

Extraction kinetics of *Leptocarpha rivularis* stalks in a packed bed extractor using supercritical carbon dioxide

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ABSTRACT

Yield and extraction rate, antioxidant capacity, and the kinetics of supercritical extraction from *Leptocarpha rivularis* using CO₂ were studied in terms of temperature (40–60°C), pressure (18–30 MPa), specific CO₂ consumption (30–50 kg/kg d.s.), and particle diameter (0.5–1.1 mm). Antioxidant capacity was also measured. The highest extraction yield (27.23 g/kg d.s.) was obtained at 40°C, 30 MPa, 30 kg CO₂/kg d.s. and 0.5 mm. The higher antioxidant capacity (3.836 mmol TE/g) was obtained at 40°C, 30 MPa, 30 kg CO₂/kg d.s. and 1.1mm. The diffusion model based on Fick's 2nd law adequately described the cumulative extraction curves, using the effective diffusion coefficient (D_e) as the adjusted parameter. Yield and operational solubility increased with pressure and decreased with temperature. The yield and D_e coefficient correlated positively ($r=0.886$, $p\leq 0.05$). When the particle size decreased, the yield increased. Modeling of the cumulative extraction curves allowed for an adequate description of the extraction kinetics behavior.

KEYWORDS

Supercritical extraction, Diffusion model, *Leptocarpha rivularis*, Carbón dioxide, Antioxidant capacity

1. Introduction

Herbaceous plants are consumed as medicinal plants because they are high in bioactive compounds, which are beneficial in disease prevention and health protection, so they have applications as an alternative therapeutic treatments for diseases. *Leptocarpha rivularis*, a medicinal plant belonging to the Asteraceae, is known as "palo negro" [1] and is used as a treatment for different illnesses [2]. It has a wide variety of bioactive compounds, such as terpenes, flavonoids, and alkaloids [3], which have functional groups that provide therapeutic properties and can act as antioxidants, anti-inflammatories, anti-microbial and anti-carcinogenic agents, so its consumption contributes to the prevention of degenerative diseases [4, 5, 6].

Antioxidants are compounds that prevent oxidative reactions by stabilizing reactive oxygen species (ROS) in the body's cells. Antioxidant capacity is an important characteristic to establish the importance of a plant and its interest for applications in the food and pharmaceutical sectors.

Obtaining extracts with an antioxidant capacity requires extraction techniques that protect the quality and bioactive properties of the extracts. Conventional extraction techniques (Soxhlet, hydrodistillation, steam stripping) have disadvantages associated with the use of dangerous liquid solvents that require prolonged periods to complete the extraction [7] in addition to the use of high temperatures, low efficiency, and degradation of thermolabile compounds [8].

Supercritical fluid extraction is an efficient and selective technique for obtaining high-quality extracts by controlling temperature and pressure conditions, which determine the density and their solvent power [9]. Carbon dioxide is the most widely used supercritical solvent for the extraction of natural compounds since it allows extraction at low temperatures and prevents the degradation of thermolabile compounds. Furthermore, carbon dioxide is innocuous and non-flammable.

Temperature and pressure are the two most studied extraction variables because they affect CO₂ solvent power and its transport properties [10]. The solvent power of the supercritical fluid is directly related to its density (ρ), which is the property most affected by changes in pressure [11]. The temperature affects the solute vapor pressure. In the case of a volatile solute, as the temperature increases, the volatility increases, and in turn the solubility increases. For a nonvolatile solute, an increase in temperature results in a lower extraction yield due to a decrease in solubility [12]. On the other hand, to prevent thermal damage of bioactive compounds, low-temperature ranges are recommended (e.g., $\leq 60^\circ\text{C}$).

A decrease in particle size increases the extraction yield due to its direct relationship with the effective mass transfer surface, where smaller particles provide larger surfaces [13]. The effect of particle size (d_p , mm) has been studied in the range of 0.35–0.75 mm for *Foeniculum vulgare* ssp. Piperitum [14], 0.802–1.29 mm for *Senecio brasiliensis* [15] and 0.30–0.90 mm for *Peganum harmala* [13].

Eggers and Lack [16] reported a range between 25 and 70 kg CO₂/kg d.s. for the extraction of species and herbs. We evaluated specific solvent consumption in a range where a certain difference in responses could be observed, focusing on the yield and extraction rate, and antioxidant capacity of the extracts.

A specific solvent consumption (kg CO₂/kg d.s.) of 32–60 for *Foeniculum vulgare* ssp. Piperitum [14], 20–38 for *Santalum album* and *Boswellia carterii* [17], 38–44 for *Satureja montana* [18], 40–50 for *Plumula nelumbinis* [19] and 20–45 for *Senecio brasiliensis* [15] has been reported in the literatura.

Supercritical extraction kinetics show three periods in a cumulative extraction curve. The first period corresponds to the constant extraction rate (CER), where the easily accessible solute is extracted and controlled by mass transfer by convection. The second period corresponds to the falling extraction rate (FER) due to the exhaustion of the easily accessible solute and the beginning of the extraction of the difficult-to-access solute. The third period corresponds to diffusion-controlled extraction (DC), where the difficult-to-access solute is extracted [20].

Modeling of the cumulative extraction curves is important to establishing mass transfer parameters that are physically significant, and extraction conditions for large-scale extraction [21]. Supercritical extraction from solid matrices is mainly controlled by the diffusion mechanism where solutes are extracted from the innermost part of the solid matrix.

The theoretical models reported in the literature include the diffusion model based on Fick's second law. This model is adequate for cases where intraparticle diffusion rather than solubility limitation controls the extraction mass transfer processes [22]. The extension of the results to the whole bed of particles is immediate since all the particles are assumed to be at the same extraction conditions [23]. This model has been used to properly model the extraction kinetics of marigold oleoresin

[24], shiitake oil [25], and macaúba palm oil [26], and to determine the effective solute diffusivity in the solid substrate (D_e).

