SUPERCRITICAL FLUID EXTRACTION FROM *Psidium guajava* L. LEAVES: MATHEMATICAL MODELING AND EXPERIMENTS

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Abstract. *Psidium guajava* L. is a fruitful tree widely spread throughout the Brazilian territory as well other South and Central American countries. Its leaves present phenolic compounds, which are attributed antioxidant activity. In this work we applied the CO_2 supercritical extraction to evaluate the use of this technique in obtaining of extracts of *Psidium guajava* L. from its leaves, in order to obtain natural products for food industries. The extracts were obtained through the use of CO_2 supercritical extraction process at 150 bar, two temperatures (313.15 and 333.15 K) and three different flow rate. Yield curves were generated for each investigated condition. 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 having to perform experiments in order to know its extraction process behavior. In view of future industrial application, the mathematical modeling was performed to determine unknown parameters present in the model. The mathematical model fitted very well the experimental data. Therefore, numerical results for the mathematical simulation and new data for the supercritical extraction in pilot scale were successfully obtained.

Keywords: *Psidium guajava* L., supercritical fluid extraction, natural product, antioxidant activity, mathematical modeling

1. Introduction

The guava tree (*Psidium guajava* L.) is a fruitful tree and widely grown in tropical and subtropical countries. The leaves of this tree are the source for extraction of the essential oil used in folk medicine and as a vegetal drug against diarrhea [1]. Reports of antioxidant effect are found and in agreement with the literature are due to the presence of phenolic acids such as ferulic acid. Recent studies have shown that foods rich in antioxidants play an essential role in preventing cardiovascular disease and cancer which justifies the use of extracts of *Psidium guajava* L. both in pharmaceutical and food industry [2].

Much attention has been focused on the use of antioxidants, especially natural anti-oxidants, to inhibit lipid peroxidation, or to protect against the damage of free radicals [3]. However, information concerning the antioxidant activity of guava leaves is scarce. *P. guajava* is widely cultivated and its fruit is popular in Brazil. The leaves and skin of the fruit have greater effects. Guava tea, the infusion of dried guava fruit and leaves, is commonly use in folk medicine. Besides, studies demonstrated the anti-diarrhoeal [4] antipyretic [5], antimicrobial [6] and bio-antimuta-genic [7] properties of guava leaf.

Supercritical fluid extraction is a technique widely used in separation processes of natural products, where the solvent usually used is not toxic, such as carbon dioxide. The supercritical fluid has properties such as high diffusivity, low viscosity and low surface tension, which gives it attractive characteristics as a solvent for the extraction of components from the solid matrix [8]. Advantages of the use of carbon dioxide under supercritical conditions are selectivity [9, 10] and solubility [11]. Supercritical fluid extraction is influenced by operational conditions. These conditions are bed porosity, solvent flow, temperature, pressure, and particle size.

The mathematical modeling of the supercritical extraction process is an inevitable step in designing industrial plants with good operational conditions. A mathematical modeling can be performed by establishing differential equations based on the mass transfer in the solvent fluid phase and in the solid phase along the bed. Models based on differential mass balance have been extensively used to describe supercritical extraction. Differential equations are obtained from mass balances achieved over an infinitesimal element of the extractor according to a hypothesis frame. On the basis of different hypotheses, several models were constructed [12, 13]. The approach, based on differential mass balances along the extraction bed, requires mass transfer mechanisms and an equilibrium relationship. In models of this kind, adjustable parameters derived from experimental extraction data are required [14, 15].

Therefore the objectives of this study are: to analyze the supercritical fluid extraction performance from leaves of *P. guajava* through process variable evaluation in order to define the appropriated solvent flow conditions with regard to the yield of the extracts, keeping all other variables constant. The leaves to be used in the supercritical fluid extraction will be submitted previously to steam distillation process for removal of the essential oil present in this plant. It also will evaluate the mass transfer aspects of supercritical fluid extraction using a mathematical model, and furthermore the study of antioxidant activity of the guava leaf extracts will be performed.

