SUPERCRITICAL CO_2 EXTRACTION OF LAVENDER FLOWER WITH ANTIOXIDANT ACTIVITY: LABORATORY TO A LARGE SCALE OPTIMIZATION PROCESS

E. Cruz-Sánchez, J. M. García-Vargas, I. Gracia, J. F. Rodriguez, M. T. García*

Department of Chemical Engineering, University of Castilla-La Mancha, Facultad de Ciencias y Tecnologías Químicas, Avda. Camilo José Cela 12, 13071 Ciudad Real, Spain.

*Corresponding author:

email: teresa.garcia@uclm.es

Phone: +34 926 05 28 51

Fax: +34 926295256

Background

The volatile compounds that comprise lavender essential oils, including linalool and linalyl acetate, have demonstrative therapeutic properties. The supercritical CO_2 extraction ($scCO_2$) has demonstrated efficiency and selectivity for the extraction of essential oils from vegetable matrices.

Methods

The solubility of lavender essential oil in $scCO_2$ was determined using a high-pressure variable-volume and modeled by semiempirical models. Supercritical extraction was carried out at a pressure of 180, 250 and 300 bar and a temperature of 40-60 °C, with and without cosolvents. kinetic curve has been modeled by broken and intact cells model developed by Sovová. The composition of the extracts was evaluated through GC-MS

and their antioxidant activity by the DPPH method.

Findings

The highest values of oil solubility and extraction yield were obtained at 250 bar and 60 °C. Furthermore, the extraction yield increases significantly with the addition of ethanol as a co-solvent (0.2% v/v). A complete set of equilibrium data and kinetic parameters has been reported on a large scale for the first time. The dominant components identified in the extract were linalool and linalyl acetate, and the extracts showed very satisfactory results for antioxidant capacity. Compared with traditional methods like Soxhlet extraction, the supercritical extracts were determined to be more interesting for the formulation of nutraceutical products or biomedical applications.

Keywords: Lavender essential oil – Supercritical CO2 – Solubility – Antioxidant – Large scale extraction

1. Introduction

The blooming plant known as lavender, which belongs to the Lamiacae family, has a long history of usage in herbal therapy. The different chemical compounds contained in Lavender plants have shown several therapeutic properties related with many illnesses; and also give this plant its remarkable properties in terms of its usage for cleaning, fragances and cosmetics [1]. Most of the applications commented come from the use of lavender essential oil (LEO), which has long been thought to have a variety of therapeutic and restorative characteristics. This essential oil has the ability to improve neurologic functions such as reducing brain edema or treating renal injuries [2,3]. This essential oil

has sedative and antidepressant qualities and is also frequently used in aromatherapy to treat insomnia [4].

One of the most important properties of lavender essential oil is its antioxidant activity, which has attracted the interest of many researchers [5]. This attribute together with the anti-inflammatory capacity is attributed to the two main components of LEO, linalool and its ester, linalyl acetate [6,7]. In this context, attention is also focused on the utilization of LEO in dermatology because of the good results observed in different works regarding its topical use for wound healing [8,9] and its positive effect on dermal collagen production [10].

LEO is obtained from lavender flowers. Steam distillation is the most popular extraction technique. However, the resulting compounds may be degraded by the high temperatures or water contact. Soxhlet is another of the most commonly used traditional techniques. This method involves the use of organic solvents that can be harmful to human health and the environment. [11]. By contrast, innovative methods that don't involve high temperatures or damaging ingredients for the environment are gaining popularity. Novel techniques include microwave extraction, ultrasound or supercritical technology, the latter being one of the most widely implemented on an industrial scale [12–14].

Due to their low viscosity and high diffusivity, supercritical fluids are the ideal alternative as extraction solvents. Supercritical carbon dioxide ($scCO_2$) is one of the most widely used substances because it is non-toxic, non-flammable, inert and its critical point is easily reached [15]. Additionally, their high solvent power may be easily controlled by changing the temperature and pressure of the extraction process, enabling selectivity towards the desired molecules [16,17]. These characteristics make it possible to quickly extract essential oils at low temperatures. It is evident that supercritical fluid extraction with CO_2 is developing as a feasible and environmentally friendly acceptable option for the

73 production of essential oil products since supercritical CO_2 has a similar polarity to liquid
74 pentane and is thus suited for the extraction of lipophilic chemicals [18].

75 The main issue with $scCO_2$ is that it has low polarity, which reduces the solubility of polar
76 chemicals and limits the spectrum of applications. To improve the polarity of $scCO_2$, a
77 co-solvent can be added, such as ethanol, ethyl acetate, acetonitrile, or isopropanol,
78 helping to overcome this problem [19].

79 A wide variety of bioactive compounds, from flavonoids, phenolic compounds to
80 essential oils, can be obtained by $scCO_2$ extraction from different raw materials such as
81 agri-food waste, fruits, plants or herbs [20]. Numerous studies have investigated the
82 extraction and fractionation conditions of natural matrices as nuts [21], food by-products
83 [22] or volatile compounds of lavender [23]. Although analysing the operating conditions
84 is important, a crucial step that should be carried out in this type of process is the analysis
85 of the equilibrium conditions and solubility of the essential oil in supercritical CO_2, which
86 will help us to optimize the extraction conditions[24].

87 In a previous preliminary study, we determined the technological and economic
88 feasibility of the supercritical extraction of lavender extracts, concluding that it was an
89 attractive technology for investment and capable of satisfying the needs of a growing
90 market niche [14]. The aim of the present research was to obtain the required parameters
91 to operate supercritical extraction of LEO on a large scale, in addition to the exhaustive
92 characterization of the extracts. Firstly, the solubility of the essential oil in supercritical
93 CO_2 was obtained using a semi-empirical model to correlate the data. In a further study,
94 the variables that most influence the extraction yield were evaluated. This information
95 was used to select the pilot plant operating conditions, in which the kinetic and mass
96 transfer parameters were calculated for the plant design. Finally, the large-scale extracts

were characterized, and their antioxidant properties were analyzed to determine their potential use, whether in nutraceutical products or biomedical applications.

2. Materials and methods

2.1. Materials

For the solubility study, lavender oil from *Lavandula angustifolia* L. (Sigma-Aldrich, Spain) and CO_2 of 99.8% purity (Carburos Metálicos, Barcelona, Spain) were used. Dried lavender flowers (Peñarrubia del Alto Guadiana S. L., Albacete, Spain), CO_2 and ethanol (EtOH) and ethyl acetate (EA) HPLC grade (Scharlab, Barcelona, Spain) were required for the extraction. For the chromatographic analysis, the same lavender essential oil was used and also linalool and linalyl acetate as standards (Sigma-Aldrich, Spain), as well as diethyl ether (Scharlab, Barcelona, Spain). For the antioxidant potential assay, EtOH absolute HPLC grade (Scharlab, Barcelona, Spain), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Alfa-Aesar, Thermo Fisher, Ward Hill, MA, USA), and L-(+) ascorbic acid (Scharlab, Barcelona, Spain) were employed. The Soxhlet extraction was carried out using n-hexane at HPLC purity (Scharlab, Barcelona, Spain).

2.2 Evaluation of solubility of LEO in supercritical CO_2

Solubility experiments were carried out using a high-pressure variable-volume cell model ProVis 500 (from Eurotechnica), which was describe elsewhere [25] (Figure 1). The apparatus consists of a variable volume cell that containsa sapphire window and light for visual observation of the phase separation. The cell has a maximum capacity of 50 cm^3 and contains a movable piston to prevent pressure drops (Valves A and C), which separates the equilibrium chamber from the pressurisation circuit. The whole system is externally heated by an air bath capable of resisting temperatures up to 80 °C. The temperature inside the equilibration chamber is measured by a thermocouple. The CO_2 is

supplied by means of a dosing pump (Dosapro Milton Roy) and a pressure meter with a digital controller.

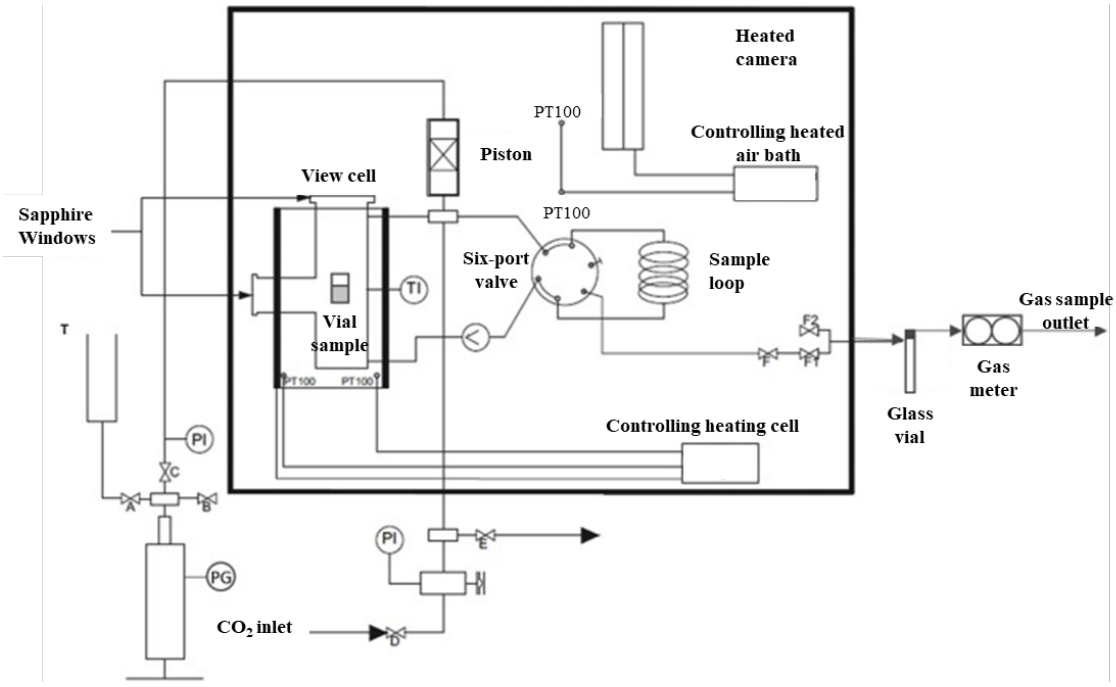


Figure 1. Experimental set-up employed to high-pressure phase equilibrium measurements. PG: pressure generator; PI-1: manometer; PI-2: pressure digital indicator; T: liquid supply tank; TI: temperature digital controller.