The objective was to study the effect of the supercritical conditions of temperature, pressure, specific solvent consumption, and particle size on the yield and extraction rate, and the antioxidant capacity of extracts from *L. rivularis* stalks using supercritical carbon dioxide, and the mathematical modeling of the cumulative extraction curve to obtain mass transfer parameters that describe the extraction kinetics.

2. Materials and methods

Ethanol 99.9% ($\text{CH}_3\text{CH}_2\text{OH}$) (CAS Number: 64-17-5), chloroform 99.8% (CHCl_3) (CAS Number: 67-66-3), sulfuric acid $\geq 96\%$ (H_2SO_4) (CAS Number: 7664-93-9), and toluene 99.5% (C_7H_8) (CAS Number: 108-88-3), all of analytical grade were procured from J.T. Baker (J.T. Baker, Phillipsburg, NJ). DPPH $\geq 90\%$ (2,2-diphenyl-1-picrylhydrazyl) ($\text{C}_{18}\text{H}_{12}\text{N}_5\text{O}_6$) (CAS Number: 1898-66-4), and NDGA (nordihydroguaiaretic acid) (CAS Number: 500-38-9) were acquired from Calbiochem Co. (San Diego, CA). Iron (II) sulfate heptahydrate $\geq 99.0\%$ ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) (CAS Number: 7782-63-0) and iron (III) chloride anhydrous $\geq 98\%$ (FeCl_3) (CAS Number: 7705-08-0) were procured from ACROS Organics (Morris, NJ). Phosphate buffer pH 7 (CAS Number: 9005-84-9) was obtained from Winkler Ltda. (Santiago, Chile). Aluminum chloride ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$) 99.99% (CAS Number: 7784-13-6), atropine $\geq 99\%$ ($\text{C}_{17}\text{H}_{23}\text{NO}_3$) (CAS Number: 51-55-8), BCG (bromocresol green) $\geq 95\%$ ($\text{C}_{21}\text{H}_{14}\text{Br}_4\text{O}_5\text{S}$) (CAS Number: 76-60-8), linalool $\geq 97\%$ ($\text{C}_{10}\text{H}_{18}\text{O}$) (CAS Number: 78-70-6), quercetin $\geq 95\%$ ($\text{C}_{15}\text{H}_{10}\text{O}_7$) (CAS Number: 6151-25-3), TROLOX 97% (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) ($\text{C}_{14}\text{H}_{18}\text{O}_4$) (CAS Number: 53188-07-1), and lipoxygenase from *Glycine max* (CAS Number: 9029-60-1) were procured from Sigma-Aldrich (St. Louis, MO).

2.1 Substrate pre-treatment

The *L. rivularis* stalks used as substrate were locally acquired in La Unión, Chile ($39^\circ 52' \text{S}$, $73^\circ 14' \text{W}$). The stalks were dried in a convection oven (model UF110, Memmert, Schwabach, Germany) at 50°C for 10 h. Then, the dry substrate was placed in a freezer (-60°C) for 2 days, and stalks were ground in a Moulinex chopper (model AD5661AR, Moulinex, Ecully, France). Substrate samples were stored in darkness and refrigerated, packed in plastic bags without oxygen until use.

2.2 Substrate characterization

The moisture content was determined in triplicate by drying the sample in a drying oven (model UF 110, Memmert, Germany) at 105°C until a constant weight was reached. The substrate was screened through a series of US sieve sizes (ASTM E11:95): 14, 18, 20, 25, 40, 50, 60, 80, 140, using a Ro-Tap testing sieve shaker (model RX-29-10, W.S. Tyler, Mentor, OH). The average particle diameter (d_p) (Eq. 1) was calculated using ASTM S319.3 [27]:

$$d_p = \log^{-1} \left[\frac{\sum_{i=1}^n w_i \log \bar{d}_i}{\sum_{i=1}^n w_i} \right] \quad (1)$$

Where \bar{d}_i (mm) is the geometric mean diameter of particles on i th sieve, or $(d_i \times d_{i+1})^{1/2}$; d_i is the nominal sieve aperture size of the i th sieve and d_{i+1} is the nominal sieve aperture size in next larger than an i th sieve. w_i is the mass of particles with an average diameter of \bar{d}_i . The particle density (ρ_p , kg/m³) was calculated as the ratio of the weight of the dry substrate (d.s.) to the corresponding volume of the total sample excluding air. A Gay-Lussac specific-gravity bottle (25.020 cm³ at 20°C, Brand GmbH, Germany) was used to determine the volume of pre-weighed samples according to the liquid displacement method using toluene. The apparent density of samples (ρ_a , kg/m³) was determined gravimetrically, weighing the material loaded in a 100 cm³ graduated cylinder using a standard tapping procedure [28]. The bed porosity ($\varepsilon = 1 - \rho_a / \rho_p$) of the sample was determined using the values of particle density (ρ_p) and apparent density (ρ_a). Measurements were taken in triplicate.

2.3 Extraction

2.3.1 Supercritical extraction

Supercritical extraction assays were carried out in a Spe-ed SFE process development unit (model Spe-ed 2, Applied Separations, Allentown, PA) loading 12 g of the substrate (feed) in a 50 cm³ extraction vessel (14 mm inner diameter). Depending on the extraction conditions, 4.1 to 4.6 L NTP/min of carbon dioxide (99.95% pure, Linde Chile S.A.) were fed into the extraction vessel to maintain a constant superficial velocity of 1.0 mm/s through the extractor vessel. The CO₂ flow rate was controlled manually by the micro-metering valve and it was measured by a CO₂ gas flow meter placed at the end of the extraction line (precision: $\pm 2\%$). The extraction pressure was controlled with an air pump to increase the pressure of the carbon dioxide and monitored by a

pressure gauge (precision: ± 0.1 MPa). The temperature of the extraction vessel was automatically controlled (precision: $\pm 0.5^\circ\text{C}$) using a convection oven with a fan for circulating hot air. Air trapped in the extraction vessel had been previously purged using a controlled flow of CO_2 . The static 20-min extraction period was followed by a dynamic extraction period, which varied between 43 and 71 min to obtain the studied levels of specific solvent consumption. During extraction, the extracted mass was collected in previously weighed glass vials (60 cm^3 capacity), and the extracted mass was measured gravimetrically by weight difference in clean and dry vials. The extraction yield was expressed in grams of extract per kilogram of the dry substrate (g/kg d.s.). With this information, cumulative extraction curves were obtained. The extraction assays were performed in duplicate.

