material (as the percolation process), eliminating complex separation steps and the issues associated with solvent absorption observed during solid-liquid extraction.
- Faster extraction times compared to solid-liquid extraction.

To enhance the yield of supercritical extraction, one strategy that can be implemented is to introduce additional portions of cosolvent into the extractor after the depletion of the initial ethanol addition to extract the residual compounds.

**Table 7**

Average recovery rate for each extraction method.

| Extraction method                        | Average solvent recovery rate (%) | Ratio mass/volume (g raw material/mL solvent) |
|--|-----------------------------------|---|
| Soxhlet                                  | 92                                | 0.083   |
| Single batch                             | 93                                | 0.025   |
|  | 81                                | 0.050   |
|  | 70                                | 0.100   |
|  | 50                                | 0.150   |
| Simulated countercurrent (1st batch)     | 54                                | 0.100   |
| Simulated countercurrent (2nd, 3rd, 4th) | 100                               | 0.100   |
| Percolation                              | -                                 | 0.02  |

### 3.4. Extraction methods discussion

#### 3.4.1. Absorption phenomena

The porous nature inherent to wood material enables it to absorb solvents, which results in an increase in its volume during the extraction process. Consequently, this phenomenon contributes to a reduction in the quantity of recovered solvent, as depicted in Table 7, subsequently impacting the overall extraction yield.

Table 7 shows the average recovery rates associated with each extraction method, demonstrating a substantial dependence on the applied extraction technique. For example, the Soxhlet method exhibits a recovery rate of 92%, whereas single batch extraction yields 70%. Notably, the solvent recovery rate is also influenced by the quantity of wood material used, with single batch extraction achieving a solvent recovery rate of 93% for a mass-to-volume ratio of 0.025 and 50% for a ratio of 0.150. Furthermore, scale-up considerations impact the solvent recovery rates, as evidenced by single batch and simulated countercurrent extraction (e.g. 12.5 fold the scale of single batch extraction), achieving recovery rates of 70% and 54%, respectively.

This observation emphasizes three crucial aspects that warrant implementation at an industrial scale:

- Incorporating a unit operation, such as centrifugation or press extraction, to recover residual solvent.
- Include a “washing step” to retrieve the extracted compounds absorbed by the wood material along with the solvent that can't be recovered by any separation methods, such as simulated countercurrent batch extraction (Section 3.2.4)
- Employing flow extraction methods, such as percolation (Section 3.2.5), as well as supercritical CO<sub>2</sub> (Section 3.3), thereby circumventing the necessity for intricate downstream techniques.

#### 3.4.2. Industrial implementation

This article underscores the potential applicability of the proposed wood extraction methods for industrial implementation, emphasizing the critical considerations of solvent recovery efficiency and implementation feasibility in the selection of an appropriate method. Table 8 presents the optimal operational conditions and corresponding results for each extraction method.

Soxhlet extraction exhibits the highest total extractives yield, however, it poses challenges for industrial implementation due to its complexity.

Single batch extraction, with a yield of 14.4 g/100 g of dry wood, ranks second in terms of extractive yield, however, its main drawback is that it requires an additional downstream step to isolate the solvent from the wood material.

Simulated countercurrent extraction was expected to yield higher extractives than single batch extraction, as it includes intermediate washing steps to extract remaining compounds. Discrepancies between theoretical and experimental yields can be attributed to the increased difficulty of handling larger quantities of wet wood during solvent-wood

**Table 8**

Best operational conditions for each extraction method.

