

Supercritical Carbon Dioxide Extraction of *Hypericum caprifoliatum* using Co-Solvents: Experiment and Mathematical Modeling

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Hypericum genus is represented in the south of Brazil by approximately 20 species, among them, *Hypericum caprifoliatum* showed in its lipophilic extract the presence of phloroglucinol derivatives such as hyperbrasilol B and uliginosin B, which biological activities are known, pointing out their antimicrobial and antidepressant properties. The supercritical fluid extraction using carbon dioxide associated with co-solvents has been reported in the literature as an efficient extraction technique to attain desired pharmacological active compounds from vegetal matrices. Aiming to promote the improvement of the knowledge about this technology an aspect to be considered is the mathematical simulation of the experimental data. Mathematical models are used to simulate processes without the need of perform experiments in order to know its extraction process behavior. Therefore, the importance of mathematical modeling of supercritical fluid extraction in presence of co-solvents is an alternative strategy to evaluate the selection of the process variable conditions. In this work, three different models were used to simulate the experimental data and as well as their adequacy to physical aspects inherent to process using supercritical fluid. The mathematical models used are available in the literature and they are based on mass balance performed in the solid-phase and in the fluid-phase along the bed. To test the feasibility of the mathematical model experiments were conducted in a pilot automated unit. The experiments were carried out at 150 bar with two temperature conditions (40°C and 50°C), using 2% (p/p) of the co-solvent in relation to carbon dioxide quantity. The co-solvents tested were ethanol, water, and water-ethanol mixtures (25%, 50%, and 75%). Finalizing a discussion about the mathematical modeling is presented together with aspects of yield as function of the process variable investigated.

INTRODUCTION

The interest in the genus *Hypericum* (Guttiferae) has increased considerably all over the world due to several factors including the chemical investigation and isolation of more than 100 compounds, such as flavonoids, xanthenes and phloroglucinol derivatives [1]. Brazilian *Hypericum* species occur predominantly at southern regions, where 20 of them have been identified. In this work is studied the *Hypericum caprifoliatum*, a native specie to South Brazil of herbaceous or shrubby plants which occur in temperate regions [2]. The lipophilic extracts obtained from *Hypericum caprifoliatum*, showed an antidepressant-like activity in mice and rat forced swimming test and in vivo experiments suggest an effect on the dopaminergic transmission [2].

The proposal was to obtain *H. caprifoliatum* extracts using supercritical solvents with different polarity. Supercritical extraction process was carried out using CO₂, CO₂+water, CO₂+ethanol, and CO₂+water+ethanol as solvent. The co-solvent

concentration, water and ethanol, was 2.0 % p/p with regard the carbon dioxide mass. The experimental procedure performed in an automated pilot-scale unit of supercritical extraction [3] carried out at 150 bar with temperature ranging from 313.15K to 323.15K, and with a constant solvent flow rate ($6.7 \times 10^{-4} \text{ kg.s}^{-1}$). For each experimental condition was determinate an extraction curve, yield *versus* time.

The mathematical modeling is an important instrument to design and simulate industrial processes. It should also be taken into account as one of the first steps in a scale-up project. A model should not be a simple mathematical instrument, but should reflect the behavior of physical phenomena. The supercritical fluid extraction modeling was developed by way of different approaches: empirical models, models based on a heat transfer analogy, and models based on differential mass balance [4]. In this work, the purpose was to evaluate co-solvent effect on supercritical process behavior of *H. caprifoliatum* extraction, using the models proposed by Sovová [5], Reverchon [6], and Tan and Liou [7]. All three mathematical models require adjustment of the parameters. These parameters were estimated for each experimental condition using the software EMSO – Environment for Modeling, Simulation and Optimization [8]

The aim was the use of the supercritical extraction process to obtain non volatile extracts from *Hypericum caprifoliatum* using carbon dioxide and carbon dioxide/(water+ethanol) supercritical solvent. The experimental data was used to perform the mathematical simulation of the supercritical extraction from *H. caprifoliatum*. The models proposed by Sovová [5], Reverchon [6], and Tan and Liu [7] were chosen to simulate the extraction process.

MATERIALS AND METHODS

Plant Material

The aerial parts of *Hypericum caprifoliatum* were collected during its flowering stage in Porto Alegre, Rio Grande do Sul state, Brazil, in December, 2010. The plant material was identified by S. Bordignon (Departamento de Botânica, Universidade Luterana do Brasil). A voucher specimen was deposited in the Herbarium of the Universidade Federal do Rio Grande do Sul (ICN). Plant material was dried at room temperature and powdered.