2.3.2 Hydroalcoholic extraction

To determine the total extract in the substrate (30.0 ± 0.6 g/kg, m_0), hydroethanolic extraction was carried out according to the methodology described by Paula et al. [29] with modifications. Ten grams of dried and milled stalks were extracted twice, each time using 50 mL of an ethanol: water mixture (80:20, v:v) at 40°C under magnetic stirring for 60 min. The extract was vacuum filtered and evaporated using a rotary evaporator (model RV10, IKA, Staufen, Germany) connected to a vacuum pump (model R-300, Boeco, Hamburg, Germany), then the remaining solvent was removed under a flow of gaseous nitrogen ($>99.99\%$ pure, Linde Chile S.A.).

2.4 Modeling of cumulative extraction curves

Cumulative supercritical extraction curves were modeled using the diffusion model [22]. Applying Fick's second law of diffusion, and assuming a homogeneous distribution of the solute inside the particles and external film mass transfer resistance neglected, the fraction of the solute extracted from the bed with respect to time can be given by (Eq. 2):

$$\frac{m_t}{m_0} = 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp\left[-\left(\frac{n\pi}{R}\right)^2 D_e t\right] \quad (2)$$

Where m_t is the total cumulative amount of the solute extracted at time t (g), m_0 is the initial amount of the extractable solute in solid phase (g), R is the radius of equivalent spherical particles (m), t is the extraction time (min), n is an integer, D_e is the effective diffusion coefficient (m^2/s), the adjustable parameter, and it was obtained using the Microsoft Excel Solver tool. The difference

between extraction yield values observed experimentally (Y_{exp}) and extraction yield values predicted by the diffusion model (Y_{pred}) was established by root mean square deviation (*RMSD*) (Eq. 3).

$$RMSD = \sqrt{\frac{\sum_1^n (Y_{exp} - Y_{pred})^2}{n}} \quad (3)$$

2.5 Analysis of extracts

2.5.1 Antioxidant capacity

The antioxidant capacity of the extracts was determined using the DPPH radical scavenging assay [30] with some modifications [31]. DPPH solution was prepared by dissolving with ethanol. TROLOX solution was prepared by dissolving with ethanol and deionized water. The absorbance was measured at 520 nm in a UV-Vis spectrophotometer Genesys 10S (Thermo Fisher Scientific Inc., Madison, WI). Antioxidant capacity was expressed as mmol of TROLOX equivalent per gram of extract (mmol TE/g). The analysis was carried out in duplicate. The FRAP assay was carried out according to the method reported by Benzie and Strain [32] with some modifications [33]. The FRAP reagent was prepared by mixing of acetate buffer, solution of TPTZ, and aqueous solution of $FeCl_3$. The absorbance was read at 593 nm by spectrophotometry. The FRAP value was expressed as millimol Fe (II) equivalents per gram of extract (mmol Fe (II)/g). The analysis was carried out in duplicate.

2.5.2. Total content of antioxidant compounds

The terpene content extract was quantified according to Truong et al. [35]. 200 μ L of the ethanolic solution of extract (10 mg/mL) was mixed with 2 mL of chloroform and left to stand for 3 minutes. 200 μ L of concentrated sulfuric acid was added along the side walls of the test tube to form a layer and incubated at room temperature for 120 minutes in the dark. The formation of a reddish-brown interface indicated the presence of terpenes in the extract samples. Then, all the liquid was carefully and gently decanted from the supernatant reaction mixture without disturbing the precipitation. 3 mL of ethanol was added and vortexed until all precipitation was completely dissolved in ethanol, and the absorbance was read at 538 nm. Ethanol was used as a blank. The total terpene content was expressed as mg of linalool equivalent per gram of extract based on the calibration curve using the

linalool standard (mg linalool/g). The analysis was carried out in duplicate the calibration curve using the linalool standard (mg linalool/g). Analysis was carried out in duplicate.

The flavonoid content was quantified according to the method reported by Liu et al. [36] with some modifications. 250 μ L of the extract solution (10 mg/mL), 1250 μ L of deionized water, and 75 μ L of NaNO₂ solution (5% w/v) were mixed and incubated at room temperature for 6 min. 150 μ L of AlCl₃ solution (10% w/v) was added and left to stand for 5 min. Then, 500 μ L of NaOH solution (1M) and 275 μ L of deionized water were added and absorbance was read at 510 nm. A blank was prepared similarly by replacing the extract solution with ethanol. The total flavonoid content was expressed as mg of quercetin equivalent per gram of extract based on the calibration curve using a quercetin standard (mg quercetin/g).

The alkaloid content was quantified according to Li et al. [37] with some modifications. 600 μ L of the ethanolic solution of extract (10 mg/mL) was mixed with 3 mL of phosphate buffer in a test tube. 3 mL of BCG solution was added, shaken, and allowed to stand for 30 min. The complex formed was extracted with (2, 2, 3, 3) mL of chloroform by vigorous stirring. The extract was collected in a 10 mL volumetric flask and diluted to volume with chloroform. The absorbance was read at 418 nm. The total alkaloid content was expressed as mg of atropine equivalent per gram of extract based on the calibration curve using the atropine standard (mg atropine/g). The analysis was carried out in duplicate.

2.5.3 Lipoxygenase inhibition assay

The procedure was measured according to Ahmed et al. [38], with some modifications reported by Uquiche and Martinez [31]. The extract was dissolved in DMSO at different concentrations. The NDGA solution was used as a positive control. The % inhibition of hydrogen peroxide production was calculated from the changes in absorbance read at 234 nm, and the % inhibition measured was plotted as a function of the extract concentration (mg/mL). The IC₅₀ value was estimated from this plot and represents the extract concentration sufficient to obtain 50% of the maximum inhibition capacity of lipoxygenase activity.