| Extraction method             | Total extractives (g/100 g of dry wood) | Hydroxymatairesinol (g/100 g of dry wood) | Operational condition                   | Ratio mass/volume (g raw material/mL solvent) |
|-------------------------------|---|---|---|---|
| Soxhlet                       | 17.4                                    | 6.3                                       | Acetone, 600 mL                         | 0.083   |
| Single batch                  | 14.4                                    | 5.3                                       | 15% Ethanol/Water, 4 g/40 mL, 40 °C     | 0.100   |
| Simulated countercurrent      | 8.3                                     | 3.4                                       | 15% Ethanol/Water, 50 g/500 mL, 40 °C   | 0.100   |
| Percolation                   | 11.6                                    | 4.8                                       | 15% Ethanol/Water, 6 g/300 mL, 25 °C    | 0.02  |
| Supercritical CO <sub>2</sub> | 5.7                                     | 2.0                                       | 7.9 g, 40 mL of ethanol, 40 °C, 220 bar | -   |

material separation. For example, a comparison between small-scale (4 g wood) and larger-scale (50 g wood) extractions revealed a recovery rate decrease from 70% to approximately 54%. This highlights the drawback of an isolation step, which can reduce extraction yield. Moreover, countercurrent extraction's requirement for numerous intermediary storage tanks and complex logistics may not be suitable for industrial-scale implementation.

Percolation extraction demonstrated a lower yield of 11.6 g/100 g dry wood compared to single batch extraction. This disparity in yield is likely due to the percolation process employing lower temperatures and shorter contact times compared to the previous extraction methods. Optimization of the percolation process could involve adjusting the superficial velocity to enhance wood compounds' diffusion into the solvent. Another strategy involves incorporating a static step, allowing solvent absorption and compound diffusion before controlled flow percolation.

Regarding supercritical extraction, SC-CO<sub>2</sub> exhibited the lowest extraction yield and displayed low sensitivity to operating parameters, such as pressure, temperature, and static time, under the chosen experimental conditions.

For successful industrial-scale implementation, considerations extend beyond extraction yield. Ease of implementation, process steps, and overall duration are critical factors for comparison. Percolation and supercritical extraction, both relying on flow processes facilitating solvent recovery, are better suited for industrial scaling. Percolation, with its higher extraction yield compared to supercritical processes, emerges as the favorable choice among the methods discussed in this article.

#### 4. Conclusions

This study aimed to provide comprehensive insights into the industrial implementation of high-added-value molecule extraction from wood, with a particular emphasis on critical factors including solvent recovery, ease of implementation, and extraction yields. A model mixture of *Picea abies* and *Abies alba* (water content of 11 wt%, length is  $0.73 \pm 0.60$  mm, width is  $0.32 \pm 0.28$  mm and equivalent diameter is  $0.34 \pm 0.23$  mm) served as raw material to this study.

Various extraction techniques were systematically explored, including single batch, simulated countercurrent, percolation, and supercritical CO<sub>2</sub> extraction. Each method presents its unique advantages and challenges, necessitating careful consideration based on specific industrial requirements when choosing the most suitable approach for industrial applications.

The article underscores the role played by the wood material's propensity to absorb solvents, resulting in diminished solvent recovery and subsequently reduced extraction yields. To mitigate this challenge, mechanical steps such as centrifugation, filtration, and manual pressing were employed to improve solvent recovery rates, albeit with limited success. Notably, the implications of scaling up the process or increasing the quantity of wood underscored the importance of these steps to mitigate adverse effects on solvent recovery rates and extraction yields.

In response to these challenges, the potential benefits of introducing the washing step have been highlighted, wherein the washing solvent is sequentially utilized in the subsequent extraction step, thus limiting solvent consumption. Furthermore, the employ of flow extraction methods was discussed, which avoids the necessity for downstream processing steps.

Soxhlet extraction offers the highest total extractive yield but presents significant complexity and impracticality for industrial applications. Single batch extraction, while offering a commendable yield, necessitates a supplementary downstream step for solvent-wood material isolation. Simulated countercurrent extraction, although theoretically promising by incorporating intermediate washing steps, faces challenges related to handling larger quantities of wet wood, potentially diminishing extraction yields. Percolation, despite a slightly lower yield, emerges as an attractive choice due to its ease of industrial integration and potential for improved efficiency at larger scales.

#### CRedit authorship contribution statement

**Laurence Muhr:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Danielle Barth:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Renan Ravetti Duran:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization.

#### Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Danielle BARTH reports financial support was provided by CNRS Délégation Centre-Est.

#### Data Availability

No data was used for the research described in the article.

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#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the



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