The experimental procedure for the determination of the equilibrium solubility is similar to the one proposed by other authors [25,26]. It starts by placing the LEO sample in a vial and introducing it into the cell. Once the temperature set has been reached, CO_2 is fed into the cell until arriving to the desired pressure value, conditions that were maintained during the selected contact time under stirring. After that, depressurisation was carried out. Samples from the top of the equilibrium cell were isobarically collected during the equilibrium experiments through a six-port valve attached to a 20 cm^3 loop through capillary lines and needle valves and decompressed to atmospheric pressure (via valves F and F1, at the same temperature of the cell). To determine the amount of LEO solubilised in CO_2, two samples were extracted from the equilibrium cell and expanded

in a glass vial, which was weighed before and after sampling on a precision analytical balance with an accuracy of 0.0001 g. Glass vials were used to collect LEO, which was then separated from CO_2 using a trap. A Ritter TG-05 gas meter was used to measure the CO_2 amount. The CO_2 density was calculated as a function of pressure and temperature with the equation of Bender [27] and Equation 1 was used to determine solubility, where the volume of CO_2 at standard conditions and the mass of LEO obtained are taken into account.

$$Solubility = \frac{kg\ LEO}{m^3 CO_2\ equilibrium} \quad \text{Eq. 1}$$

Regarding the operation conditions, the influence of 3 variables in the solubility was studied. First of all, a contact time study was carried out. Tests were carried out considering 6, 15, 24, 48 and 72 h at a pressure of 120 bar and at a temperature of 40 °C to determine the time it took to reach equilibrium conditions for the solubility of LEO in supercritical CO_2. After contact time, the influence of pressure was analysed by applying 120, 140, 180 and 250 bar Finally, in order to study how the working temperature affects the solubility, experiments were carried out at 40, 50, 60 and 80 ºC at different pressure values.

2.3. Modelling of equilibrium system

For the semi-empirical modelling of the equilibrium system formed by LEO and supercritical CO_2, different experimental correlations using density as an independent variable were evaluted. The correlations used in this work are those proposed by Chrastil (1982), del Valle and Aguilera (1988) and Adachi and Lu (1983).

i. Chrastil's equation (1982)

158 Chrastil proposed the following exponential relationship between the solubility of a solute
159 S (g/dm^3), the solvent density ρ (g/cm^3) and the temperature T (K) [28].

$$\ln S = q - \ln\left[\frac{(1000\,M_1)^k}{(M_2 + kM_1)}\right] + \frac{\Delta H}{RT} + \mathrm{k}\ln\rho \qquad \text{Eq. 2}$$

160 Where q is a dimensionless constant, M_1 and M_2 are the molecular weights of the solvent
161 and solute respectively, ΔH is the sum of the heats of solvation and vaporization (J/mol),
162 and k is the average number of solvent molecules in the solvato-complex.

163 The above expression can be simplified to Eq. 3 by regrouping the constants.

$$\ln S = C_1 + \frac{C_2}{T} + C_3 \ln\rho \qquad \text{Eq. 3}$$

164 Therefore, C_1 would be related with the molecular weights, C_2 with the total heat of
165 reaction (solvation and vaporization) and C_3 is the association number.

166 ii. Equation of del Valle and Aguilera (1988)

167 This equation introduces into the Chrastil equation an additional quadratic dependence
168 on the inverse of temperature [29].

$$\ln S = C_1 + \frac{C_2}{T} + \frac{C_3}{T^2} + C_4 \ln\rho \qquad \text{Eq. 4}$$

169 This expression has been used by many authors to estimate the solubility of different
170 species in different supercritical fluids [30].

171 iii. Adachi and Lu equation (1983)

172 This is another modification of the Chrastil equation [31]. In this case, the authors assume
173 that the association number k of this equation depends quadratically on the density.

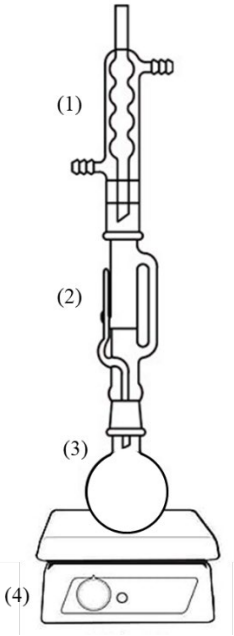
$$\ln S = C_1 + C_2 T + K\ln\rho \qquad \text{Eq. 5}$$

174 Where $K = C_3 + C_4\,\rho + C_5\rho^2$.

175 The C parameters were calculated by minimizing the sum of the squares of the differences
176 between experimental and calculated solubility using the Solver tool in Microsoft Excel.
177

178 **2.4. Soxhlet extraction**

179 Soxhlet extraction of LEO with n-hexane was carried out as a way of comparing this
180 process with supercritical CO_2 extraction. The conventional set-up was configured for
181 this type of extraction (Figure 2) [11].



182

183 **Figure 2.** Soxhlet extractor. (1) Condenser, (2) Glass vessel, (3) Round-bottomed flask,
184 (4) Heating plate.

185 Lavender flowers (20 g) were placed in a cartridge made of filter paper, which was placed
186 in a glass vessel connected to a round-bottomed flask. A condenser was placed at the top
187 of the installation. For the extraction, 250 ml of hexane were placed in the flask and the
188 heating was switched on. Once the hexane reached boiling point (70 °C), its vapours
189 condensed in the coolant and fell into the cartridge, where the essential oil was extracted
190 due to the contact with the lavender. When the mixture of hexane and extracted oil

exceeded a certain level in the glass container, it fell into the flask by siphon effect and the process was repeated. The Soxhlet extractions were carried out for 3 hours. At the end, a liquid mixture of solvent and oil was obtained and separated in a rotary evaporator. The experiments were carried out at atmospheric pressure.

2.5. Small-Scale Supercritical CO_2 extraction

All processes of supercritical extraction of solids consist of two stages: the extraction and the separation of the extract from the solvent. In the extraction, the supercritical CO_2 flows through the solid and dissolves the extractable components. The solvent charged with the extract is evacuated from the extractor and fed to the separator where the pressure is reduced so that the solute is not soluble and precipitates.

The main part of the experimental setup is the supercritical fluid extractor (Spe-ed SFE-Basic, Applied Separations, Allentown, PA, USA), which is shown in Fig. 3. Briefly, this unit contains a pneumatic pump for CO_2, an HPLC pump for the use of co-solvents and a cooler to reduce CO_2 temperature. The extraction section is made up of a cylindrical metal container (50 ml) which is equipped with a heating jacket to reach the desired temperature. In the exit of the extraction vessel there is a micrometering valve that controls the CO_2 flow rate. In addition, there is also another temperature controller for the micrometric valve at the system outlet. Regarding the collection of the extracts, as they are volatile compounds, they have to be collected in low temperature conditions, so the system has a cryocollector (Applied Separations) which uses CO_2 to cool the extract collection flask. After collection of the extract, it was introduced into a rotary evaporator, if necessary, to eliminate the co-solvent. The extraction yield was calculated with Equation 6 and the experiments were performed in triplicate.

$$(\%)\ Extraction\ Yield = \frac{weight\ of\ collected\ extract}{weight\ of\ fed\ lavender} \times 100 \qquad \text{Eq. 6}$$

Optimization of the main variables influencing the extraction performance, pressure and temperature, was carried out. We worked with pressures of 180, 250 and 300 bar and temperatures of 40, 60 and 80 ºC, in extraction experiments of 90 minutes long and with a CO_2 flow rate of 500 ml/min. In some experiments, extraction cosolvent, EtOH or EA, was added at a percentage of 0.2% v/v [32].

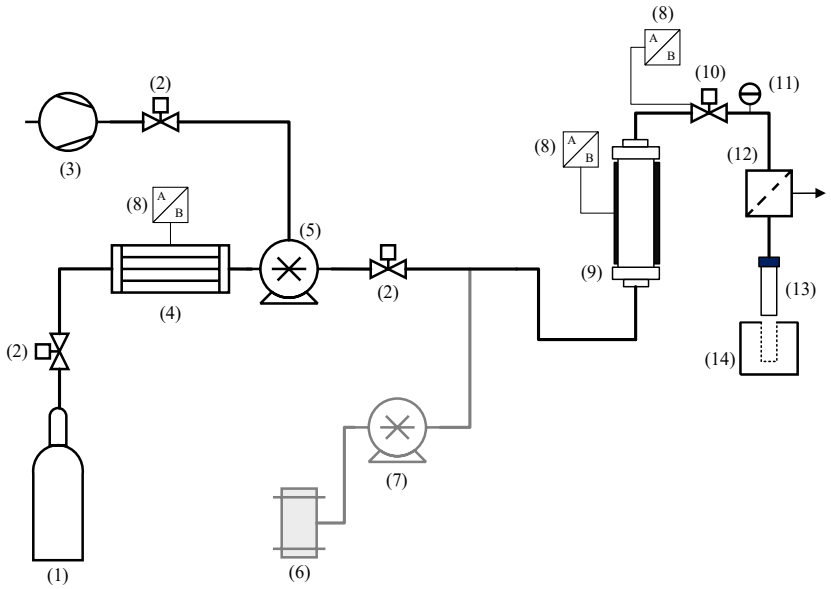


Figure 3. Diagram of SFE equipment. (1) CO_2 cylinder, (2) Blocking valve, (3) Air compressor, (4) Cooling bath, (5) CO_2 pump, (6) Co-solvent tank, (7) Co-solvent pump, (8) Temperature controller, (9) Extraction vessel and oven, (10) Micrometric valve, (11) Flowmeter, (12) Separator, (13) Collection vessel, (14) Cryo-collector.

2.6. Large Scale Supercritical CO_2 extraction.

Extraction experiments at pilot plant scale were also carried out (Figure 4). The plant used (Ainia Technological Centre in Valencia, Spain) consists basically of the same equipment as the laboratory extraction equipment. The main difference is the volume of

the extraction vessel, which was 20 L. Furthermore, in this case a separator was used, by means of which, in addition to selectively obtaining the products, the CO_2 can be recirculated to the storage tank.