2. Material and Methods

2.1 Supercritical Extraction

The raw material was collected in the state of Rio Grande do Sul in Southern Brazil. The plant previously used in the steam distillation process to extract the essential oil was dried at 313.15 K during 48 h. A sample of 100 g of dried and milled material with an average particle diameter of $2.4\text{x}10^{-3}$ m was used for extraction of compounds in a supercritical extraction pilot plant [16]. The solvent used was 99.9% carbon dioxide (Air Products) with flow rate of 1.59×10^{-4} kg s^{-1}, 2.77×10^{-4} kg s^{-1}, and 4.16×10^{-4} kg s^{-1} through the extraction vessel. The extractor pressure was 15 MPa and the temperatures set were 313.15 K and 333.15 K. The temperature of the separator vessel was 273.15 K.

A schematic diagram of the experimental apparatus is shown in Figure 1.

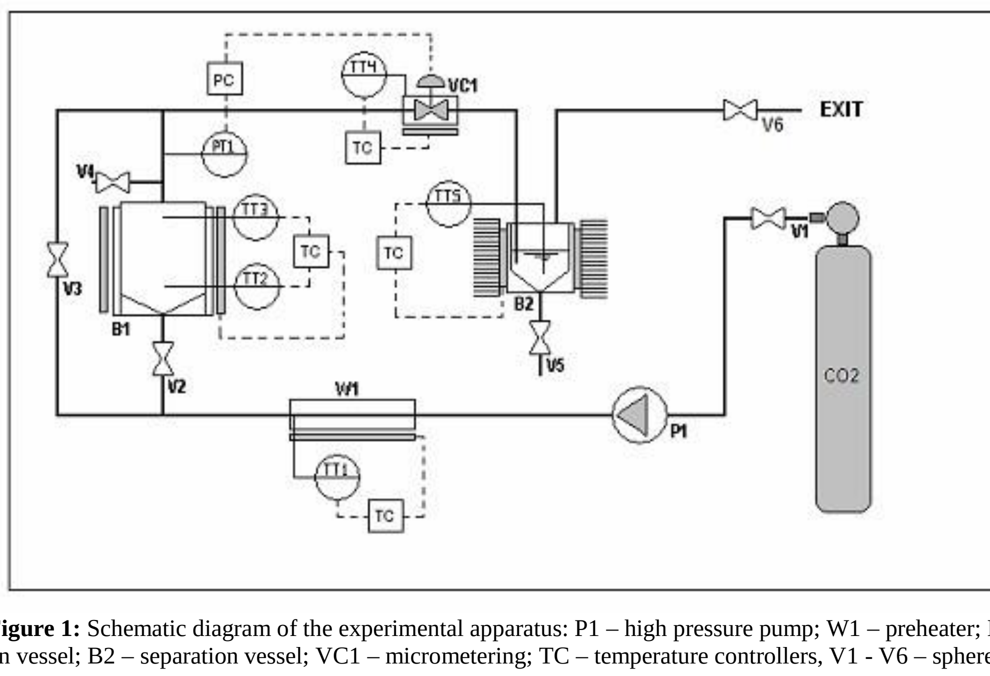

Figure 1: Schematic diagram of the experimental apparatus: P1 – high pressure pump; W1 – preheater; B1 – extraction vessel; B2 – separation vessel; VC1 – micrometering; TC – temperature controllers, V1 - V6 – sphere valves.

The experimental unit includes an air driven liquid pump (Maximator G35-CO_2) for solvent delivery, a 1000 mL high-pressure extraction vessel, and a separator flask. The extraction vessel is supplied with a heating jacket and an automated temperature controller. Heating tapes were used throughout the apparatus to maintain a constant temperature in the extraction section. Flow rates and accumulated gas volumes passing through the apparatus were measured using a mass flowmeter. Micrometering valves were used for flow control throughout the apparatus. Heating tapes with automated temperature control were also used around this valve to prevent both freezing of the solvents and solid solute precipitation following depressurization. Pressure in the extractor was monitored with a digital transducer system (Novus 8800021600) acquired from Novus Produtos Eletrônicos (Brazil) with a precision of ±1.0 bar and the temperature controller was connected to thermocouples (PT-100, with an accuracy of 0.5K).