Extraction methods

Supercritical extractions were carried out on a pilot-scale equipment (Figure 1) according to procedures previously described [8, 9]. Powdered plant material (120 g DW) was extracted at constant temperature (313.15 and 323.15 K) while the pressure was 150 bar. In this study samples were collected every 10 min in order to evaluate yield of the extracts *versus* extraction time.

The supercritical carbon dioxide flow rate was $6.7 \times 10^{-4} \text{ kg.s}^{-1}$ (through the extraction vessel) using a flowmeter assay (Sitraus F C Massflo 2100 - Siemens) with accuracy of < 0.1%. The co-solvent concentration, water and ethanol, was 2.0 % p/p with regard the carbon dioxide mass. In the Table 1 are presented the experimental conditions with regard to co-solvent concentration, as well as the experimental plan applied in this study.

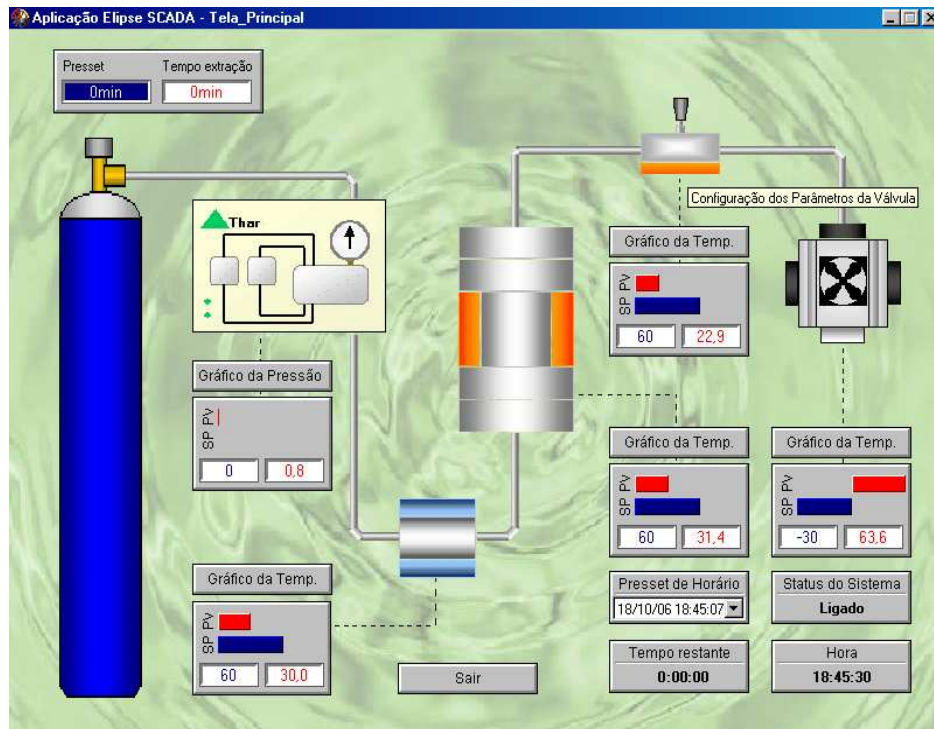


Figure 1: Pilot scale supercritical extraction equipment

Pressure in the extractor was monitored with a digital transducer system, Novus 8800021600, acquired from Novus Produtos Eletrônicos (Brazil) with precision of ± 1.0 bar. The temperature controller was connected to thermocouples (PT-100) with accuracy of < 0.5 .

Table 1: CO₂ and CO₂+co-solvent supercritical extraction conditions of *H. caprifoliatum* at 150 bar (co-solvent – 2.0 % p/p).

