Vol.56, n.3: pp. 513-519, May-June 2013
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
A N I N T E R N A T I O N A L J O U R N A L

Supercritical CO_2 Extraction of *Schinus molle* L with Co-Solvents: Mathematical Modeling and Antimicrobial Applications

Rodrigo Scopel, Roberto Góes Neto, Manuel Alves Falcão, Eduardo Cassel and Rubem Mário Figueiró Vargas*

Laboratório de Operações Unitárias; Faculdade de Engenharia; Pontifícia Universidade Católica do Rio Grande do Sul; Av. Ipiranga, 6681; 90619-900; Porto Alegre - RS - Brasil

ABSTRACT

This work investigates the antimicrobial activity of the Schinus molle L. leaves extracts obtained under supercritical conditions using carbon dioxide and co-solvents. Antimicrobial qualitative evaluation was carried out through the bioautography technique and the microorganisms studied were Staphylococcus aureus, Pseudomonas aeruginosas, Escherichia coli, Micrococcus luteus, and Salmonella choleraesuis. The supercritical fluid extraction was carried out in a pilot scale equipment using carbon dioxide modified by the addition of co-solvents, such as ethanol and water at 150 bar and 333 K. A mathematical modeling of the process was also performed.

Key words: Supercritical extraction, co-solvents, *Schinus molle* L, antimicrobial applications

INTRODUCTION

The pepper tree (*Schinus molle*) is native from the Peruvian Andes (Huerta et al. 2010) and it is widely grown in the tropical and subtropical countries (Wimalaratne et al. 1996). The *S. molle* leaves are used for the extraction of an essential oil widely used in the popular medicine as a repellent and bioinsecticide (Huerta et al. 2010, Ferrero et al. 2007). The volatile oil is reported to have antimicrobial, antispasmodic, antipyretic, antifungal and cicatrizing properties (Marongiu et al. 2004, Ferrero et al. 2006, Hayouni et al. 2008), while regarding the nonvolatile compounds, extensive literature is available (Ferrero et al. 2007, Huerta et al. 2010).The chemical composition *S. molle* extracts depends on the factors such as the plant geographical localization, the genetic variability and of the technology used in the extraction process (Bandoni 2000).

One of the main parameters of supercritical extraction is the type of co-solvent that is proposed to be used (Taylor 1996). The role of the co-solvent is to increase the polarity and solvent strength as well as also to promote the improvement of the selectivity of separation of the solute without significantly changing the density and compressibility of the original supercritical fluid solvent (Mukhopadhyay 2000).

The implementation of industrial processes requires forecasting to promote the adequate roll out of the process; in this sense, mathematical modeling becomes a basic tool. Different modeling of the physical problem is regulated by the equations which will describe the behavior of the system according to a set of hypotheses assumed in the phase of physical description of the

*Author for correspondence: rvargas@pucrs.br

process. Mathematical models enable to generalize the experimental results, which can be applied to a new process conditions and to materials other than those investigated. Moreover, they are useful in the future scale-up process (Reverchon, 1997). In the literature, simple mathematical models are available as well as complexes ones due to the higher inclusion of information about the physical system (Reverchon and Marrone 2001). Different approaches have been proposed for mathematical modeling of the supercritical fluid extraction, but those based on mass transfer have been largely used.

The aim of this work was to study the effect of different co-solvents in the supercritical fluid extraction (SFE) of aerial parts of *Schinus molle* on the antimicrobial activity of the obtained extracts. The antimicrobial assay applied was the bioautography on thin-layer chromatography (Choma and Grzelak 2011). The microorganisms evaluated were *Staphylococus aureus, Pseudomonas aureginosas, Escherichia coli, Micrococus luteus* and *Salmonella enterica* subs. *enterica* Sorovar Choleraesuis. Experimental models were developed for the supercritical extraction of *Schinus molle* using carbon dioxide and co-solvents (water and ethanol).