2.2 Antioxidant Analysis

The antioxidant activities of extracts from guava leaves were determined by the DPPH method [17] based in the capture of the free radical DPPH (2,2-difenil-1-picril-hidrazil) by antioxidants, decreasing the absorbance at 515 nm. The analyses were performed in a Biospectro SP-220 spectrophotometer where the absorbance of the extracts was measured at the concentration of 100%, 50% and 25%. The dilutions were made in a solution of absolute ethyl alcohol PA and free radical DPPH (2,2-Diphenyl-1-picryl-hydrazyl).

The evaluation of the absorbance for each concentration was performed until the stabilization, which was observed after 1h, for subsequent construction of the curve of absorbance versus time, which allowed the determination of oxidative action of the compound under analysis.

Each dilution corresponds to a point in a new curve of absorbance *versus* dilution used in the determination of the parameter EC50 (amount of oil required to reduce by 50% the initial absorbance).

2.3 Mathematical Modeling

Several mathematical models have been employed to simulate supercritical fluid extraction from vegetable matrixes [18,19,1]. In this work, the mathematical model used to fit the experimental data was that proposed by Xavier et al. [20] based on the discussion provided by Sovová [21]. 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 [21]. This model is based on the concept of broken and intact cells and it was developed to fit experimental data and simulates two extraction periods. The first period is governed by phase-equilibrium and the second period by internal diffusion in the particles. Basically the two periods assumed to describe the extraction curve is based on mass balance of the solute. The solute is the extracted material. The extract is assumed to be a pseudo component in terms of the mass balance. The extract recovered is considered the same in inner of the particles.

The choice of the adequate model was done by analysis of the experimental behavior. The solution for this model is presented as follow

$$\frac{M(t)}{M(\infty)} = \begin{cases} \dfrac{K_1 t}{M(\infty)} \quad \text{for the first period} \\ \text{and} \\ (1-e^{-K_2 t}) \quad \text{for the second period} \end{cases} \tag{1}$$

These results are expressed in terms of the maximum value for the extract obtained in each extraction, $M(\infty)$, for the two periods in the extraction. Where:

$$K_1 = \dot{m} Y^* \tag{2}$$

$$K_2 = \frac{k_s a_0}{(1-\varepsilon)} \tag{3}$$

and M(t) is the mass of extract, k_s is solid-phase mass transfer coefficient, a_0 is specific surface area per unit volume of extraction bed, Y^* is equilibrium fluid-phase mass fraction and x_k is easily accessible solute in solid-phase, ε is bed porosity.

3. Results and Discussions

The unknown parameters in the model presented above were adjusted by using the experimental data from the pilot supercritical extraction equipment. The numerical values for these parameters are presented in Table 1.

Table 1: Parameters of extraction curves fitted to experimental data for *P. guajava* at 313.15 K and 333.15 K.

Temperature	Flow Rate	K_1 (g/min)	K_2 (min^{-1})
313.15 K	1.59×10^{-4} kg s^{-1}	0.001659	0.016202
	2.77×10^{-4} kg s^{-1}	0.002591	0.019019
	4.16×10^{-4} kg s^{-1}	0.011096	0.026017
333.15 K	1.59×10^{-4} kg s^{-1}	0.001415	0.033273
	2.77×10^{-4} kg s^{-1}	0.005304	0.028602
	4.16×10^{-4} kg s^{-1}	0.017287	0.025912

These parameters were estimated by minimization of the sum of squares of errors between the experimental data and mathematical results. The experimental data and the results for the mathematical modeling of the supercritical extraction at 313.15 K and 333.15K at the pressure of 15MPa are shown in Figure 2 and Figure 3, respectively.