T (K)	without	co-solvent* - % ethanol (v/v)				
	co-solvent	0.0	25.0	50.0	75.0	100
313.15	Exp1	Exp3	Exp5	Exp7	Exp9	Exp11
323.15	Exp2	Exp4	Exp6	Exp8	Exp10	Exp12

* binary mixture – ethanol/water

Mathematical modeling

Three mathematical models of the extractive process were evaluated and applied in this work. The first model used to represent the supercritical extraction of *H. caprifoliatum* with CO₂ and CO₂+co-solvent was proposed by Sovová [5]. In this model, the solute is assumed to be homogeneously distributed in the solid particles. The readily accessible solute from broken cells near the surface is transferred directly to the fluid-phase, while the solute from intact cells diffuses internally and then to the fluid-phase [10]. Based on the hypothesis that extraction curves are associated with the non-existence of interaction between solute and matrix, the model used is described by the following equation:

$$e = qy_s \quad \text{for} \quad 0 \leq q \leq q_c \quad (1)$$

$$e = x_u [1 - C_1 \exp(-C_2 q)] \quad \text{for} \quad q > q_c \quad (2)$$

where e is the extraction yield ($\text{kg}_{\text{extract}}/\text{kg}_{\text{insoluble solid}}$); q is the relative amount of the solvent passed ($\text{kg}_{\text{solvent}}/\text{kg}_{\text{insoluble solid}}$); y_s is the solubility ($\text{kg}_{\text{extract}}/\text{kg}_{\text{solvent}}$); x_u is the concentration in the untreated solid ($\text{kg}_{\text{extract}}/\text{kg}_{\text{insoluble solid}}$). In this formulation, the fitting of the first part of equilibrium extraction curve is carried out with a straight line of slope y_s . The curve described by equation (2) is associated with the second period of the extraction and the estimated grinding efficiency, r , and solid phase coefficient, $k_s a_s$, depend on the constants C_1 , C_2 and the coordinate q_c at the crossing point:

$$r = x_u \left[1 - C_1 \exp\left(-C_2 \frac{q_c}{2}\right) \right] \quad (3)$$

$$k_s a_s = (1 - r)(1 - \varepsilon) \dot{Q} C_2 / N_m \quad (4)$$

where ε is the bed porosity, \dot{Q} is the solvent flow rate (kg/s) and N_m is the charge of insoluble solid (kg). The adjustable parameters (C_1 , C_2 and y_s) were estimated using the software EMSO - Environment for Modeling, Simulation and Optimization [11].

The second model used to represent the extraction curves, yield *versus* extraction time, of *H. caprifoliatum* was proposed by Reverchon [6]. In this model, the solvent density and solvent flow rate are constant throughout the bed. The oil extracted is assumed to be one component. Based on the above assumptions, the model is composed by a one-dimensional mass balance for the *H. caprifoliatum* extracts as presented by Equations 5 and 6.

Fluid phase

$$\frac{\partial C}{\partial t} = -v \frac{\partial C}{\partial z} - \frac{(1 - \varepsilon)}{\varepsilon} \cdot \rho \frac{\partial q}{\partial t} \quad (5)$$

Solid phase:

$$\frac{\partial q}{\partial t} = -k_{TM} (q - q^*) \quad (6)$$

where C is the oil concentration in the fluid phase, q is the oil content in aromatic plant, v is the interstitial velocity, ε is the bed porosity; k_{MT} is the overall mass transfer coefficient (s^{-1}); ρ is aromatic plant density. Mass balances can be solved if the solid-liquid phase relationship $q^*(q)$ is known. As a rule, a linear relationship is used due to the lack of experimental phase equilibrium data.

$$c = k_p q^*, \quad (7)$$

where k_p is the volumetric partition coefficient of the extract between the solid and the fluid phase at equilibrium.

The third model employed was developed by Tan and Liou [7] based on mass balance differential equations for the fluid-phase and for the solid-phase associated to desorption process. The result for the mass of extract is written as follow

$$M(t) = \dot{m} \frac{(1-\varepsilon) \rho_s}{\varepsilon \rho_f} \frac{x_0}{k} \left\{ \left(1 - e^{-kt} \right) \left(e^{\frac{kH}{u}} - 1 \right) \right\} \quad (8)$$

where $M(t)$ is the mass of extract, \dot{m} is steam flow rate and H is extraction bed length, where ρ_f is steam density, ρ_s is solid density, ε is bed void fraction, u is superficial fluid velocity, x_0 is the initial mass fraction of solute in vegetal material and k is the desorption coefficient.

The extraction degree, $e(t)$, is the relation among the removed mass in a time t and the mass collected in an infinite time of extraction. Thus, the following result is established by desorption model here developed

$$e(t) = \frac{M(t)}{M(\infty)} = 1 - e^{-kt} \quad (9)$$

RESULTS

The estimated parameters in the models presented above were adjusted by using the experimental data from the pilot supercritical equipment (Table 1). The numerical values for these parameters are presented in Tables 2, 3, and 4, respectively to models proposed by Sovová [5], Reverchon [6], and Tan and Liou [7]. These parameters were estimated by minimization of the sum of squares of errors between the experimental data and mathematical results [11] obtained by means of the model using the software EMSO [12].