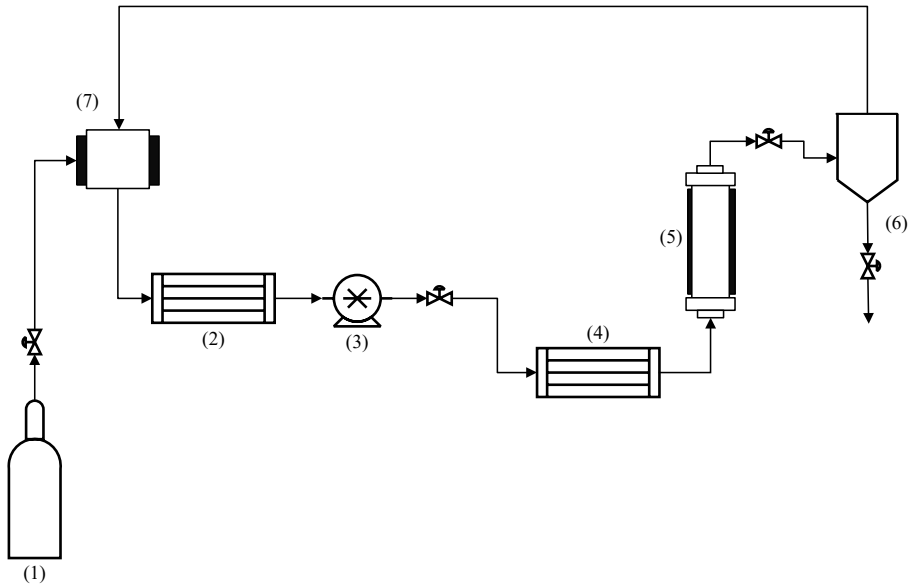


Figure 4. Supercritical extraction pilot plant schematic. (1) CO_2 cylinder, (2) Cooler, (3) CO_2 pump, (4) Heater, (5) Extraction vessel, (6) Separator, (7) Storage tank.

In this work, large scale kinetic experiments were carried out preserving the extraction temperature and pressure range as those used in the small-scale supercritical extraction and the CO_2 flow was optimized with the objective to obtain the maximum overall yield.

Mathematical modelling

Mathematical modelling of the extraction process is necessary to interpolate or extrapolate results to conditions other than those tested and to extend the extend the experimental data to different materials. It is also necessary for the scaling up of the

results obtained in the laboratory and pilot plant and for the implementation and sizing of the possible industrial process.

One of the most commonly used models is that of Sovová et col. [30,33] based on the mass transfer dynamics between the solid and fluid phases. This mathematical model considers that solid particles contain easily accessible solutes from broken cells, leading to a fast extraction rate, and hardly accessible models leading to a slow extraction rate related to the diffusion capacity in the solid phase.

The concentration profile in the solid phase is divided in three sections given by the following equations (Eq.7-Eq.9). In the first extraction step, the solubilization and external transport from the broken cells to the fluid are dominant. The second is the falling extraction rate period in which extraction relies also on more inaccessible regions, namely intact cells. The third and last branch is the diffusion-controlled period where usually only small amounts of extract are generated due to the prevalence of slow internal diffusion.

$$C_s = C_0[1 - Z\psi \exp(-Zh^*)] \quad \text{for} \quad \psi < m/Z \qquad \text{Eq. 7}$$

$$C_s = C_0\{1 - mexp[-Z(h^* - h_k)]\} \quad \text{for} \quad m/Z \leq \psi < \psi_k \text{ and } h^* > h_k \qquad \text{Eq. 8}$$

$$C_s = \frac{C_0}{1+\left\{\frac{exp[Y(\psi - m/Z]}{1-m} - 1\right\}\exp(-Yh^*)} \quad \text{for} \quad \psi \geq \psi_k \text{ and } m/Z \leq \psi \leq \psi_k \text{ and } h^* \leq h_k \qquad \text{Eq. 9}$$

Ψ is the dimensionless time defined by the equation 10.

$$\psi = \frac{WS\rho_s}{W_s C_o \rho} t \qquad \text{Eq. 10}$$

Where W is the solvent mass flow rate (kg/s) and W_s (kg) is the mass of the solid particles constituting the bed.

For the extraction yield the above equations lead to:

$$Y = 100\psi(1 - \exp(-Z)) \quad \text{for} \quad \psi < m/Z \qquad \text{Eq. 11}$$

$$Y = 100\left\{\psi - \frac{m}{Z} exp[Z(h_k - 1)]\right\} \quad \text{for} \quad m/Z \leq \psi \leq \psi_k \qquad \text{Eq. 12}$$

$$Y = 100\left[1 - \frac{ln\left\{1+[\exp(y)-1]exp\left[y\left(\frac{m}{Z}-\psi\right)\right](1-m)\right\}}{y}\right] \quad \text{for} \quad \psi \geq \psi_k \qquad \text{Eq. 13}$$

Where m represents the grinding efficiency ($0 < m < 1$), h^* the dimensionless axial coordinate, h_k time-varying bed level, ψ dimensionless time, ψ_k the dimensionless time at which the "free" solute is exhausted from the whole bed, and Z e y parameters proportional to mass transfer coefficients k_s and k_f. These variables and the dimensionless parameters are expressed by the following equations where H is the total bed height.

$$m = {C_{S,EASY}}/{C_0} \qquad \text{Eq. 14}$$

$$h^* = {h}/{H} \qquad \text{Eq. 15}$$

$$h_k = \frac{1}{y}\langle 1 + \frac{\left\{exp\left[y\left(\psi - \frac{m}{Z}\right)\right] - 1\right\}}{m}\rangle \quad \text{for} \quad m/Z \leq \psi \leq \psi_k \qquad \text{Eq. 16}$$

$$\psi_K = \frac{m}{Z} + \frac{1}{y}\ln\{1 - m[1 - \exp(y)]\} \qquad \text{Eq. 17}$$

$$Z = \frac{W_s k_f a \rho}{W(1-\varepsilon)\rho_s} \quad \text{Eq. 18}$$

276

$$y = \frac{W_s k_s a C_0 \rho}{W(1-\varepsilon)\rho_s} \quad \text{Eq. 19}$$

277

278 Model parameters values were estimated using the least squares method with Solver in
279 Microsoft Excel representing the error by the standard deviation (s.d.).

280

281 **2.7. Extracts characterization: composition and antioxidant potential**

282 Composition of the lavender extracts was evaluated through Gas chromatography coupled
283 to mass spectrometry (GC-MS), following the method reported by Daferera et al. [34].
284 The equipment used was a Varian Saturn 2000 GC-MS equipped with a HP-5 capillary
285 column (30 m, 0.25 mm i.d., 0.25 μm film thickness). The column temperature was set to
286 60 °C for 5 min. Then, it was increased to 160 °C at a rate of 4 °C/min. Finally,
287 temperature was raised to 240 °C at 15 °C/min. The injector temperature was 250 °C and
288 the detector temperature was 280 °C during the whole analysis. An electron ionisation
289 system with an energy of 70 eV was used. The samples were analysed after dilution with
290 diethyl ether (1:100 v/v) and 1 μL of the diluted samples was injected in splitless mode.
291 The carrier gas was helium at a flow rate of 1 mL/min.

292 Regarding the antioxidant capacity, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was
293 used to estimate the antioxidant capacity of the extracts [35,36]. DPPH• is a stable
294 nitrogen-centred free radical which is conventionally used to determine the free radical
295 scavenging activities of antioxidants present in plant extracts or synthetic compounds.
296 The reduction capability of DPPH• radical is determined by the decrease in absorbance

297 at 517 nm induced by the antioxidant. The procedure described in the work of L. T. Danh
298 et al. [5] was followed in the present study. Briefly, 40 μL of extracted essential oil was
299 mixed with 0.4 mL of 0.5 mM DPPH• solution in EtOH and the final volume was adjusted
300 to 1.5 mL with EtOH. The control solution was prepared with 0.4 mL of the ethanolic
301 DPPH solution and 1.1 mL of EtOH. In addition, a sample of the standard antioxidant
302 ascorbic acid was prepared to compare the antioxidant potential of the supercritical
303 extracts. The absorbance of the solutions was measured at 517 nm, with three replicates,
304 after 30 min of stirring in darkness to allow a complete reaction.

305 To quantify the antioxidant potential of the supercritical extracts, the inhibition
306 percentage (I%), which corresponds to the radical scavenging activity of the extracts, was
307 calculated considering Equation 7, where Ao corresponds to the absorbance of the control
308 sample and As to that of the extracted oil samples.

$$(\%)\ Inhibition = \frac{A_o - A_s}{A_o} \times 100 \qquad \text{Eq. 7}$$

309 **2.8. Data reproducibility analysis**

310 In the solubility study and the supercritical extraction experiments, the experiments were
311 performed in triplicate, with a maximum difference in values of 1%. Regarding the
312 characterisation of the samples obtained from the extraction, GC-MS analysis and a
313 DPPH assay was performed for two different extract samples from each experiment.
314 Concentration and absorbance were measured in triplicate to check reproducibility.

315 **3. Results and discussion**

316 The first group of experiments is focused on the study of the solubility of LEO in
317 supercritical CO_2 and the influence of contact time, pressure, and working temperature
318 on it. The solubility data were correlated using empirical equations that related the

319 solubilities to the density of pure gas. Once the process conditions that maximize the
320 solubility of LEO were determined, experiments were carried out on the supercritical
321 extraction of LEO from lavender flowers on a small scale. The main objective was to
322 determine the influence of pressure, temperature, and cosolvent addition on the extraction
323 yield.
324 Subsequently, kinetic experiments were conducted on a pilot plant and modeled using
325 Sovova's model. Moreover, their composition and antioxidant capacity were
326 characterized to determine their properties. The results obtained in the supercritical LEO
327 extraction were compared with the extracts obtained by the conventional Soxhlet
328 extraction method to see not only the differences in yield but also in content or quality of
329 the essential oil obtained.