The ordinate represents the extract mass quantity, and the abscissa represents the extraction time. Agreement between the mathematical model used and the experimental data was observed. The lesser variations can be the result of the numerical method applied to adjust the parameters, as well as the variations inherent to experimental data.

The mathematical model used here is based on the concept of broken and intact cells; it is suited to fit experimental data whose behavior is described by two temporal phases, the first one governed by phase equilibrium and the second one governed by internal diffusion from particles. In the first phase, a straight line reproduces the behavior of the extraction curve. The solubility is established from the slope of this line for all extraction conditions carried out, and it is observed to increase with the pressure. In the second phase, a curve represents the extraction and the mathematical modeling involves the parameters K_2 is associated with the solid-phase coefficient. It is important to remember that the crossing point is a fictional time when mass transfer from intact cells begins, also known as the shift of the second part of extraction curve.

3.1 Antioxidant Activity

The results for the activity antioxidant were obtained by the methodology presented previously and they were presented in Table 3.

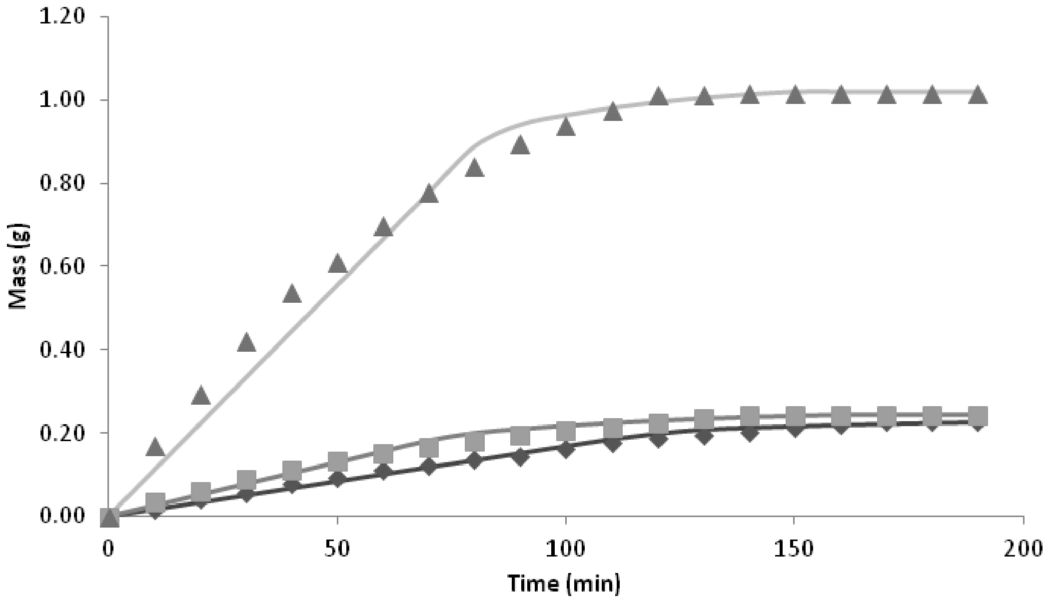

Figure 2: CO_2 supercritical extraction mass *vs*. time curves at 313.15 K and 15 MPa. Flow rate conditions: (♦) $1.59{\times}10^{-4}$ kg s^{-1}; (■) $2.77{\times}10^{-4}$ kg s^{-1} ; (▲) $4.16{\times}10^{-4}$ kg s^{-1} ;(—) Eqs. (1) with parameters from Table 3.