MATERIALS AND METHODS

Material

Schinus molle leaves and twigs were collected in the Rio Grande do Sul State (Southern Brazil). The plant material was dried at 313.15 K during 48 h. A sample of 150 g of dried and milled material, with an average particle thickness of 1.93×10^{-4} m, was used for the extraction.

Method of extraction

The extraction was performed in a supercritical extraction pilot plant (Cassel et al. 2010, Cassel et al. 2007). The solvent used was 99.9% carbon dioxide (Air Products) with a flow rate of 1.38×10^{-4} kg s^{-1} through the extraction vessel. The extractor temperature was 333.15 K and the pressure was 150 bar. The temperature was chosen on the basis of previous results on SFE for similar matrixes (Marongiu et al. 2004, Barroso et al. 2011). The temperature of the separator vessel was 273.15 K. The stages of the extraction equipment are shown in Figure 1.

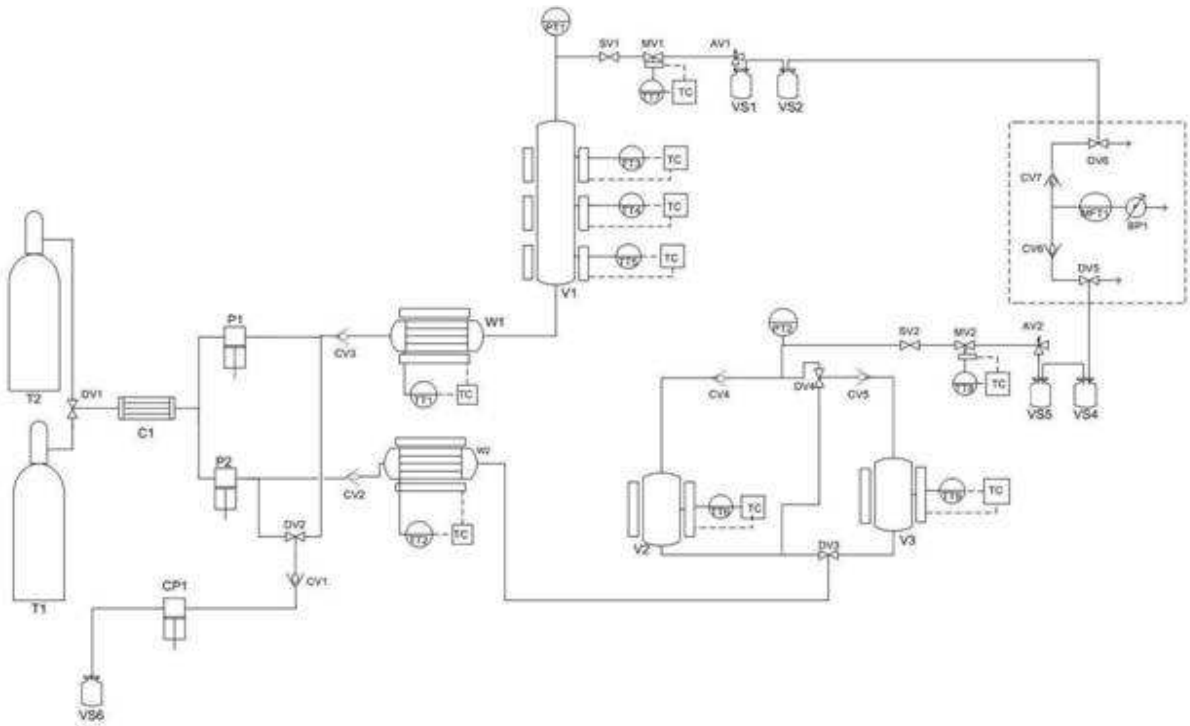

Figure 1 - Flowchart of the supercritical extraction pilot scale equipment – (DV) Directional Valve; (CV) Check Valve; (TT) Temperature Transmitter; (W) Pre-heater; (PT) Pressure Transmitter; (AV) "Alluvium" Valve; (CP) Co-solvent Pump; (VS) Separation Vessel; (SV) Shut-off Valve; (MV) "Micrometric" Valve; (TC) Temperature Controller; (BP) Back Pressure Regulator; (MFT) Mass Flow Transmitter; (P) CO_2 Pump; (V) Extraction Vessel.