330 **3.1. Study of solubility**

331 Determining the solubility of the solute of interest in the supercritical fluid is essential in
332 any transfer process, whether one wishes to know the final composition of a separation
333 or to determine the most favorable conditions for a given process. The solubility studies
334 were conducted over a temperature range of 40–80 °C, and a pressure range of 120–300
335 bar, which are generally considered to be suitable operating conditions for antioxidant
336 compounds extractions.

337 First, a set of preliminary experiments was performed to determine the contact time in the
338 equilibrium cell (range of 6–72 h) needed between the solute and CO_2 to reach the
339 saturation state. Figure 5 shows the variation of the kg of LEO in light phase per cubic
340 meter of CO_2 as a function of the contact time between them. For times shorter than 48
341 hours, there is a very significant increase in oil in the vapor phase, and from this time on,

this value stabilizes. Therefore, a time of 48 hours was chosen for performing subsequent solubility experiments.

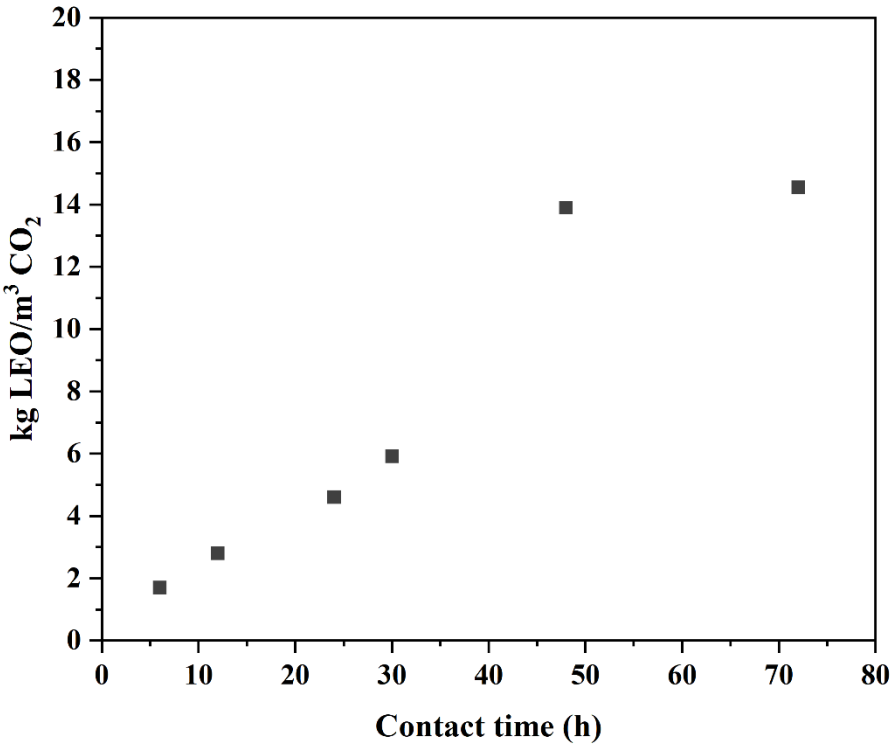


Figure 5. Study of the equilibrium time of LEO-supercritical CO_2 system (P=120 bar, T=40 ºC).

Table 1 shows the solubility results for the different operating conditions. Operating pressure in supercritical extraction processes is one of the main factors contributing to the extraction efficiency and yield of the desired component. An increase in the solubility of LEO in supercritical CO_2 with an increase in pressure is clearly observed at each of the temperatures studied [37]. This is because the density of supercritical CO_2 increases with pressure and, with it, its solvating power. An increase in pressure decreases the average intermolecular distance between solutes, which increases the interaction between solute and solvent [38,39].

355 Regarding the influence of temperature on solubility, it can be seen from Table 1 that its
356 increase does not contribute to the increase in solubility. An increment in temperature at
357 the same pressure decreases the density of the solvent and increases the vapour pressure
358 of the solute. The solvent's density effect is predominant in the region where pressure is
359 lower than the crossover point, while in the high pressure region, the vapor pressure effect
360 of the solute is dominant, so as the temperature increases in this region, solubility also
361 increases [40,41].

362

Table 1. Experimental solubility of LEO in supercritical CO_2

Temperature (ºC)	**Pressure (bar)**	**CO_2 density (kg/m^3)**	**Experimental solubility (kg LEO/m^3 CO_2)**
40	120	653.59	13.90
60	120	355.69	6.59
80	120	266.91	3.90
40	140	652.64	16.59
60	140	589.23	16.61
80	140	389.82	9.35
40	180	788.92	22.69
60	180	712.79	21.80
80	180	590.88	11.15
40	250	798.09	24.80
60	250	703.25	24.98
80	250	607.03	22.80

363

These results can provide valuable information for the development of lavender flowers extraction.

3.2. Semiempirical modelling

With the aim of generalizing the behaviour of lavender essential oil in supercritical CO_2, the solubility data were correlated using three semi-empirical models used in other studies that correlate the solubility with the density of pure gas, $scCO_2$: the model of Chrastil (Eq. 3), the model of Del Valle and Aguilera (Eq. 4) and, finally, the model of Adachi and Lu (Eq. 5).

Table 2 shows the calculated values for each one of the constants used in the different models. All constants were calculated by minimizing the sum of the squares of the differences between the experimental and calculated solubility. In Figure 6, it can be seen that the solubility prediction was compared to the experimental results. It is evident that all the empirical models fit the experimental results with exactitude and that standard deviations (s.d.) are low and identical in the Chrastil and Del Valle and Aguilera models. In the case of the third empirical model, Adachi and Lu, s.d. is slightly lower but similar despite the modifications of the first model. Therefore, it could be concluded that the Chrastil model can correctly estimate the LEO solubility in supercritical CO_2. These results are in line with the studies of De Lucas et al., focused on the prediction of vegetable oils solubility [42].

Table 2. Values of constant used for solubility model.

Empirical model	C_1	C_2	C_3	C_4	C_5	s.d.
Chrastil	-6.789	$-4.551\ 10^{-02}$	1.486			0.150
Del Valle y Aguilera	-7.910	$-5.309\ 10^{-02}$	$-4.914\ 10^{-04}$	1.664		0.139
Adachi y Lu	-18.308	10.000	3.865	$-1.540\ 10^{-03}$	8.24010^{-07}	0.237

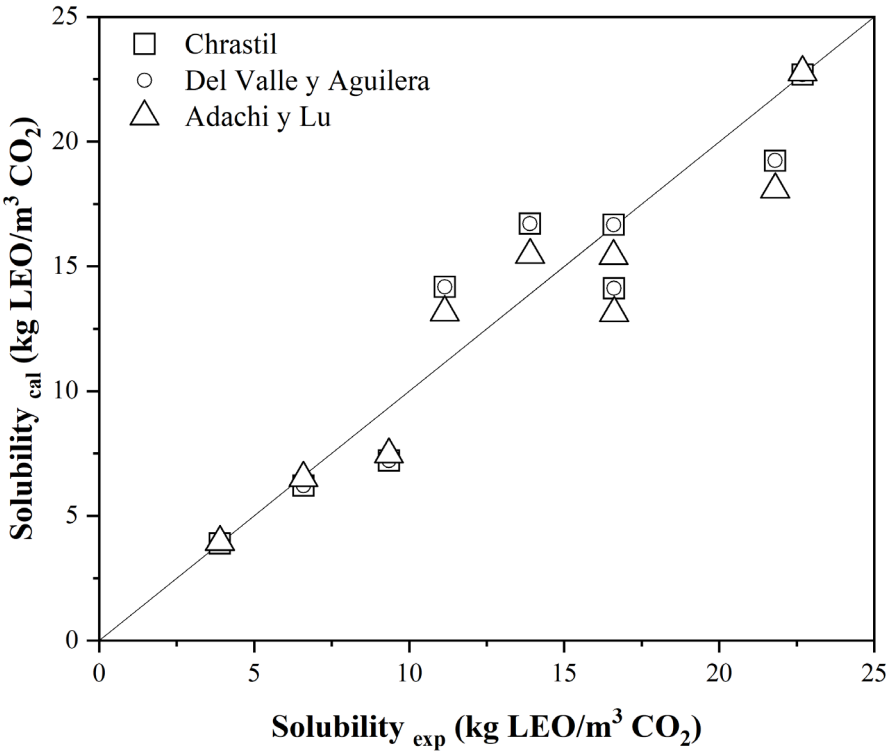


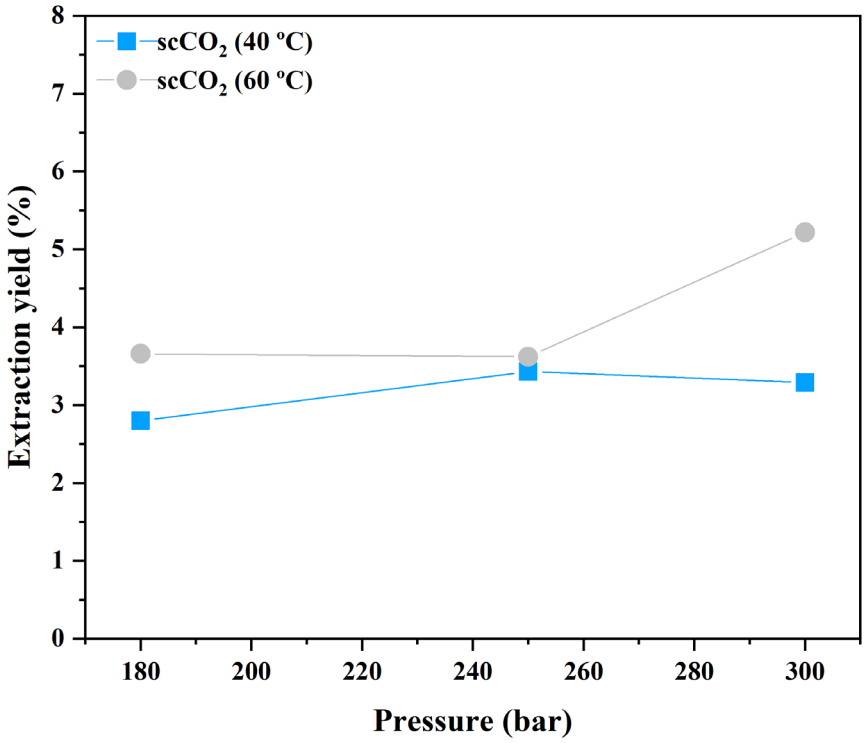
Figure 6. Comparison between experimental and calculated solubilities of LEO in supercritical CO_2.