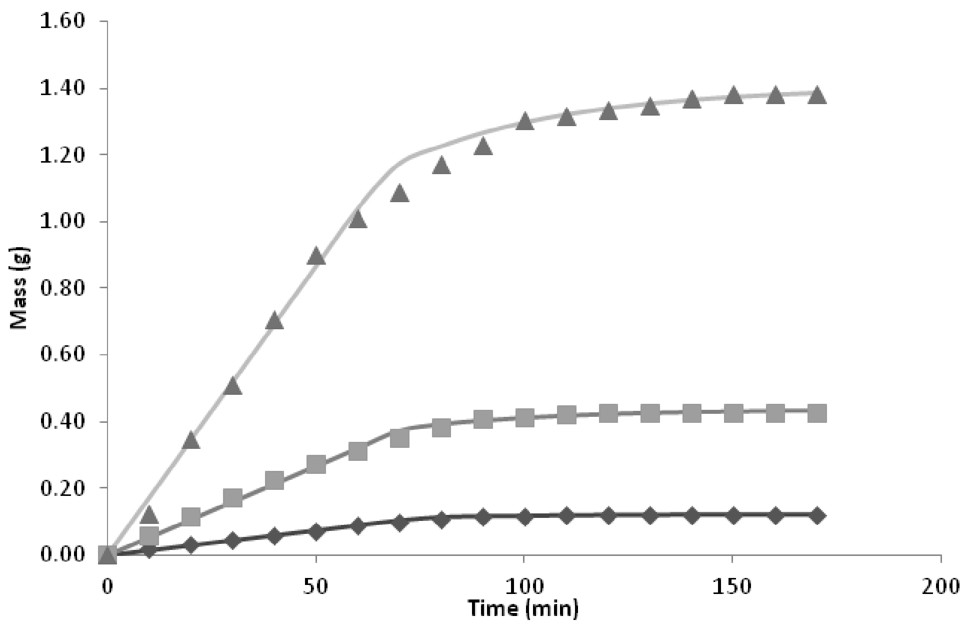

Figure 3: CO_2 supercritical extraction mass *vs*. time curves at 333.15 K and 15 MPa. Flow rate conditions: (♦) 1.59×10−4 kg s−1; (■) 2.77×10−4 kg s−1 ; (▲) 4.16×10−4 kg s−1 ; (—) Eqs. (1) with parameters from Table 3.

Table 2: Antioxidant activity of the extracts obtained by supercritical extraction.

	EC_{50} (g/L)	**g extract/g DPPH**
313.15 K	0.2171	19.1347
333.15 K	0.1970	17.4707

4. Conclusions

Steam distillation enables preliminary purification of the leaves from volatile to abstaining of extracts volatiles and compounds of low antioxidant activity. Subsequent supercritical extraction leads to obtaining of extracts with non-volatile compounds and high DPPH radical scavenging properties.

According to the results obtained, traditionally used solvents (i.e. methanol) can be successfully replaced by a nontoxic solvent such as CO_2. The use of supercritical fluid extraction is part of a proposal for a sustainable use of this plant that exists in abundance in Southern Brazil and whose extracts have proven activities.

The model used in this study describes satisfactorily the experimental extraction curve enabling the determination of the values of the adjustable parameters which provides important knowledge about the supercritical extraction of *Psidium guajava* L.. Finally, the model parameter evaluation reported in this work on the pilot scale permits one to evaluate the extraction time required to obtain a given mass and should be useful for the scale-up of the extraction process.

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References

[1] Silva, J.D., Luz, A.I.R., Silva, M.H.L., Andrade, E.H.A., Zoghbi, M.G.B., Maia, J.G.S. Essential oils of the leaves and stems of four *Psidium* spp. Flavour and Fragrance Journal 18 (2003) p. 240-243.

[2] Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M.Binkoski, A.E., Hilpert, K.F. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. American Journal of medicine 113 (2002) 71S-88S.

[3] Vendemiale, G., Grattagliano, I., Altomare, E. An update on the role of free radicals and antioxidant defense in human disease. International Journal of Clinical and Laboratory Research, 29 (1999) p. 49-55.

[4] Lutterodt, G. D. Inhibition of gastrointestinal release of acetylcholine by quercetin as a possible mode of action of Psidium guajava leaf extracts in the treatment of acute diarrhoeal disease. Journal of Ethnopharmacology, 25 (2989) 235–247.