2.6 Experiments and statistical analysis

The effect of extraction temperature (40, 50, and 60°C), pressure (18, 24, and 30 MPa), average particle size (0.5, 0.8, and 1.1 mm), and specific solvent consumption (30, 40, and 50 kg CO₂/kg

d.s.) on the yield and extraction rate, and the antioxidant capacity, were evaluated. Results of experiments were analyzed by analysis of variance established with 95% confidence.

3. Results and discussion

3.1 Substrate

The substrate moisture was 7.43 ± 0.65 g/100 g d.s., which we consider adequate for the extraction assays with supercritical carbon dioxide. Fornari et al. [39] recommended that the moisture should not be greater than 12%, since the water could cause ice formation in the pipes of the extraction equipment due to rapid decompression and also cause the hydrolysis of some compounds present in the substrate.

Table 1

Table 1 shows the physical characteristics of the substrate samples used for the supercritical extraction assays. To obtain an average particle size (d_p), fractions of substrate samples retained on each sieve were collected, so that the size distribution of the total collected resulted in the d_p selected for the study (0.5 or 0.8 or 1.1 mm) (Eq. 1). Since it is the same substrate, ρ_p was a constant value ($=769.59$ kg/m³). Substrate samples with different particle sizes affect the characteristics of a packed bed [15] due to differences in the packed grade of the particles within the extraction vessel, resulting in different apparent density and packed bed porosity values, as shown in Table 1. When the d_p increases, the values of apparent density (ρ_a) and porosity (ϵ) decrease and increase, respectively. These physical characteristics affect the hydrodynamics of the supercritical fluid through the extractor vessel. In effect, porosity measures the fraction of void volume within the packed bed, through which supercritical CO₂ flows and which affects the hydrodynamics of extraction and mass transfer (interstitial velocity).

3.2 Extraction yield

Table 2 shows the effect of temperature, pressure, specific solvent consumption, and particle diameter (d_p) on the extraction yield of *L. rivularis*, which was found to range from 17.30 to 27.23 g/kg d.s., an increase of 1.6-fold. Cumulative extraction curves (g solute/kg d.s.) versus specific solvent consumption (kg CO₂/kg d.s.) were constructed to evaluate the effect of temperature, pressure, specific solvent consumption, and particle size on yield and extraction rate. The cumulative extraction curves were modeled and the effective diffusion coefficient (D_e , m²/s) was obtained by adjustment, which was in a range between 3.50 and 19.26×10^{-12} m²/s (Table 2).

For a better interpretation of the results, Biot number values ($B_i=k_f d_p/D_e$) were calculated for each extraction condition. For this purpose, the values of k_f (m/s) were calculated using the Puigenné equation [40]. The coefficient k_f depends on the conditions T , p , and the hydrodynamic characteristics of the packed bed. The k_f value was in a range between 14.81 and 2.34×10^{-5} m/s. The CO_2 density was obtained with the Bender equation of state reported by Brunner [9]. Biot number represents the ratio between internal and external mass transfer resistance. If the Biot number is greater than 10, internal diffusion may be the controlling factor in the extraction process. Biot number values were in a range between 275 and 2669. Due to the values found, in our study the internal diffusion possibly controls the extraction process [22].

On the other hand, when experimental data from the extraction yield (g solute/kg d.s.) is plotted against specific CO_2 consumption (kg CO_2 /kg d.s.), the initial slope represents the “operational” solubility (g solute/kg CO_2). Operational solubility values were in a range between 2.73 and 6.92 g solute/kg CO_2 . Next, the effect of extraction conditions on yield and extraction rate will be shown, and its behavior in terms of diffusion coefficient, Biot number, and operational solubility will be discussed.

Table 2

3.3 Temperature effect

The cumulative extraction curves (Figure 1) show the negative effect ($p \leq 0.05$) of temperature on the extraction yield (Figure 1). When the temperature increased from 40 to 60°C, the extraction yield decreased from 23.31 to 17.30 g/kg d.s. (at 30 MPa, 30 kg CO_2 /kg d.s., 0.8 mm). Likewise, the increase in temperature decreased the operational solubility (5.67–2.73 g solute/kg CO_2).

When the temperature increases at constant pressure, the CO_2 density and its solvent capacity decrease. However, the solute vapor pressure increases, facilitating the mass transfer of the solute to the supercritical phase [9]. According to the results, the decrease in CO_2 density due to an increase in temperature prevailed over the increase in the solute vapor pressure. This explains the decrease in yield and extraction rate with increasing temperature.

When the extraction temperature increased, the coefficient D_e decreased ($9.97-3.50 \times 10^{-12}$ m²/s). The increase in temperature reduced the CO_2 viscosity ($8.67-7.37 \times 10^{-5}$ Pa·s) and increased the k_f coefficient ($1.93-2.34 \times 10^{-5}$ m/s), while the Biot number was found in a range between

(773–2669), indicating a prevalence of internal control to mass transfer resistance. Therefore, the reduction of the operational solubility and the coefficient D_e with the increase in temperature, explain the decrease in the extraction yield. A negative effect of temperature (40–60°C) on supercritical CO₂ extraction yield has been reported for *Rosmarinus officinalis* L. [41] and *Tetraclinis articulata* leaves [42].

Figure 1

3.4 Pressure effect

The cumulative extraction curves (Figure 2) show the influence of pressure on extraction yield. The pressure had a positive effect ($p \leq 0.05$) on the extraction yield (Figure 2). When the pressure was from 18 to 30 MPa, the extraction yield increased 1.17-fold from 19.94 to 23.31 g/kg d.s. (at 40°C, 30 kg CO₂/kg d.s., 0.8 mm). The increase in pressure increased the operational solubility (2.78–5.67 g solute/kg CO₂). The increased pressure results in an increase in the CO₂ density, which increases the solvent power and the higher extraction yield [9].