3.3. Supercritical fluid extraction: small scale study

With the aim of optimizing the extraction process based on the extraction yield, the influence of pressure and temperature as well as the addition of an extraction co-solvent were evaluated. Figure 7 shows all the data on LEO extraction performance at pressures of 180-300 bar and temperatures of 40 and 60 °C.

Firstly, extraction yield increases with increasing temperature at constant pressure. In this way, the efficiency of the extraction process is higher at 60 ºC than in the tests carried out at 40 ºC. This can be explained by the increase in vapour pressure at higher temperatures, as discussed in previous sections. It should be noted that experiments were also carried out at 80 ºC but the yield was very low. For this reason, this working temperature was discarded.

Regarding the influence of pressure, it can observed that extraction yield increases with increasing pressure at a constant temperature. Since the variation of solvent density with pressure also follows this trend, it is obvious that the results obtained are related to the value of this parameter. It should be noted that the trend of increasing performance with pressure was not observed in the experiment conducted at 300 bar and 40 °C. In the work of Díaz-Reinoso et al. [43] they obtained a similar behaviour and attributed this phenomenon to the fact that the crossover point for this system, relative to the increase of the vapour pressure of the solute in the extraction, could be around 250 bar [43,44].



411

412 **Figure 7.** Influence of pressure on lavender flowers extraction yield of at 40ºC and

413 60ºC.

414 Figure 8 shows the results corresponding to the study of the influence of the addition of

415 cosolvent (0.2% v/v). It shows that the extraction yield is higher when EtOH is used as a

416 co-solvent, reaching yield values two times higher than when extracting only with CO_2.

417 CO_2, being intrinsically non-polar, allows excellent extraction of both non-polar

418 compounds and some low molecular weight volatile polar compounds. Nevertheless, it is

419 less efficient at extracting polar compounds that are embedded in the cell wall. The

420 addition of organic co-solvents can enhance the solvation power of CO_2, improving the

421 recovery of bioactive compounds. This solubility enhancement is mainly caused by the

422 formation of special interactions between the solute and the cosolvent molecules

[45]. The results obtained agree with those obtained in other studies with polar cosolvents for the extraction of vegetable oil [42,46,47].

Figure 8 also shows that the pressure favors the extraction yield, reaching the maximum value at 300 bar for both working temperatures (13.22% at 40 º C and 12.85% at 60 º C). Regarding the influence of temperature, in this case, extraction yields were slightly higher in the case of the experiments carried out at 40 º C, which shows that the importance of the vapor pressure is lower when a co-solvent is added.

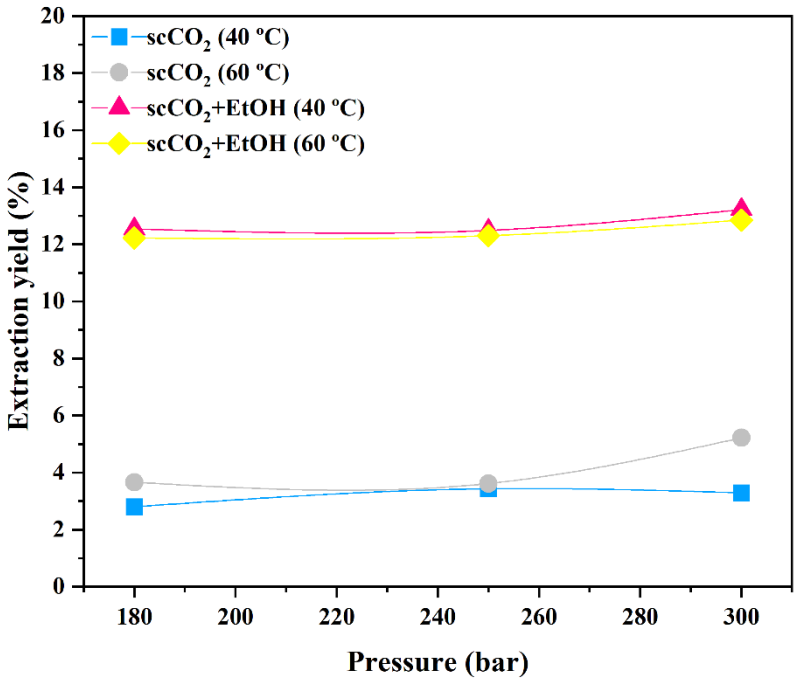
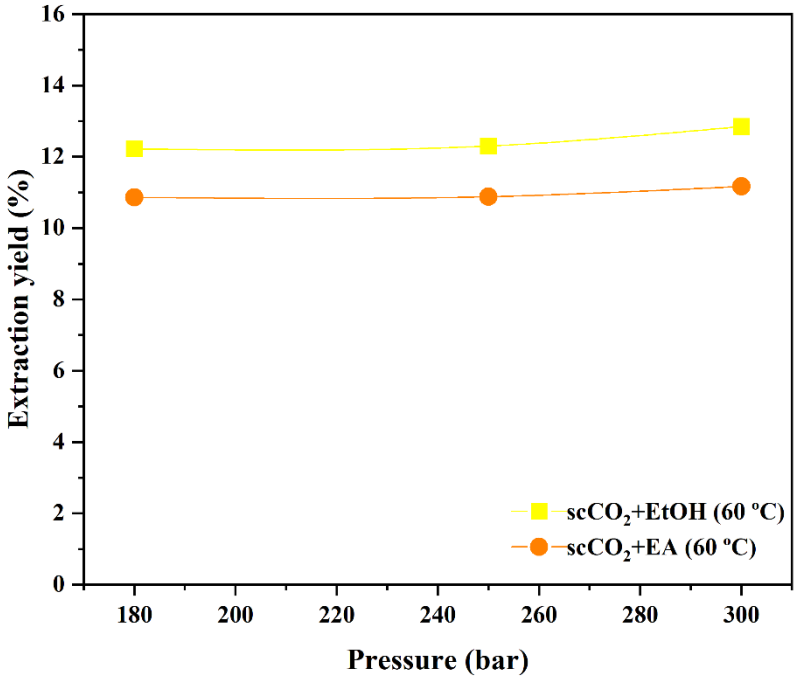


Figure 8. Influence of cosolvent addition on lavender flowers extraction yield of at 40ºC and 60ºC.

In addition, ethyl acetate (EA) was also studied as a co-solvent to test whether the performance offered by EtOH could be improved. Figure 9 shows a comparison of the results of the co-solvent extractions at 60º C. It can be observed that the use of EA as a

436 cosolvent does not improve the results (in terms of the extraction yield) obtained with
437 EtOH, as reported in previous work [48,49].



438

439 **Figure 9**. Influence of type of cosolvent on lavender flowers extraction yield at 60ºC
440 ( ) EtOH; ( ) ethyl acetate EA

441 **3.4. Supercritical fluid extraction: large scale study**

442 Lab scale experiments showed that optimal extraction working ranges were 180-250 bar
443 and 40-60 ºC, respectively. Hence, a further pilot scale study was carried out to study the
444 supercritical extraction of LEO in this range, to validate previous equilibrium data, and
445 to determine kinetic parameters (solid and liquid phases mass transfer coefficients) for
446 the extraction curves.

447 Table 3 shows the conditions and overall extraction yield results for pilot plant
448 experiments. From Table 3, it can be seen that the pressure and temperature have a

449 positive effect on the extraction yield in all cases in the range tested, confirming the trends
450 observed in previous laboratory research.

451 **Table 3.** LEO extraction experiments in pilot plant.

Pressure (bar)	180	250	180	180	250
Temperature (ºC)	60	60	40	60	60
Mass Flow CO_2 (kg/h)	60	60	60	60	60
Cosolvent EtOH (%)	-	-	0.2	0.2	0.2
Time (min)	120	120	120	120	120
Ext. Yield (%)	**6.2**	**6.9**	**4.6**	**11.2**	**14.7**

452

453 Figure 10 shows the extraction curves for all the experiments performed in the pilot plant.
454 The kinetics correspond to a classical profile in which the first linear stage corresponds
455 to the fast-extraction of the easily accessible solute located on the particle surface
456 (governed by solubility and k_f parameter in Eq. 18), followed by a slow-extraction period
457 in which there is an increase in the solute internal resistance diffusion from the matrix to
458 the surface (represented by k_s, in Eq. 19). Moreover, Figure 10 compares the
459 experimental extraction yields (solid points) and linear predictions by Sovová's
460 mathematical model [30,33].

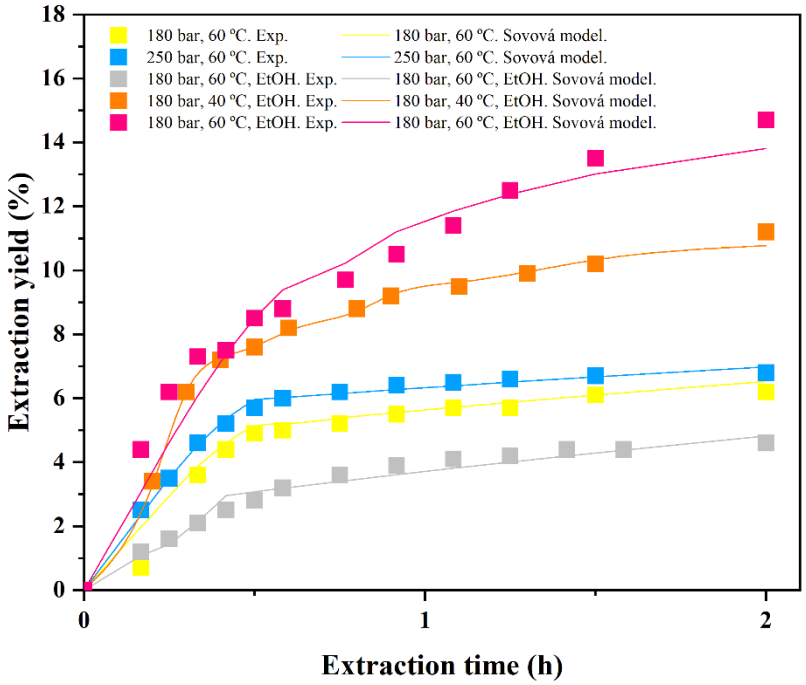


Figure 10. Kinetic study of lavender flowers extraction at large scale. Experimental results (closed symbols) and sovova's model predictions (solid lines).