Table 2: Parameters of extraction curves fitted to experimental data for *H. caprifoliatum* for the Sovová model [5].

T = 313.15 K				T = 323.15 K			
Conditions	y_s	C_1	C_2	Conditions	y_s	C_1	C_2
Exp1	0.0028	4.523	0.459	Exp2	0.0020	6.246	0.618
Exp3	0.0034	10.588	0.626	Exp4	0.0023	4.010	0.333
Exp5	0.0019	1.153	0.163	Exp6	0.0016	2.042	0.275
Exp7	0.0028	1.194	0.176	Exp8	0.0024	1.183	0.158
Exp9	0.0033	2.584	0.325	Exp10	0.0022	10.130	0.524
Exp11	0.0045	5.543	0.520	Exp12	0.0021	6.864	0.510

Table 3: Parameters of extraction curves fitted to experimental data for *H. caprifoliatum* for the Reverchon model [6].

Conditions	T = 313.15 K		Conditions	T = 323.15 K	
	k_p ($m^3 kg^{-1}$)	$k_{TM}(s^{-1})$		k_p ($m^3 kg^{-1}$)	$k_{TM}(s^{-1})$
Exp1	0.00497	0.00257	Exp2	0.00477	0.04605
Exp3	0.00001	0.00057	Exp4	0.00001	0.00032
Exp5	0.00001	0.00032	Exp6	0.00415	0.00299
Exp7	0.00001	0.00034	Exp8	0.00002	0.00031
Exp9	0.00411	0.01000	Exp10	0.00001	0.00040
Exp11	0.00001	0.00060	Exp12	0.00001	0.00045

Table 4: Parameters of extraction curves fitted to experimental data for *H. caprifoliatum* for the Tan and Liu model [7].

Conditions	T = 313.15 K		Conditions	T = 323.15 K	
	k (s^{-1})			k (s^{-1})	
Exp1	0.00025		Exp2	0.00026	
Exp3	0.00033		Exp4	0.00021	
Exp5	0.00022		Exp6	0.00027	
Exp7	0.00023		Exp8	0.00021	
Exp9	0.00028		Exp10	0.00025	
Exp11	0.00033		Exp12	0.00028	

The curves for the experimental and mathematical model implementation are shown in Figures 2 to 7. The first figures, Figure 2a and 2b present the extraction curves to carbon dioxide solvent without co-solvent at 150 bar, respectively to T = 313.15 K and T = 323.15 K.

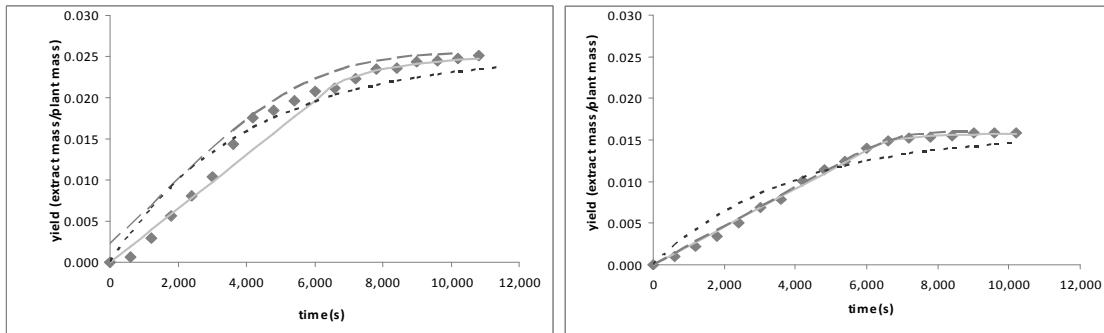


Figure 2: CO₂ supercritical extraction yield curves vs. time at 150 bar. (a) T = 313.15 K; (b) T = 323.15 K; (◆) experimental data ; (—) Sovová model; (==) Reverchon model; (-.-) Tan and Liou model with parameters from Tables 2 to 4.