The equipment constituted of three extractions vessels: 1000mL (V1), 500mL (V2) and 100mL (V3); two high pressure pumps for carbon-dioxide (P1, P2), one co-solvent pump (CP1); two storage cylinders of CO_2 (T1,T2); two pre-heaters (W1,W2); one condenser (C1); one system to measure the flow of CO_2, two separation vessels, and one automated control system. The co-solvent was added to the system using the pump ISCO 260D with the flux of 5.0 g/min. The operative conditions (150 bar and 60°C) were selected according to available information in the literature relative to the extraction of non-volatile compounds from this plant (Barroso et al. 2011). In order to prepare the extraction curve (extract yield *versus* extraction time), samples were collected every 10 min.

Mathematical modeling

The mathematical model was based on two periods to describe the extraction curve and the equations explained the mass balance of the solute. The extract was considered as a single component in terms of mass balance. The mass transfer properties of extract recovered were considered the same throughout the process. The first period of the extraction curve was associated to the extraction of the free solute from the broken cells. In this extraction step, it was assumed that the fluid phase was in equilibrium with the solid phase throughout the extractor. Then, the following expression could be written for the extracted mass flow which migrated to the fluid-phase

$$\dot{m}_A = C_A^* Q \qquad 1)$$

where, C_A^* was the equilibrium fluid-phase concentration and Q the volumetric solvent flow. To evaluate the extraction curve for this step, the following equation was calculated

$$M_A(t) = \int_0^t \dot{m}_A \, dt \qquad 2)$$

The following result was obtained from the Eqs. (1) and (2)

$$\frac{M_A(t)}{M_\infty} = \frac{F\,t}{M_\infty} \qquad 3)$$

where the parameters $F = C_A^* Q$ and M_∞ was the maximum value for the extract obtained in the extraction. It is important to mention that result presented in Eq. (3) was valid for $t \le t^*$, being t^* the time associated to beginning of the second step.

The second period was controlled by the solute diffusion from the inner cells of the vegetal structure. This step corresponded to the diffusion from intact cells. The diffusion was modeled by the Fick second law written for one-dimensional rectangular system

$$\frac{\partial^2 C_A}{\partial x^2} = \frac{1}{D}\frac{\partial C_A}{\partial t} \qquad 4)$$

where $-a < x < a$ and $t > t^*$ subject to the following boundary conditions

$$in \quad x = 0, \quad \frac{\partial C_A}{\partial x} = 0 \qquad 5)$$

$$in \quad x = a, \quad -D\frac{\partial C_A}{\partial x} = k_c(C_A - C_{A\infty}) \qquad 6)$$

and the temporal condition for $t = t^*$, $C_A = C_{A0}$. Using the solution presented by Crank (1975) for the diffusion equation in a slab subject to convective boundary condition, the following result can be written

$$\frac{M(t)}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{2L^2\, exp(-\beta_n^2 Dt / a^2)}{\beta_n^2(\beta_n^2 + L^2 + L)} \qquad 7)$$

where βn are zeros of the following equation

$$\beta\, tg\beta = L \,. \qquad 8)$$

The parameter $L = \frac{a\,k_c}{D}$, where a is the half thickness of slab; k_c, is the superficial coefficient of mass transfer, D is the effective diffusion coefficient, $M(t)$ is the recovered mass at time t and M_∞ is the maximum value for the extract obtained in the extraction.