Table 4 lists the parameters obtained from the mathematical analysis of the data. In all cases, it can be observed a good fit with the model, confirming Sovova's hypotheses. As regards k_f, this value, it increases with pressure in all cases. However, the k_s slightly decreases with pressure. This last effect can be explained since pressure has two opposite effects: pressure increases viscosity and increases solute-solvent interactions [50,51]. k_f is not influenced by viscosity because this parameter gives information about external diffusion to the fluid phase, while for k_s the diffusion effect, the inner matrix is also affected by viscosity.

This behaviour can be confirmed when considering the variation of temperature. As observed, in the 180 bar, 40 °C and 60 °C tests with co-solvent, increasing temperature, decreasing viscosity, maintaining k_f value, while increasing k_s. Last, it can be observed

475 that the fitting parameter in the table represents the easy-accessible oil fraction, remaining
476 almost constant in all cases.

477 **Table 4.** Sovová model parameters.

Pressure (bar)	180	250	180	180	250
Temperature (ºC)	60	60	40	60	60
Cosolvent EtOH (%)	-	-	0.2	0.2	0.2
$k_f a$ (s^{-1})	0.007	0.008	0.002	0.002	0.009
$k_s a$ (s^{-1})	$1.22\ 10^{-5}$	$1.04\ 10^{-5}$	$1.05\ 10^{-5}$	$7.69\ 10^{-5}$	$7.00\ 10^{-5}$
m	0.50	0.58	0.301	0.58	0.58
s.d.	0.016	0.003	0.005	0.069	0.016

478

479 **3.5. Extracts characterization**

480 The results obtained from the GC-MS characterization of the extracts are shown in Table
481 5. These results correspond to two extractions performed at the same temperature in order
482 to check how much pressure variation influences the composition. In the case of
483 extractions with pure CO_2, it is clear that the two main compounds are linalool and linalyl
484 acetate, with mass percentages of 32 and 43% respectively. These results are similar to
485 the mass percentages of the commercial oil (Sigma-Aldrich) which gave a mass
486 percentage of linalool of 28% and linalyl acetate of 31%. This is therefore an indication
487 that the supercritical CO_2 extraction technology is suitable for obtaining LEO and can
488 also be used for applications requiring the therapeutic properties of these compounds [7].
489 Other minor components were also identified, like eucalyptol, camphor, endoborneol,
490 terpinen-4-ol, α-terpineol, nerol acetate, caryophyllene and β-famesene, which are also

present in many other essential oils. Concerning the influence of operating pressure on the composition of extracts, no clear trend is observed, and the changes are very subtle.

From Table 5, it can also be seen that the presence of these components clearly decreased in the experiments where EtOH was used as a cosolvent. This is probably due to the fact that EtOH favours the extraction of waxes or substances with higher molecular weights present in natural oils [52,53].

Table 5. GC-MS analysis of obtained LEO under supercritical and Soxhlet extraction conditions.

% wt	**180 bar, 60 ºC**		**250 bar, 60 ºC**		**Soxhlet**
	$scCO_2$	**$scCO_2$+EtOH**	**$scCO_2$**	**$scCO_2$+EtOH**	**Hexane 69 ºC**
Eucalyptol	3.48	1.85	4.11	1.90	3.25
Linalool	32.07	12.95	32.20	14.74	27.76
Camphor	4.24	2.42	4.58	2.55	7.68
Endoborneol	4.07	1.81	3.91	2.06	2.21
Terpinen-4-ol	3.60	1.54	3.58	1.76	4.05
α-Terpineol	1.49	0.42	1.43	0.48	0.26
Linalyl acetate	43.03	17.53	43.01	20.46	49.55
Nerol acetate	2.44	1.05	2.34	1.19	2.95
Caryophyllene	2.95	0.93	2.58	1.14	2.60
β-Famesene	2.64	1.16	2.25	1.34	0

Figure 11 shows the inhibition provided by the standard substance, ascorbic acid, and % compared to LEO supercritical extract. In the samples obtained at 250 bar the inhibition percentage is higher, above 85%, than in the samples obtained at 180 bar, whose values do not exceed 82%. On the other hand, in the experiments in which EtOH was added as a co-solvent, no decrease in antioxidant capacity was observed. So, it could be concluded that the essential oil obtained has a great potential for antioxidant applications.

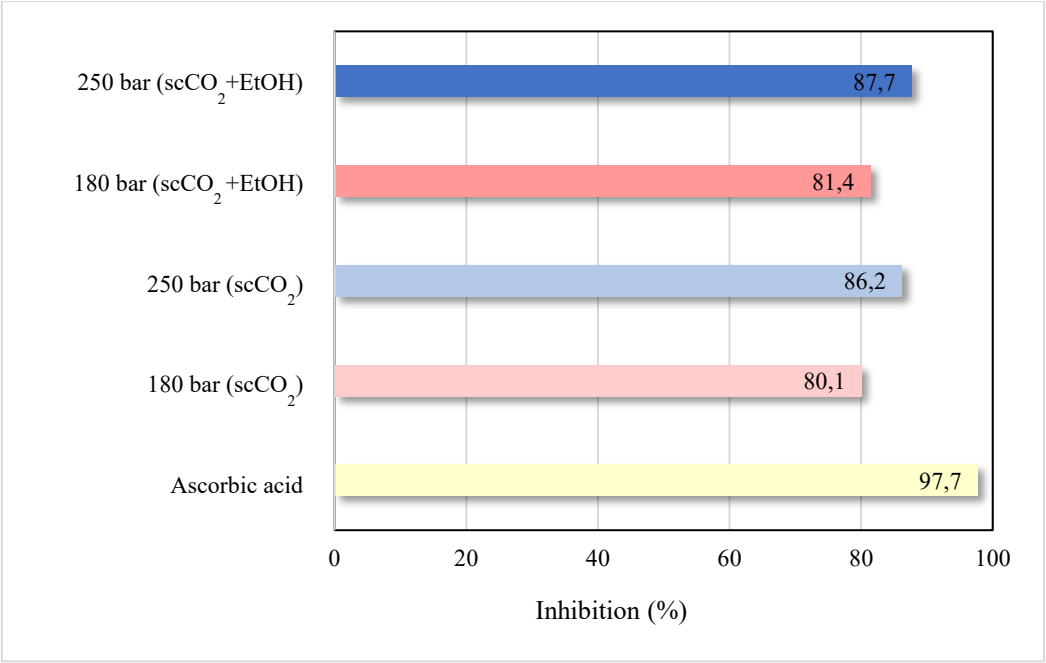


Figure 11. Inhibition of ascorbic acid and LEO supercritical extracts.

3.6. Soxhlet

Hexane extractions were carried out for 150 minutes at atmospheric pressure and 69 °C. Analyses of the antioxidant potential of the LEO obtained as well as the calculation of the extraction yield were carried out (Figure 12). Table 5 also shows the composition of the extracts obtained by this technique.

In terms of extraction yield, it can be observed that the extraction yield is higher than in the extraction with supercritical CO_2, something that was also observed in previous

similar studies [54]. In contrast, the inhibition potential of Soxhlet extracts is 20%, which is much lower than in the case of $scCO_2$ extractions, so it can be deduced that their antioxidant potential is lower.

Regarding composition (Table 5), in other studies, it is considered that, despite the good results of the Soxhlet extraction process in terms of yield, supercritical extracts are still of higher quality and more interesting for the industrial implementation of the process [55]. In the case of this study, Soxhlet extraction with hexane is less selective towards linalool which, due to its therapeutic properties, is considered the most interesting compound for LEO application.

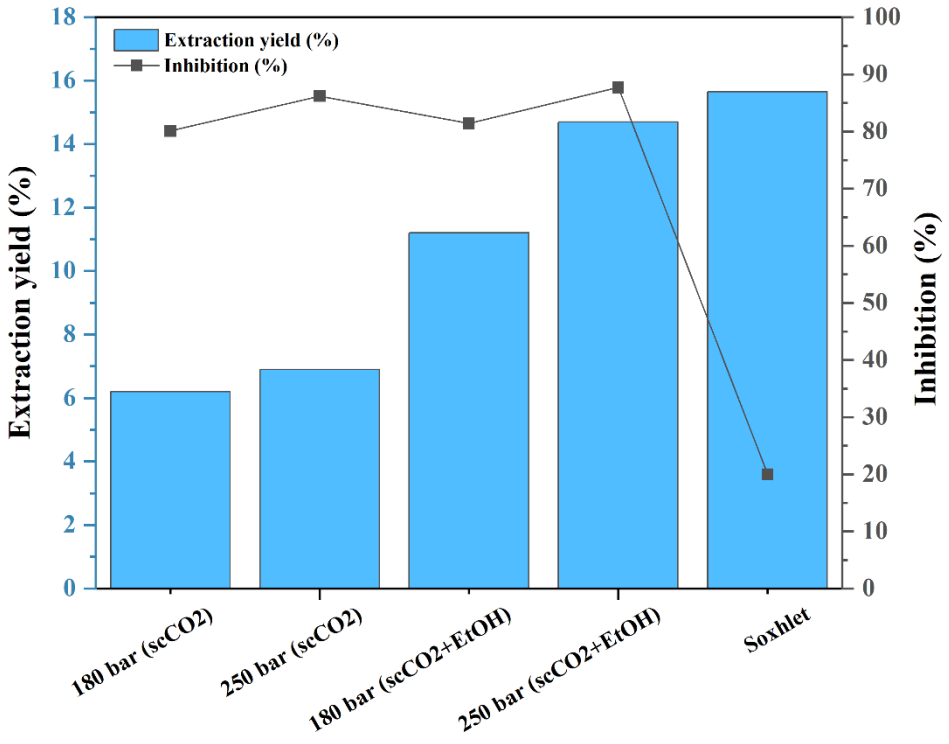


Figure 12. Comparison of $scCO_2$ and Soxhlet extraction: large scale extracition yield from lavender flowers (primary axys) and Inhibition (secondary axis) results.