When the extraction pressure increased, the coefficient D_e increased ($4.92\text{--}9.97 \times 10^{-12}$ m/s). The increase in pressure increased the viscosity ($7.13\text{--}8.67 \times 10^{-5}$ Pa·s) and reduced the k_f coefficient ($2.30\text{--}1.93 \times 10^{-5}$ m/s). The Biot number decreased in a range between 1868 and 773, but still remained in the internal control region for mass transfer resistance. Therefore, the increase in operational solubility and increase in the D_e coefficient with increasing pressure explain the increase in extraction yield. Indeed, the extraction yield correlated positively with the operational solubility ($r=0.877$, $p \leq 0.05$) and the D_e coefficient ($r=0.886$, $p \leq 0.05$). A positive effect of pressure on supercritical CO₂ extraction yield has been reported for *Rosmarinus officinalis* L. [41], *Tetraclinis articulata* [42], *Senecio brasiliensis* [15], *Salvia fruticosa* [43], *Peganum Harmala* [13], *Carum carvi* L. [44].

Figure 2

3.5 Specific solvent consumption effect

The cumulative extraction curves (Figure 3), show a slight positive effect ($p \leq 0.05$) of the specific solvent consumption on the extraction yield. When the specific solvent consumption increased from 30 to 50 kg CO₂/kg d.s., the extraction yield changed from 23.31 to 25.99 g/kg d.s. (40°C, 30 MPa, 0.8mm). At 50 kg CO₂/kg d.s. a higher extraction yield was reached. The increase in the

specific solvent consumption decreased the operational solubility (5.67–4.88 g solute/kg CO₂). The extraction curves almost overlap for the different consumptions up to <10 kg CO₂/kg d.s. When the specific solvent consumption increased, the coefficient D_e had close values (9.97–10.12×10⁻¹² m²/s). The Biot number was kept in a narrow range (761–780), being in a region of the prevalence of internal control to mass transfer resistance.

Brunner [9] points out that high consumption of CO₂ results in a high extraction yield. Grosso et al. [18] observed a higher extraction yield of *Santolina chamaecyparissus* when CO₂ consumption increased. An increase in the specific solvent consumption is related to a longer extraction time, which can favor the solvent having enough time to diffuse into the solid particle, dissolve the extract compounds, and diffuse towards the surface [45, 46]. Therefore, if a larger quantity of solvent is consumed, mass transfer is favored and the extraction oil yield increases.

Figure 3

3.6 Particle size effect

Cumulative extraction curves (Figure 4) show the negative effect of particle size on extraction yield ($p \leq 0.05$). When d_p decreased from 1.1 to 0.5 mm, the extraction yield increased 1.35-fold from 20.10 to 27.23 g/kg d.s. (at 40°C, 30 MPa, 30 kg CO₂/kg d.s.). The increase in d_p decreased the operational solubility (6.92–4.02 g solute/kg CO₂), and the coefficient D_e decreased (19.26–5.32×10⁻¹² m²/s). The Biot number decreased from 1868 to 275 with increasing d_p , corresponding to internal control to mass transfer resistance.

Therefore, the reduction of the coefficient D_e and operational solubility with the increase of d_p , explain the decrease in the extraction yield. On the other hand, when the particle size is reduced, the contact surface of the particles with the solvent increases, which makes it possible to entrain a higher content of solute from the substrate [41]. A positive effect of particle size reduction on extraction yield with supercritical CO₂ has been reported for *Rosmarinus officinalis* L. [41].

Figure 4

3.7 Antioxidant capacity

Table 2 shows the antioxidant capacity (mmol TE/g) of extracts obtained as a function of extraction conditions. The values were in a range between 1.83 and 3.82 (mmol TE/g), where the highest value was obtained at 40°C, 30 MPa, consumption 50 kg CO₂/kg d.s. and 1.1 mm, and the lowest

value was obtained at 50°C, 30 MPa, consumption 30 kg CO₂/kg d.s. and 0.8 mm. The antioxidant capacity did not follow a clear behavior with respect to changes in temperature, pressure, and specific solvent consumption. The antioxidant capacity increased as the particle size decreased. Pereira et al. [47], using *Myrtus communis* L., pointed out that the factors with the greatest influence on antioxidant capacity were CO₂ consumption, followed by pressure and temperature. Additionally, extract samples were obtained at the highest extraction yield condition (at 40°C, 30 MPa, 30 kg CO₂/kg d.s., 0.5 mm) for extract characterization.

Reactive oxygen species (ROS) produce oxidative damage at the cellular level and constitute the starting point of various neurodegenerative, cardiovascular, and inflammatory diseases, among others. Quantification of antioxidant compounds in the selected extract resulted in 445.4±21.7 mg linalool/g for terpenes, 101.0±0.9 mg quercetin/g for flavonoids, and 4.34±0.69 mg atropine/kg for alkaloids. Flavonoids have the ability to inhibit reactive oxygen species (ROS) and quench the formed ROS [4]. The antioxidant capacity of flavonoids depends on the presence of hydroxyl groups [48]. Also, terpenes also act as antioxidants against the excessive production of ROS [49]. Alkaloids have been shown to behave as powerful antioxidants, scavenging ROS [50].

In addition, the antioxidant capacity of the selected extract was measured by FRAP assay, obtaining 68.48 mmol Fe⁺²/g. FRAP value represents the reducing capacity of antioxidant compounds in a redox reaction, in which one reactive species is reduced at the expense of the oxidation of another [32]. Lipoxygenase is an oxidative enzyme associated with the biosynthesis of proinflammatory mediators, such as leukotrienes, which have been related to inflammatory diseases. Wisastra and Dekker [51] have pointed out that lipoxygenases and their reaction products are associated with cancer. We measured the inhibition of 5-lipoxygenase as anti-inflammatory capacity, obtaining an IC₅₀=1.12 mg/mL, a value close to that obtained for the positive control (NDGA), with IC₅₀=0.75 mg/mL.