Antimicrobial Test

The antibacterial activity of the *S. molle* SFE extracts was evaluated against *Staphylococcus*

aureus (ATCC 25923), *Pseudomonas aeruginosas* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Salmonella enterica* subs. *enterica* Sorovar Choleraesuis (ATCC 10708) and *Micrococcus luteus* (ATCC 9341) using the bioautographic method. *S. aureus*, *P. aeruginosas*, *E. coli* and *M. luteus* were inoculated at $1.0x10^6$ CFU/mL, while *S.* Choleraesuis at $2.0x10^6$ CFU/mL in Mueller-Hinton agar. Firstly, a thin-layer chromatography (TLC) of 3.0 μL (10 mg/mL in toluene) of the *S. molle* supercritical extract was made in silica gel GF_{254} plates (Farmacopéia Brasileira, 1988) and developed using toluene/ethyl acetate (93:7). After elution, the plates were overlaid with Mueller-Hinton agar inoculates in Petri dishes. Next, the plates were removed and the agar layers were incubated for 24 h at 37°C. Then a solution of INT "p-iodonitrotetrazolium violet" was added for a better visualization of inhibition halos (Pereira 2010, Valgas 2002).

RESULTS AND DISCUSSION

Experimental Data for Supercritical Fluid Extraction

The results for the supercritical fluid extraction of *S. molle* aerial parts are presented in Table 1 (extracted mass as the function of the extraction time).

The fitting of the experimental data presented in Table 1 was carried out by the mean of least square errors. This procedure estimated the numerical values of the unknown parameters of the mathematical model. The results for the parameters are presented in Table 2 and the compatibility among the experimental data and the mathematical model can be observed in Figure 2. The obtained correlation coefficient, R^2, was 0.9922 for CO_2, 0.9821 for CO_2 with water as co-solvent, and 0.9582 for CO_2 with ethanol as co-solvent.

The unknown parameters presented in the mathematical model were the effective diffusivity, the convective mass transfer coefficient and the parameter F, which was associated with the solubility of the extracts. These parameters were estimated by the minimization of the difference of the sum of squares of errors between the experimental data and the prediction using the model.

Table 1 - Experimental data for supercritical fluid extraction of *Schinus molle* using co-solvents at 333.15 K and 150 bar.

t (min)	M_1(g)	M_2(g)	M_3(g)
0	0.00	0.00	0.00
10	0.05	0.02	0.01
20	0.22	0.05	0.10
30	0.44	0.09	0.18
40	0.86	0.13	0.33
50	1.32	0.18	0.40
60	1.81	0.26	0.65
70	2.13	0.31	0.79
80	2.46	0.37	1.18
90	2.85	0.42	1.42
100	3.27	0.45	1.58
110	3.47	0.50	1.87
120	3.80	0.55	2.05
130	3.89	0.57	2.20
140	4.03	0.60	2.28
150	4.05	0.61	2.47
160	4.06	0.61	2.59
170	4.06	0.61	2.68
180	-	-	2.75
190	-	-	2.82
200	-	-	2.83
210	-	-	2.84
220	-	-	2.84

Solvents used: M_1, CO_2; M_2, CO_2 +H_2O; M_3, CO_2+C_2H_5 OH

Table 2 – Parameter of extraction curves fitted to experimental data for *S. molle* at 333.15K and 150 bar using CO_2 and co-solvents.

	F **(g/s)**	***D*** **(m²/s)**	k_c **(m/s)**
CO_2	$6.80x10^{-4}$	$1.72x10^{-9}$	$7.68x10^{-6}$
CO_2 + ethanol	$2.63x10^{-4}$	$4.76x10^{-10}$	$7.58x10^{-6}$
CO_2 + water	$7.33x10^{-5}$	$4.19x10^{-9}$	$4.87x10^{-6}$