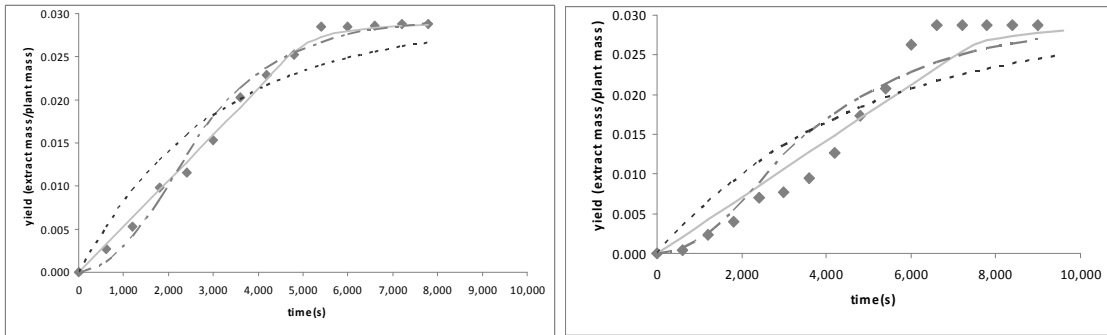


Figure 3: CO₂ + co-solvent (water) supercritical extraction yield curves vs. time at 150 bar. (a) T = 313.15 K; (b) T = 323.15 K; (◆) experimental data ; (—) Sovová model; (==) Reverchon model; (---) Tan and Liou model with parameters from Tables 2 to 4.

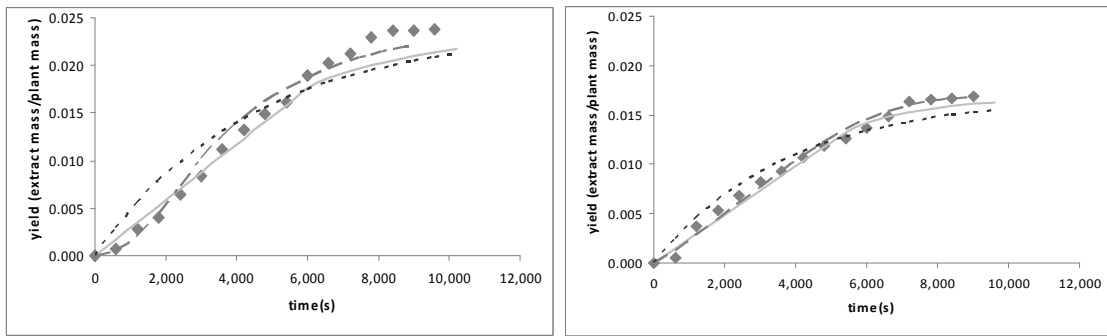


Figure 4: CO₂ + co-solvent (25% ethanol) supercritical extraction yield curves vs. time at 150 bar. (a) T = 313.15 K; (b) T = 323.15 K; (◆) experimental data ; (—) Sovová model; (==) Reverchon model; (---) Tan and Liou model with parameters from Tables 2 to 4.

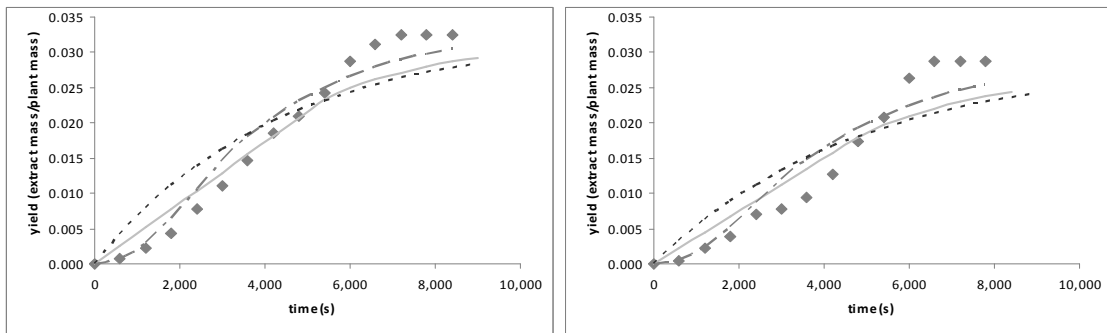


Figure 5: CO₂ + co-solvent (50% ethanol) supercritical extraction yield curves vs. time at 150 bar. (a) T = 313.15 K; (b) T = 323.15 K; (◆) experimental data ; (—) Sovová model; (==) Reverchon model; (---) Tan and Liou model with parameters from Tables 2 to 4.