4. Conclusions

In this study, it was performed an overall lab-to-pilot plant study for $scCO_2$ extraction of lavender flowers, being obtained for the first time the kinetic mass transfer parameters in the large scale. The solubility study showed that the time to reach equilibrium was 48 h, and that an increase in pressure favours the solubility of LEO, while an increase in temperature decreases its value. The data obtained best fitted the semi-empirical model of Del Valle and Aguilera. These data were used as a basis for the extraction of LEO with CO_2 from lavender flowers, and the results indicated that the yield was favoured by pressure and temperature. Furthermore, the use of cosolvent, produced a two-fold of extraction yield. Once the most suitable extraction conditions were established, extraction was carried out on a larger scale, pilot plant size. The results reproduced those previously obtained in laboratory setup and were in good agreement with Sovova's mathematical model. Extracts were analysed by GC-MS, being the major compounds identified linalool, and linalyl acetate. It was observed that the supercritical extracts presented an antioxidant potential higher than 80 % (referred to ascorbic acid) in all operating conditions.

5. Acknowledgements

This research was funded by the Project Ref. SBPLY/17/180501/000311 from Consejería de Educación, Cultura y Deportes of Junta de Comunidades de Castilla La Mancha.

548 **6. References**

549 [1] D. Sabara, A. Kunicka-Styczyńska, LAVENDER OIL − FLAVOURING OR
550 ACTIVE COSMETIC INGREDIENT?, Food Chem. Biotechnol. 73 (2009) 33–
551 41.

552 [2] A. Vakili, S. Sharifat, M.M. Akhavan, A.R. Bandegi, Effect of lavender oil
553 (Lavandula angustifolia) on cerebral edema and its possible mechanisms in an
554 experimental model of stroke, Brain Res. 1548 (2014) 56–62.
555 https://doi.org/https://doi.org/10.1016/j.brainres.2013.12.019.

556 [3] N. Aboutaleb, H. Jamali, M. Abolhasani, H. Pazoki Toroudi, Lavender oil
557 (Lavandula angustifolia) attenuates renal ischemia/reperfusion injury in rats
558 through suppression of inflammation, oxidative stress and apoptosis., Biomed.
559 Pharmacother. 110 (2019) 9–19. https://doi.org/10.1016/j.biopha.2018.11.045.

560 [4] Y. Xu, L. Ma, F. Liu, L. Yao, W. Wang, S. Yang, T. Han, Lavender essential oil
561 fractions alleviate sleep disorders induced by the combination of anxiety and
562 caffeine in mice, J. Ethnopharmacol. 302 (2023) 115868.
563 https://doi.org/https://doi.org/10.1016/j.jep.2022.115868.

564 [5] L.T. Danh, N.D.A. Triet, L.T.N. Han, J. Zhao, R. Mammucari, N. Foster,
565 Antioxidant activity, yield and chemical composition of lavender essential oil
566 extracted by supercritical CO2, J. Supercrit. Fluids. 70 (2012) 27–34.
567 https://doi.org/https://doi.org/10.1016/j.supflu.2012.06.008.

568 [6] E. Barocelli, F. Calcina, M. Chiavarini, M. Impicciatore, R. Bruni, A. Bianchi, V.
569 Ballabeni, Antinociceptive and gastroprotective effects of inhaled and orally
570 administered Lavandula hybrida Reverchon “Grosso” essential oil, Life Sci. 76
571 (2004) 213–223. https://doi.org/https://doi.org/10.1016/j.lfs.2004.08.008.

[7] A. Zanotti, L. Baldino, M. Scognamiglio, E. Reverchon, Post-processing of a lavender flowers solvent extract using supercritical CO2 fractionation, J. Taiwan Inst. Chem. Eng. 147 (2023) 104901. https://doi.org/https://doi.org/10.1016/j.jtice.2023.104901.

[8] H.-M. Mori, H. Kawanami, H. Kawahata, M. Aoki, Wound healing potential of lavender oil by acceleration of granulation and wound contraction through induction of TGF-β in a rat model, BMC Complement. Altern. Med. 16 (2016) 144. https://doi.org/10.1186/s12906-016-1128-7.

[9] E. Cruz Sánchez, M.T. García, J. Pereira, F. Oliveira, R. Craveiro, A. Paiva, I. Gracia, J.M. García-Vargas, A.R.C. Duarte, Alginate-Chitosan Membranes for the Encapsulation of Lavender Essential Oil and Development of Biomedical Applications Related to Wound Healing, Molecules. 28 (2023). https://doi.org/10.3390/molecules28093689.

[10] D. Andrys, M. Adaszyńska-Skwirzyńska, D. Kulpa, Essential oil obtained from micropropagated lavender, its effect on HSF cells and application in cosmetic emulsion as a natural protective substance., Nat. Prod. Res. 32 (2018) 849–853. https://doi.org/10.1080/14786419.2017.1361950.

[11] Y. Rajesh, N.M. Khan, A. Raziq Shaikh, V.S. Mane, G. Daware, G. Dabhade, Investigation of geranium oil extraction performance by using soxhlet extraction, Mater. Today Proc. 72 (2023) 2610–2617. https://doi.org/https://doi.org/10.1016/j.matpr.2022.07.276.

[12] V. Harish, M.M. Ansari, D. Tewari, A.B. Yadav, N. Sharma, S. Bawarig, M.-L. García-Betancourt, A. Karatutlu, M. Bechelany, A. Barhoum, Cutting-edge advances in tailoring size, shape, and functionality of nanoparticles and

596 nanostructures: A review, J. Taiwan Inst. Chem. Eng. 149 (2023) 105010.
597 https://doi.org/https://doi.org/10.1016/j.jtice.2023.105010.

598 [13] E.G.O. Menezes, A.P. de Souza e Silva, K.R.P. de Sousa, F. de F.M. de Azevedo,
599 R.M. Morais, R.N. de Carvalho Junior, Development of an innovative strategy
600 capable of describing the large-scale extraction of tucumã-of-Pará oil
601 (Astrocaryum vulgare Mart.) using supercritical CO2 as solvent, J. Supercrit.
602 Fluids. 193 (2023) 105825.
603 https://doi.org/https://doi.org/10.1016/j.supflu.2022.105825.

604 [14] E. Cruz Sánchez, J.M. García-Vargas, I. Gracia, J.F. Rodríguez, M.T. García,
605 Pilot-Plant-Scale Extraction of Antioxidant Compounds from Lavender:
606 Experimental Data and Methodology for an Economic Assessment, Processes. 10
607 (2022). https://doi.org/10.3390/pr10122708.

608 [15] M.A. Ayub, N. Choobkar, M.A. Hanif, M. Abbas, Q.U. Ain, M. Riaz, A.D.
609 Garmakhany, Chemical composition, antioxidant, and antimicrobial activities of
610 P. roxburghii oleoresin essential oils extracted by steam distillation, superheated
611 steam, and supercritical fluid CO2 extraction, J. Food Sci. 88 (2023) 2425–2438.
612 https://doi.org/10.1111/1750-3841.16597.

613 [16] E. Reverchon, I. De Marco, Supercritical fluid extraction and fractionation of
614 natural matter, J. Supercrit. Fluids. 38 (2006) 146–166.
615 https://doi.org/10.1016/J.SUPFLU.2006.03.020.

616 [17] M. Leila, D. Ratiba, A.-H. Al-Marzouqi, Experimental and mathematical
617 modelling data of green process of essential oil extraction: Supercritical CO2
618 extraction, Mater. Today Proc. 49 (2022) 1023–1029.
619 https://doi.org/https://doi.org/10.1016/j.matpr.2021.08.125.

620 [18] T. Fornari, G. Vicente, E. Vázquez, M.R. García-Risco, G. Reglero, Isolation of
621 essential oil from different plants and herbs by supercritical fluid extraction, J.
622 Chromatogr. A. 1250 (2012) 34–48.
623 https://doi.org/https://doi.org/10.1016/j.chroma.2012.04.051.

624 [19] B. Hashemi, F. Shiri, F. Švec, L. Nováková, Green solvents and approaches
625 recently applied for extraction of natural bioactive compounds, TrAC Trends Anal.
626 Chem. 157 (2022) 116732.
627 https://doi.org/https://doi.org/10.1016/j.trac.2022.116732.

628 [20] O. Wrona, K. Rafińska, C. Możeński, B. Buszewski, Supercritical Fluid Extraction
629 of Bioactive Compounds from Plant Materials, J. AOAC Int. 100 (2017) 1624–
630 1635. https://doi.org/10.5740/jaoacint.17-0232.

631 [21] J.C. Kessler, V. Vieira, I.M. Martins, Y.A. Manrique, P. Ferreira, R.C. Calhelha,
632 A. Afonso, L. Barros, A.E. Rodrigues, M.M. Dias, The potential of almonds,
633 hazelnuts, and walnuts SFE-CO2 extracts as sources of bread flavouring
634 ingredients, Food Chem. 417 (2023).
635 https://doi.org/10.1016/j.foodchem.2023.135845.

636 [22] J. Villacís-Chiriboga, S. Voorspoels, M. Uyttebroek, J. Ruales, J. V Camp, E. Vera,
637 K. Elst, Supercritical CO2 extraction of bioactive compounds from mango
638 (Mangifera indica L.) peel and pulp, Foods. 10 (2021).
639 https://doi.org/10.3390/foods10092201.

640 [23] J. Vladić, I. Jerković, S. Svilović, V. Pavić, K. Pastor, A. Paiva, S. Jokić, S.
641 Rebocho, A.R. Duarte, Evaluation of the volatiles' chemical profile and
642 antibacterial activity of Lavandula stoechas L. extracts obtained by supercritical
643 carbon dioxide, Sustain. Chem. Pharm. 33 (2023).

644 https://doi.org/10.1016/j.scp.2023.101126.