4. CONCLUSIONS

The extraction kinetics of *L. rivularis* stalks using supercritical carbon dioxide were studied in terms of temperature, pressure, specific solvent consumption, and particle size. Cumulative extraction curves were adequately described by the diffusion model based on Fick's 2nd law, obtaining the effective diffusion coefficient (D_e) and estimating the Biot number. Additionally, the “operational solubility” was obtained from the cumulative extraction curves. The coefficient D_e

varied between 3.5 and 19.3×10^{-12} m²/s. The Biot number for the extraction conditions remained greater than 275, indicating internal control for mass transfer. The increase in operational solubility and coefficient D_e , both with increasing pressure and decreasing temperature, explain the effect of both factors on the increase in extraction yield. As the particle size decreased, the extraction yield increased. The highest extraction yield was reached at 40°C, 30 MPa, 30 kg CO₂/kg d.s., and 0.5 mm. The extraction yield did not correlate with antioxidant capacity. It would be interesting to evaluate the composition of the extracts based on the extraction conditions. The extract characteristics at the selected condition with the highest yield highlight its potential as an ingredient for the development of functional foods that aid in health care.

CONFLICT OF INTEREST

None.

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REFERENCES

- [1] H.M. Niemeyer, Composition of essential oils from five aromatic species of Asteraceae, J. Essent. Oil Res. 21 (2009) 350–353, <https://doi.org/10.1080/10412905.2009.9700189>.
- [2] R. Martínez, V. Kesternich, E. Gutierrez, H. Dölz, H. Mansilla, Conformational analysis and biological activity of Leptocarpin and Leptocarpin acetate, Planta Med. 61 (1995) 188–189, <https://doi.org/10.1055/s-2006-958048>.
- [3] A. Jiménez-González, C. Quispe, J. Bórquez, B. Sepúlveda, F. Riveros, C. Areche, E. Nagles, O. García-Beltrán, M.J. Simirgiotis, UHPLC-ESI-ORBITRAP-MS analysis of the native Mapuche medicinal plant palo negro (*Leptocarpha rivularis* DC.–*Asteraceae*) and evaluation of its antioxidant and cholinesterase inhibitory properties, J. Enzyme Inhib. Med. Chem. 33 (2018) 936–944, <https://doi.org/10.1080/14756366.2018.1466880>.
- [4] G. Agati, E. Azzarello, S. Pollastri, M. Tattini, Flavonoids as antioxidants in plants: Location and functional significance, Plant Sci, 196 (2012) 67–76, <https://doi.org/10.1016/J.plantsci.2012.07.014>.
- [5] B. Debnath, W.S. Singh, M. Das, S. Goswami, M.K. Singh, D. Maiti, K. Manna, Role of

- plant alkaloids on human health: A review of biological activities, *Mater. Today Chem.* 9 (2018) 56–72, <https://doi.org/10.1016/j.mtchem.2018.05.001>.
- [6] T. Nuutinen, Medicinal properties of terpenes found in *Cannabis sativa* and *Humulus lupulus*, *Eur. J. Med. Chem.* 157 (2018) 198–228, <https://doi.org/10.1016/j.ejmech.2018.07.076>.
- [7] R.P.F.F. da Silva, T.A.P. Rocha-Santos, A.C. Duarte, Supercritical fluid extraction of bioactive compounds, *TrAC- Trends Anal. Chem.* 76 (2016) 40–51, <https://doi.org/10.1016/j.trac.2015.11.013>.
- [8] G. Brusotti, I. Cesari, A. Dentamaro, G. Caccialanza, G. Massolini, Isolation and characterization of bioactive compounds from plant resources: The role of analysis in the ethnopharmacological approach, *J. Pharm. Biomed. Anal.* 87 (2014) 218–228, <https://doi.org/10.1016/j.jpba.2013.03.007>.
- [9] G. Brunner, Gas extraction: An introduction to fundamentals of supercritical fluids and the application to separation processes. Springer Science & Business Media, New York (1994).
- [10] B. Damjanović, Ž. Lepojević, V. Živković, A. Tolić, Extraction of fennel (*Foeniculum vulgare* Mill.) seeds with supercritical CO₂: Comparison with hydrodistillation, *Food Chem.* 92 (2005) 143–149, <https://doi.org/10.1016/j.foodchem.2004.07.019>.
- [11] M.M.R. de Melo, A.J.D. Silvestre, C.M. Silva, Supercritical fluid extraction of vegetable matrices: Applications, trends and future perspectives of a convincing green technology, *J. Supercrit. Fluids.* 92 (2014) 115–176, <https://doi.org/10.1016/J.SUPFLU.2014.04.007>.
- [12] L.C. Soto-Armenta, J.C. Sacramento-Rivero, C.A. Ruiz-Mercado, M.C. Lope-Navarrete, J.A. Rocha-Urbe, Extraction yield and kinetic study of *Lippia graveolens* with supercritical CO₂, *J. Supercrit. Fluids.* 145 (2019) 205–210, <https://doi.org/10.1016/J.SUPFLU.2018.12.018>.
- [13] S. Melloul, R. Zehioua, A.H. Meniai, Supercritical CO₂ extraction of bioactive compounds from local *Peganum Harmala* plant seeds and optimization of the extraction yield and the antioxidant activities, *Sustain. Chem. Pharm.* 28 (2022) 100729, <https://doi.org/10.1016/j.scp.2022.100729>.
- [14] J.A.P. Coelho, A.P. Pereira, R.L. Mendes, A.M.F. Palavra, Supercritical carbon dioxide

extraction of *Foeniculum vulgare* volatile oil, *Flavour Fragr. J.* 18 (2003) 316–319, <https://doi.org/10.1002/FFJ.1223>.