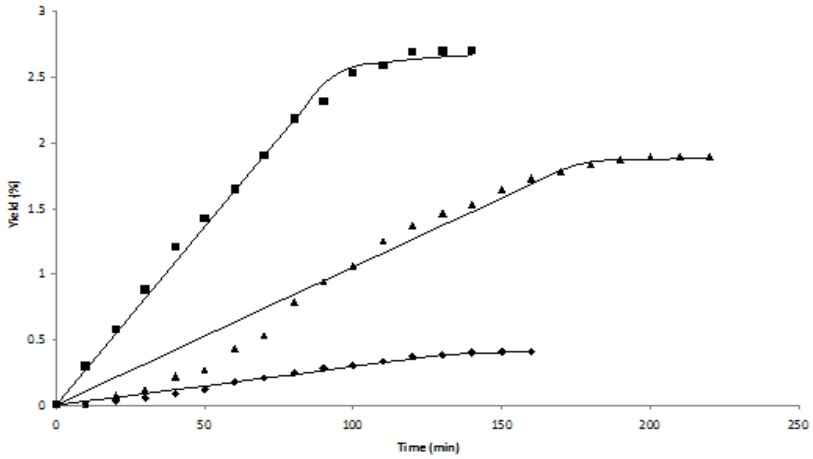

Figure 2 - Supercritical extraction yields curves *versus* time for *Schinus molle* obtained at 333.15 K and 150 bar: (■) CO_2, (▲) CO_2/ethanol, and (♦) CO_2/water; (—) mathematical model with parameters from Table 2.

Antimicrobial Activity Test Results

Using the bioautography procedure under the experimental conditions described, the extracts of *S. molle* presented antimicrobial activity only against *E. coli* and *M. luteus*. The qualitative results are presented in Figures 3 and 4. Each figure was constructed according to following sequence: the first result was the bioautography for *S. molle* extract obtained with carbon dioxide and water, the second one for the extract obtained with only carbon dioxide, and the third one for the extract obtained with carbon dioxide and ethanol as co-solvent.

Figure 4 refers to *Micrococcus luteus* incubated in the chromatography plate. The only assay that exhibited the zones of inhibition growth was the plate with the extract obtained using water as co-solvent.

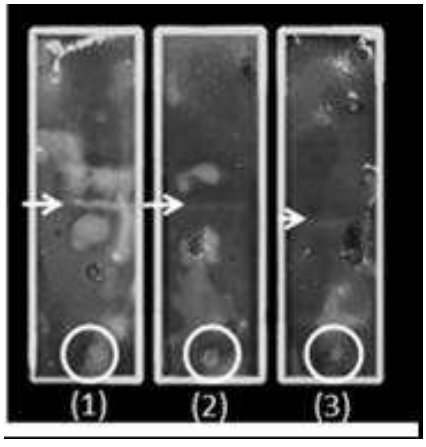

Figure 3 - TLC separation of *S. molle* non volatile extract obtained with (1) CO_2/water; (2) CO_2, and (3) CO_2/ethanol as solvent against *Escherichia coli*.

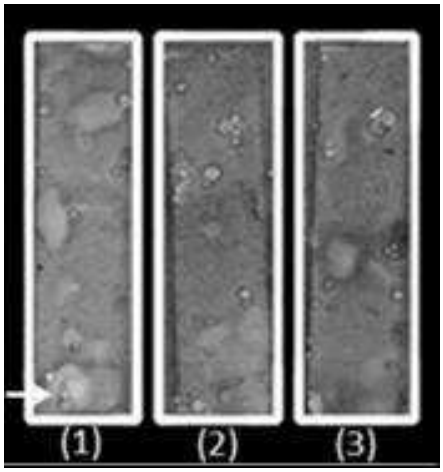

Figure 4 - TLC separation of *S. molle* non volatile extract obtained with (1) CO_2/water; (2) CO_2, and (3) CO_2/ethanol as solvent against *Micrococcus luteus*.

CONCLUSION

The mathematical model used showed feasibility to fit the experimental data for the process of extraction in supercritical conditions, either for the use of carbon dioxide as solvent or for the situation where the co-solvents were used, so the model parameters could facilitate a future process of scale-up.

On the microbiological aspects, the results showed that the obtained extracts of *S. molle* with carbon dioxide presented antimicrobial action for *M. luteous* and *E. coli*. The bioautography procedures for *E. coli* showed antimicrobial action for the obtained extracts using CO_2, CO_2+water and CO_2+ethanol whereas for *M. luteus* the antimicrobial action was only observed for the extract for *S. molle* using the supercritical CO_2+water.

ACKNOWLEDGMENTS

The authors are grateful to the Brazilian agencies CNPq, CAPES and FAPERGS for financial support.