645 [24] A. Mouahid, I. Bombarda, M. Claeys-Bruno, S. Amat, E. Myotte, J.-P. Nisteron,
646 C. Crampon, E. Badens, Supercritical CO2 extraction of Moroccan argan (Argania
647 spinosa L.) oil: Extraction kinetics and solubility determination, J. CO2 Util. 46
648 (2021) 101458. https://doi.org/https://doi.org/10.1016/j.jcou.2021.101458.

649 [25] C. Gutiérrez, J.F. Rodríguez, I. Gracia, A. de Lucas, M.T. García, High-pressure
650 phase equilibria of Polystyrene dissolutions in Limonene in presence of CO2, J.
651 Supercrit. Fluids. 84 (2013) 211–220.
652 https://doi.org/10.1016/J.SUPFLU.2013.08.017.

653 [26] I. Álvarez, C. Gutiérrez, A. de Lucas, J.F. Rodríguez, M.T. García, Measurement,
654 correlation and modelling of high-pressure phase equilibrium of PLGA solutions
655 in CO2, J. Supercrit. Fluids. 155 (2020) 104637.
656 https://doi.org/https://doi.org/10.1016/j.supflu.2019.104637.

657 [27] B. Platzer, G. Maurer, Application of a generalized Bender equation of state to the
658 description of vapour-liquid equilibria in binary systems, Fluid Phase Equilib. 84
659 (1993) 79–110. https://doi.org/10.1016/0378-3812(93)85118-6.

660 [28] J. Chrastil, Solubility of solids and liquids in supercritical gases, J. Phys. Chem. 86
661 (1982) 3016–3021. https://doi.org/10.1021/j100212a041.

662 [29] J.M. Del Valle, J.M. Aguilera, An improved equation for predicting the solubility
663 of vegetable oils in supercritical carbon dioxide, Ind. Eng. Chem. Res. 27 (1988)
664 1551–1553. https://doi.org/10.1021/ie00080a036.

665 [30] H. Sovová, Rate of the vegetable oil extraction with supercritical CO2—I.
666 Modelling of extraction curves, Chem. Eng. Sci. 49 (1994) 409–414.

667 https://doi.org/10.1016/0009-2509(94)87012-8.

668 [31] Y. Adachi, B.C.-Y. Lu, H. Sugie, Three-parameter equations of state, Fluid Phase
669 Equilib. 13 (1983) 133–142. https://doi.org/https://doi.org/10.1016/0378-
670 3812(83)80087-9.

671 [32] I. Gracia, Obtención de aceite de orujo mediante extracción con fluidos
672 supercríticos. PhD Thesis., Ediciones de la Universidad de Castilla-La Mancha,
673 2001. https://books.google.dj/books?id=VThRgbxzvgkC.

674 [33] H. Sovová, J. Jez, M. Bártlová, J. St'astová, Supercritical carbon dioxide extraction
675 of black pepper, J. Supercrit. Fluids. 8 (1995) 295–301.
676 https://doi.org/https://doi.org/10.1016/0896-8446(95)90004-7.

677 [34] D.J. Daferera, B.N. Ziogas, M.G. Polissiou, GC-MS analysis of essential oils from
678 some Greek aromatic plants and their fungitoxicity on Penicillium digitatum., J.
679 Agric. Food Chem. 48 (2000) 2576–2581. https://doi.org/10.1021/jf990835x.

680 [35] W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to
681 evaluate antioxidant activity, LWT - Food Sci. Technol. 28 (1995) 25–30.
682 https://doi.org/https://doi.org/10.1016/S0023-6438(95)80008-5.

683 [36] B.-Y. Chen, C.-C. Hsueh, P.-W. Tsai, Y.-H. Lin, P.-S. Tsai, T.-K. Lien, C.-W.
684 Yang, L.-D. Jiang, Deciphering biotransformation of anthraquinone electron
685 shuttles in Rheum palmatum L. for value-added production, J. Taiwan Inst. Chem.
686 Eng. 139 (2022) 104508.
687 https://doi.org/https://doi.org/10.1016/j.jtice.2022.104508.

688 [37] G. Di Giacomo, V. Brandani, G. Del Re, V. Mucciante, Solubility of essential oil
689 components in compressed supercritical carbon dioxide, Fluid Phase Equilib. 52

690 (1989) 405–411. https://doi.org/https://doi.org/10.1016/0378-3812(89)80346-2.

691 [38] L.G.R. King J. W., Supercritical fluid technology in oil and lipid chemistry, in: Ed.
692 AOCS P, 1996.

693 [39] A. Ray, K.K. Dubey, S.J. Marathe, R. Singhal, Supercritical fluid extraction of
694 bioactives from fruit waste and its therapeutic potential, Food Biosci. 52 (2023)
695 102418. https://doi.org/10.1016/J.FBIO.2023.102418.

696 [40] V.M. y T.M.T. Luque de Castro, M. D., Extracción con fluidos supercríticos en el
697 proceso analítico, Ed. Revert, Barcelona, 1993.

698 [41] G. Park, T. Kim, Y.-W. Lee, A method for measuring the solubility of Disperse
699 Red 60 in supercritical carbon dioxide using variable-volume view cell with in-situ
700 UV–Vis spectrometer, J. Supercrit. Fluids. 176 (2021) 105302.
701 https://doi.org/https://doi.org/10.1016/j.supflu.2021.105302.

702 [42] A. de Lucas, I. Gracia, J. Rincón, M.T. García, Solubility Determination and
703 Model Prediction of Olive Husk Oil in Supercritical Carbon Dioxide and
704 Cosolvents, Ind. Eng. Chem. Res. 46 (2007) 5061–5066.
705 https://doi.org/10.1021/ie061153j.

706 [43] B. Díaz-Reinoso, A. Moure, H. Domínguez, ETHANOL-MODIFIED
707 SUPERCRITICAL CO2 EXTRACTION OF CHESTNUT BURS
708 ANTIOXIDANTS, Chem. Eng. Process. - Process Intensif. 156 (2020) 108092.
709 https://doi.org/10.1016/J.CEP.2020.108092.

710 [44] I.M. Prado, G.H.C. Prado, J.M. Prado, M.A.A. Meireles, Supercritical CO2 and
711 low-pressure solvent extraction of mango (Mangifera indica) leaves: Global yield,
712 extraction kinetics, chemical composition and cost of manufacturing, Food

713 Bioprod. Process. 91 (2013) 656–664. https://doi.org/10.1016/J.FBP.2013.05.007.

714 [45] Ö. Güçlü Üstündağ, F. Temelli, Solubility behavior of ternary systems of lipids,
715 cosolvents and supercritical carbon dioxide and processing aspects, J. Supercrit.
716 Fluids. 36 (2005) 1–15. https://doi.org/10.1016/j.supflu.2005.03.002.

717 [46] T.M. Young, W.J. Weber, Equilibrium and Rate Study of Analyte−Matrix
718 Interactions in Supercritical Fluid Extraction, Anal. Chem. 69 (1997) 1612–1619.
719 https://doi.org/10.1021/ac961014j.

720 [47] K.-Y. Khaw, M.-O. Parat, P.N. Shaw, J.R. Falconer, Solvent Supercritical Fluid
721 Technologies to Extract Bioactive Compounds from Natural Sources: A Review,
722 Molecules. 22 (2017). https://doi.org/10.3390/molecules22071186.

723 [48] D.V. Bermejo, E. Ibáñez, G. Reglero, T. Fornari, Effect of cosolvents (ethyl
724 lactate, ethyl acetate and ethanol) on the supercritical CO2 extraction of caffeine
725 from green tea, J. Supercrit. Fluids. 107 (2016) 507–512.
726 https://doi.org/10.1016/J.SUPFLU.2015.07.008.

727 [49] K.A. Santos, P.C. Frohlich, J. Hoscheid, T.S. Tiuman, J.E. Gonçalves, L. Cardozo-
728 Filho, E.A. da Silva, Candeia (Eremanthus erythroppapus) oil extraction using
729 supercritical CO2 with ethanol and ethyl acetate cosolvents, J. Supercrit. Fluids.
730 128 (2017) 323–330. https://doi.org/https://doi.org/10.1016/j.supflu.2017.03.029.

731 [50] S.S.T. Ting, D.L. Tomasko, N.R. Foster, S.J. Macnaughton, Solubility of naproxen
732 in supercritical carbon dioxide with and without cosolvents, Ind. Eng. Chem. Res.
733 32 (1993) 1471–1481. https://doi.org/10.1021/ie00019a022.

734 [51] N.R. Foster, H. Singh, S.L.J. Yun, D.L. Tomasko, S.J. Macnaughton, Polar and
735 nonpolar cosolvent effects on the solubility of cholesterol in supercritical fluids,

736 Ind. Eng. Chem. Res. 32 (1993) 2849–2853. https://doi.org/10.1021/ie00023a056.

737 [52] M. Guan, X. Xu, X. Tang, Y. Li, Optimization of supercritical CO2 extraction by
738 response surface methodology, composition analysis and economic evaluation of
739 bamboo green wax, J. Clean. Prod. 330 (2022) 129906.
740 https://doi.org/https://doi.org/10.1016/j.jclepro.2021.129906.

741 [53] S. Ahmadkelayeh, S.K. Cheema, K. Hawboldt, Supercritical CO2 extraction of
742 lipids and astaxanthin from Atlantic shrimp by-products with static co-solvents:
743 Process optimization and mathematical modeling studies, J. CO2 Util. 58 (2022)
744 101938. https://doi.org/https://doi.org/10.1016/j.jcou.2022.101938.

745 [54] L.-B. Gu, G.-J. Zhang, L. Du, J. Du, K. Qi, X.-L. Zhu, X.-Y. Zhang, Z.-H. Jiang,
746 Comparative study on the extraction of Xanthoceras sorbifolia Bunge (yellow
747 horn) seed oil using subcritical n-butane, supercritical CO2, and the Soxhlet
748 method, LWT. 111 (2019) 548–554.
749 https://doi.org/https://doi.org/10.1016/j.lwt.2019.05.078.

750 [55] A. Lucas, J. Rincón, I. Gracia, Influence of operating variables on yield and quality
751 parameters of olive husk oil extracted with supercritical carbon dioxide, J. Am. Oil
752 Chem. Soc. 79 (2002) 237–243. https://doi.org/10.1007/s11746-002-0467-9.

753

754