- [15] T.C. Confortin, I. Todero, N.I. Canabarro, L. Luft, G.A. Ugalde, J.R.C. Neto, M.A. Mazutti, G.L. Zobot, M.V. Tres, Supercritical CO₂ extraction of compounds from different aerial parts of *Senecio brasiliensis*: Mathematical modeling and effects of parameters on extract quality, *J. Supercrit. Fluids.* 153 (2019) 104589, <https://doi.org/10.1016/J.SUPFLU.2019.104589>.
- [16] R. Eggers, E. Lack, *Supercritical Processes*, in: R. Eggers (Eds.), *Industrial high pressure applications: Processes, equipment, and safety*, Wiley-VCH Verlag & Co. KGaA, Weinheim, Germany. (2012) 169–209.
- [17] B. Marongiu, A. Piras, S. Porcedda, E. Tuveri, Extraction of *Santalum album* and *Boswellia carterii* Birdw. volatile oil by supercritical carbon dioxide: influence of some process parameters, *Flavour Fragr. J.* 21 (2006) 718–724, <https://doi.org/10.1002/FFJ.1718>.
- [18] C. Grosso, A.C. Figueiredo, J. Burillo, A.M. Mainar, J.S. Urieta, J.G. Barroso, J.A. Coelho, A.M. Palavra, Enrichment of the thymoquinone content in volatile oil from *Satureja montana* using supercritical fluid extraction, *J. Sep. Sci.* 32 (2009) 328–334, <https://doi.org/10.1002/JSSC.200800490>.
- [19] D. Jia, S. Li, L. Xiao, Supercritical CO₂ extraction of *Plumula nelumbinis* oil: Experiments and modeling, *J. Supercrit. Fluids.* 50 (2009) 229–234, <https://doi.org/10.1016/J.SUPFLU.2009.06.005>.
- [20] A. Meireles, Extraction of bioactive compounds from Latin American plants, in: J. Martínez (Eds.), *Supercritical fluids extraction of nutraceuticals and bioactive compounds*, CRC Press-Taylor and Francis Group, Boca Raton, (2007), 243–262.
- [21] J.M. del Valle, J.C. Germain, E. Uquiche, C. Zetzl, G. Brunner, Microstructural effects on internal mass transfer of lipids in prepressed and flaked vegetable substrates, *J. Supercrit. Fluids.* 37 (2006) 178–190, <https://doi.org/10.1016/j.supflu.2005.09.002>.
- [22] Z. Huang, X.H. Shi, W.J. Jiang, Theoretical models for supercritical fluid extraction, *J. Chromatogr. A.* 1250 (2012) 2–26, <https://doi.org/10.1016/j.chroma.2012.04.032>.

- [23] E. Reverchon, Supercritical fluid extraction and fractionation of essential oils and related products, *J. Supercrit. Fluids.* 10 (1997) 1–37, [https://doi.org/10.1016/S0896-446\(97\)00014-4](https://doi.org/10.1016/S0896-446(97)00014-4).
- [24] L.M.A.S. Campos, E.M.Z. Michielin, L. Danielski, S.R.S. Ferreira, Experimental data and modeling the supercritical fluid extraction of marigold (*Calendula officinalis*) oleoresin, *J. Supercrit. Fluids.* 34 (2005) 163–170, <https://doi.org/10.1016/J.SUPFLU.2004.11.010>.
- [25] C.S.G. Kitzberger, R.H. Lomonaco, E.M.Z. Michielin, L. Danielski, J. Correia, S.R.S. Ferreira, Supercritical fluid extraction of shiitake oil: Curve modeling and extract composition, *J. Food Eng.* 90 (2009) 35–43, <https://doi.org/10.1016/J.jfoodeng.2008.05.034>.
- [26] A.D.P. do Nascimento, L.A.L. Soares, L. Stragevitch, L. Danielski, Extraction of *Acrocomia intumescens* Drude oil with supercritical carbon dioxide: Process modeling and comparison with organic solvent extractions, *J. Supercrit. Fluids.* 111 (2016) 1–7, <https://doi.org/10.1016/j.supflu.2016.01.013>.
- [27] ASAE S319.3– Method of determining and expressing fineness of feed materials by sieving, ASAE Standards, American Society of Agricultural Engineers, ST. Joseph, MI, USA (2010)
- [28] J.M. del Valle, M. Jiménez, J.C. de la Fuente, Extraction kinetics of pre-pelletized Jalapeño peppers with supercritical CO₂, *J. Supercrit. Fluids.* 25 (2003) 33–44, [https://doi.org/10.1016/S0896-8446\(02\)00090-6](https://doi.org/10.1016/S0896-8446(02)00090-6).
- [29] J.T. Paula, L.C. Paviani, M.A. Foglio, I.M.O. Sousa, F.A. Cabral, Extraction of anthocyanins from *Arrabidaea chica* in fixed bed using CO₂ and CO₂/ethanol/water mixtures as solvents, *J. Supercrit. Fluids.* 81 (2013) 33–41, <https://doi.org/10.1016/j.supflu.2013.04.009>.
- [30] W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of free radical methods to evaluate antioxidant activity, *LWT-Food Sci. Technol.* 28 (1995) 25–30, [https://doi.org/10.1016/s0023-6438\(95\)80008-5](https://doi.org/10.1016/s0023-6438(95)80008-5).
- [31] E. Uquiche, M. Martínez, Glandular trichome disruption by rapid CO₂ depressurization as pretreatment for recovery of essential oil from *Leptocarpha rivularis* leaves, *Ind. Crops*

Prod. 83 (2016) 522–528, <https://doi.org/10.1016/j.indcrop.2015.12.075>.