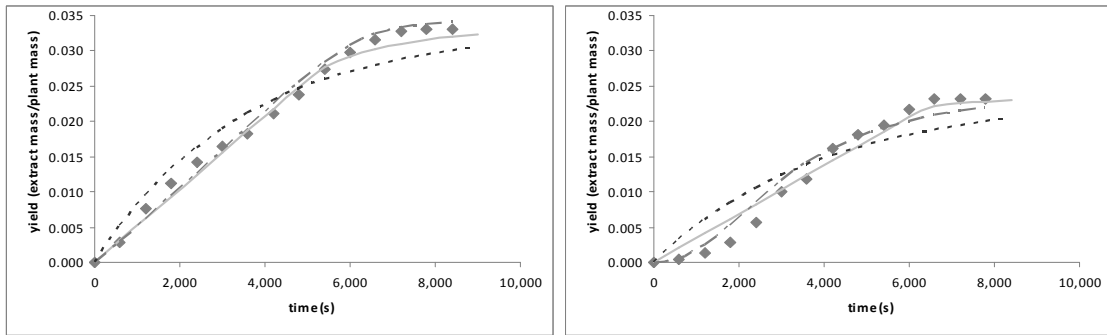


Figura 6: CO₂ + co-solvent (75% ethanol) supercritical extraction yield curves vs. time at 150 bar. (a) T = 313.15 K; (b) T = 323.15 K; (♦) experimental data ; (—) Sovová model; (—) Reverchon model; (—) Tan and Liou model with parameters from Tables 2 to 4.

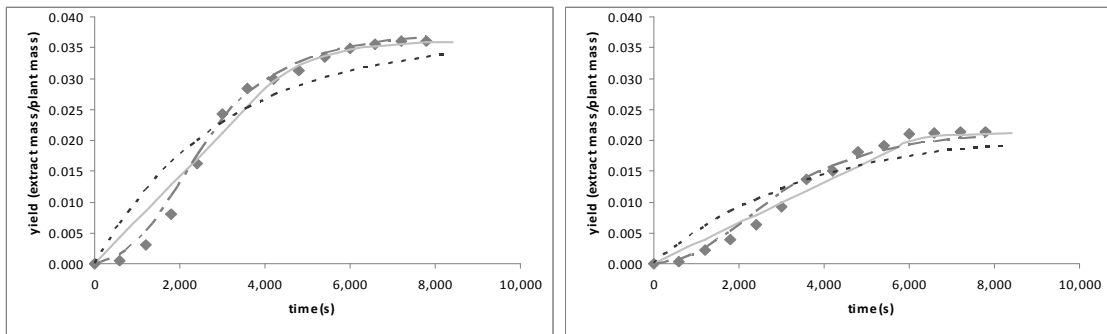


Figura 7: CO₂ + co-solvent (ethanol) supercritical extraction yield curves vs. time at 150 bar. (a) T = 313.15 K; (b) T = 323.15 K; (♦) experimental data ; (—) Sovová model; (—) Reverchon model; (—) Tan and Liou model with parameters from Tables 2 to 4.

From Figures 2 to 7, it is possible to verified the influence of temperature on the global yield (extract mass/plant mass). It is also possible to observe that a higher extract yield was obtained at 313.15 K for high ethanol concentration in the co-solvent mixture (75% and 100%). Regarding the thermodynamic modeling, Reverchon model [6] showed the best behavior to represent the experimental yield curve, while the Tan and Liou model [7] presented the highest deviations from experimental data. This fact can be attributed to Reverchon model to be more complex in terms of the hypotheses about the solid-phase balance which generates more one parameter to be adjusted in comparison with TanLiou model.

CONCLUSION

The use of ethanol/water mixtures as co-solvent in the supercritical fluid extraction to obtain non-volatile extracts of *H. caprifoliatum* promotes an increase in the extract yield when compared with the process without the co-solvent use. This study demonstrated that the higher extract yield were obtained at 313.15K, 15 MPa and ethanol as co-solvent (2% with regard to carbon dioxide mass) by supercritical fluid extraction

Good results were observed with respect to the simulated extraction curves obtained from the Reverchon model when compared with the experimental data. The determination of the values of the adjustable parameters of the model provides important knowledge about the supercritical extraction of *H. caprifoliatum* using carbon dioxide and carbon dioxide + ethanol/water mixture as solvent.

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