REFERENCES

Bandoni A. Los recursos vegetales aromáticos en Latinoamérica. Argentina: Editorial de La Universidad Nacional de La Plata.; 2000.

Barroso MST , Villanueva G, Lucas AM , Perez GP, Vargas RMF, Brun GW et al. Supercritical fluid extraction of volatile and non-volatile compounds from *Schinus molle* L. *Braz. J. Chem. Eng.* 2011; 28: 305-312.

Cassel E, Vargas RMF, Brun GW, Almeida DE, Cogoi, L, Ferraro G et al. Supercritical fluid extraction of alkaloids from *Ilex paraguariensis* St. Hil. *J. Food Eng.* 2010; 100: 656-661.

Cassel E, Vargas RMF, Bedinot C. Unidad Piloto de Extracción Supercrítica Automatizada. In: Cassel E., Vargas RMF. Aplicaciones Industriales de los Taninos Vegetales: Productos y Procesos. Porto Alegre: EDIPUCRS; 2007. p. 95-102.

Choma IM, Grzelak EM. Bioautography detection thin-layer chromatography. *J. Chromatogr. A* 2011; 1218: 2684-2691.

Crank J. The Mathematics of Diffusion. New York: Oxford University Press; 1975.

Farmacopéia Brasileira. 4 ed. São Paulo: Atheneu; 1988.

Ferrero AA, Sánchez Chopa C, Werdin González JO, Alzogaray RA. Repellence and toxicity of *Schinus molle* extracts on *Blattella germanica*. *Fitoterapia* 2007; 78: 311–314.

Ferrero AA, Werdin González JO, Sánchez Chopa C, Alzogaray RA. Biological activity of *Schinus molle* on *Triatoma infestans*. *Fitoterapia* 2006; 77: 381–383.

Hayouni EA, Chraief I, Abedrabba M, Bouix M, Leveau JY, Mohammed H et al. Tunisian *Salvia officinalis* L. and Schinus molle L. essential oils: Their chemical compositions and their preservative effects against Salmonella inoculated in minced beef meat. *Internat. J. Food Microbiol*. 2008; 125: 242–251.

Huerta A, Chiffelle I, Puga K, Azúa F, Araya JE. Toxicity and repellence of aqueous and ethanolic extracts from *Schinus molle* on elm leaf beetle *Xanthogaleruca luteola*. *Crop Protect* 2010; 29: 1118-1123.

Marongiu B, Piras A, Porcedda S, Casu R, Pierucci P. Chemical composition of the oil and supercritical CO_2 extract of *Schinus molle* L.. *Flavour Fragr. J.* 2004; 19: 554-558.

Mukhopadhyay M. Natural Extracts using Supercritical Carbon Dioxide. Florida: CRC Press LLC; 2000.

Pereira MAA. Estudo da atividade antimicrobiana de óleos essenciais extraídos por destilação por arraste a vapor e por extração supercrítica[Dissertação de mestrado em Engenharia e Tecnologia de Materiais]. Porto Alegre: Pontifícia Universidade Católica do Rio Grande do Sul; 2010.

Reverchon E. Supercritical fluid extraction and fractionation of essential oils and related products. *J. Supercrit. Fluid* 1997; 10: 1-37.

Reverchon E, Marrone C. Modeling and simulations of the supercritical CO2 extraction of vegetable oils. *J. Supercrit. Fluid* 2001; 19: 165-175.

Taylor LT. Supercritical fluid extraction. USA: Wiley-Interscience Publication; 1996.

Valgas C. Avaliação de método de triagem para determinação de atividade antibacteriana de produtos naturais [Dissertação de mestrado em Farmácia]. Florianópolis: Universidade Federal de Santa Catarina; 2002.

Wimalaratne P, Slessor K, Borden J, Chong L, Abate T. Isolation and identification of house fly, *Musca domestica* L., repellents from pepper tree, *Schinus molle* L. *J. Chem. Ecol.* 1996; 22: 49-59.

Received: January 10, 2012;
Revised: September 10, 2012;
Accepted: May 02, 2013.