- [32] I. Benzie, J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay, *Anal. Biochem.* 239 (1996) 70–76, <https://doi.org/10.1006/abio.1996.0292>.
- [33] S. Millao, E. Uquiche, Antioxidant activity of supercritical extracts from *Nannochloropsis gaditana*: Correlation with its content of carotenoids and tocopherols, *J. Supercrit. Fluids.* 111 (2016) 143–150, <https://doi.org/10.1016/J.SUPFLU.2016.02.002>.
- [34] I.I. Koleva, T.A. Van Beek, J.P.H. Linssen, A. de Groot, L.N. Evstatieva, Screening of plant extracts for antioxidant activity: A comparative study on three testing methods, *Phytochem. Anal.* 13 (2002) 8–17, <https://doi.org/10.1002/PCA.611>.
- [35] D.H. Truong, N.T.A. Ta, T.V. Pham, T.D. Huynh, Q.T.G. Do, N.C.G. Dinh, C.D. Dang, T.K.C. Nguyen, A.V. Bui, Effects of solvent—solvent fractionation on the total terpenoid content and in vitro anti-inflammatory activity of *Serevenia buxifolia* bark extract, *Food Sci. Nutr.* 9 (2021) 1720–1735, <https://doi.org/10.1002/FSN3.2149>.
- [36] S.C. Liu, J.T. Lin, C.K. Wang, H.Y. Chen, D.J. Yang, Antioxidant properties of various solvent extracts from lychee (*Litchi chinensis* Sonn.) flowers, *Food Chem.* 114 (2009) 577–581, <https://doi.org/10.1016/j.foodchem.2008.09.088>.
- [37] L. Li, W. Long, X. Wan, Q. Ding, F. Zhang, D. Wan, Studies on quantitative determination of total alkaloids and berberine in five origins of crude medicine “Sankezhen”, *J. Chromatogr. Sci.* 53 (2015) 307–311, <https://doi.org/10.1093/chromsci/bmu060>.
- [38] A.S. Ahmed, E.E. Elgorashi, N. Moodley, L.J. McGaw, V. Naidoo, J.N. Eloff, The antimicrobial, antioxidative, anti-inflammatory activity and cytotoxicity of different fractions of four South African Bauhinia species used traditionally to treat diarrhoea, *J. Ethnopharmacol.* 143 (2012) 826–839, <https://doi.org/10.1016/J.JEP.2012.08.004>.
- [39] T. Fornari, G. Vicente, E. Vázquez, M.R. García-Risco, G. Reglero, Isolation of essential oil from different plants and herbs by supercritical fluid extraction, *J. Chromatogr. A.* 1250 (2012) 34–48, <https://doi.org/10.1016/j.chroma.2012.04.051>.
- [40] J. Puiggené, M.A. Larrayoz, F. Recasens, Free liquid to supercritical fluid mass transfer in packed beds, *Chem. Eng. Sci.* 52 (1997) 195–212, [https://doi.org/10.1016/S0009-2509\(96](https://doi.org/10.1016/S0009-2509(96)

00379-X.

- [41] O. Bensebia, D. Barth, B. Bensebia, A. Dahmani, Supercritical CO₂ extraction of rosemary: effect of extraction parameters and modelling, *J. Supercrit. Fluids.* 49 (2009) 161–166, <https://doi.org/10.1016/j.supflu.2009.01.007>.
- [42] N. Herzi, S. Camy, J. Bouajila, P. Destrac, M. Romdhane, J.S. Condoret, Supercritical CO₂ extraction of *Tetraclinis articulata*: Chemical composition, antioxidant activity and mathematical modeling, *J. Supercrit. Fluids.* 82 (2013) 72–82, <https://doi.org/10.1016/j.supflu.2013.06.007>.
- [43] D. Kavoura, K. Kyriakopoulou, G. Papaefstathiou, E. Spanidi, K. Gardikis, V. Louli, N. Aliogiannis, M. Krokida, K. Magoulas, Supercritical CO₂ extraction of *Salvia fruticosa*, *J. Supercrit. Fluids.* 146 (2019) 159–164, <https://doi.org/10.1016/J.SUPFLU.2019.01.010>.
- [44] B. Shrirame, S.R. Geed, S.Z. Hassan, J.S. Verma, K. Samal, A. Namdeo, B.N. Rai, Supercritical CO₂ extraction of caraway (*Carum carvi* L.) seed: Optimization and parametric interaction studies using design of experiments, *J. CO₂ Util.* 65 (2022) 102195, <https://doi.org/10.1016/J.JCOU.2022.102195>.
- [45] A. Bogdanovic, V. Tadic, M. Stamenic, S. Petrovic, D. Skala, Supercritical carbon dioxide extraction of *Trigonella foenum-graecum* L. seeds: Process optimization using response surface methodology, *J. Supercrit. Fluids.* 107 (2016) 44–50, <https://doi.org/10.1016/j.supflu.2015.08.003>.
- [46] A. Daraee, S.M. Ghoreishi, A. Hedayati, Supercritical CO₂ extraction of chlorogenic acid from sunflower (*Helianthus annuus*) seed kernels: modeling and optimization by response surface methodology, *J. Supercrit. Fluids.* 144 (2019) 19–27, <https://doi.org/10.1016/j.supflu.2018.10.001>.
- [47] P. Pereira, M.G. Bernardo-Gil, M.J. Cebola, E. Mauricio, A. Romano, Supercritical fluid extracts with antioxidant and antimicrobial activities from myrtle (*Myrtus communis* L.) leaves. Response surface optimization, *J. Supercrit. Fluids.* 83 (2013) 57–64, <https://doi.org/10.1016/j.supflu.2013.08.010>.
- [48] M. Moalin, G.P.F. Van Strijdonck, M. Beckers, G.J. Hagemen, P.J. Borm, A. Bast, G.R.M.M. Haenen, A planar conformation and the hydroxyl groups in the B and C rings

play a pivotal role in the antioxidant capacity of quercetin and quercetin derivatives, *Mol.* 16 (2011) 9636–9650, <https://doi.org/10.3390/MOLECULES16119636>.

- [49] E. Gonzalez-Burgos, M.P. Gomez-Serranillos, Terpene compounds in nature: A review of their potential antioxidant activity, *Curr. Med. Chem.* 19 (2012) 5319–5341, <https://doi.org/10.2174/092986712803833335>.
- [50] K. Macáková, R. Afonso, L. Saso, P. Mladěnka, The influence of alkaloids on oxidative stress and related signaling pathways, *Free Radic. Biol. Med.* 134 (2019) 429–444. <https://doi.org/10.1016/j.freeradbiomed.2019.01.026>.
- [51] R. Wisastra, F.J. Dekker, Inflammation, cancer and oxidative lipoxygenase activity are intimately linked, *Cancers*, 6 (2014) 1500–1521, <https://10.3390/cancers6031500>.