[5] Olajide, O. A., Awe, S. O., & Makinde, J. M. Pharmacological studies on the leaf of Psidium guajava. Fitoterapia 70 (1999) 25–31.

[6] Jaiarj, P., Khoohaswan, P., Wongkrajang, Y., Peungvicha, P., Suriyawong, P., Sumal Saraya, M. L., et al. Anticough and antimicrobial activities of Psidium guajava Linn, leaf extract. Journal of Ethnopharmacology 67 (1999) 203–212.

[7] Matsuo, T., Hanamure, N., Shimoi, K., Nakamura, Y., & Tomita, I. Identification of (+)-gallocatechin as a bio-antimutagenic compound in Psidium guava leaves. Phytochemistry 36 (1994) 1027–1029.

[8] Taylor, L.T., Supercritical Fluid Extraction. John Wiley & Sons Inc. New York, 1996, 181p.

[9] Cardozo Jr E.L., Cardozo-Filho L., Ferrarese-Filho O. and Zanoelo E.F., Selective liquid CO2 extraction of purine alkaloids in different Ilex paraguariensis progenies grown under environmental influences. J. Agric. Food Chem. 55 (2007) 6835-6841.

[10] Azevedo A.B.A., Mazzafera P., Mohamed R.S., Melo S.A.B.V., Kieckbusch T.G., Extraction of caffeine, chlorogenic acids and lipids from green coffee beans using supercritical carbon dioxide and co-solvents, BJChE, 25 (2008) 543-552.

[11] Brennecke J.F., Eckert C.A., Phase equilibria for supercritical fluid process design, AIChE J. 35 (09) (1989) 1409-1427.

[12] Reverchon, E. Mathematical Modeling of Supercritical Extraction of Sage Oil. AIChE Journal 42 (1996) 1765.

[13] Martinez J, Monteiro A.R., Rosa P.T.V., Marques M.O.M. and Meireles M.A.A., Multicomponent model to describe extraction of ginger oleoresin with supercritical carbon dioxide. Ind. Eng. Chem. Research 42 (2003) 1057-1063.

[14] Marrone C., Poletto M., Reverchon E. and Stassi A., Almond oil extraction by supercritical CO_2: experiments and modelling. Chem. Eng. Science, 53 (1998) 3711-3718.

[15] Vargas, R.M.F.; Cassel, E.; Gomes, G.M.F.; Longhi, L.G.; Atti-Serafini, L.; Atti-Santos, A.C. Supercritical Extraction of Carqueja Essential Oil: Experiments and Modeling, Brazilian Journal of Chemical Engineering, 23 (3) (2000) 375-382.

[16] Cassel E., Vargas R.M.F., Brun G.W., Almeida D.E., Cogoi L., Ferraro G. and Filip R., Supercritical fluid extraction of alkaloids from *Ilex paraguariensis* St. Hil. J. Food Eng. 100 (2010) 656-661.

[17] Brand-Williams, W.; Cuvelier, M, E.; Berset, C. Use of free radical method to evaluate antioxidant activity. Food Science and Technology, 28 (1995) 25-30.

[18] Grosso C., Coelho J.P., Pessoa F.L.P, Fareleira J.M.N.A., Barroso J.G., Urieta J.S., Palavra A.F. and Sovova H., Mathematical modeling of supercritical CO_2 extraction of volatile oils from aromatic plants, Chem. Eng. Science 65 (2010) 3579–3590.

[19] Steffani E., Atti-Santos A.C., Atti-Serafini L. and Pinto L.T., Extraction of Ho-Sho (*Cinnamomum camphora* Nees and Eberm var. *Linaloolifera fujita*) essential oil with supercritical CO_2: experiments and modeling, BJChE, 23 (2006) 259-266.

[20] Xavier, V.B., Vargas, R.M.F., Cassel, E., Lucas, A.M., Santos, M.A., Mondin, C.A.,Santarem, E.R., Astarita, L.V., Sartor, T. Mathematical modeling for extraction of essential oil from Baccharis ssp. by steam distillation, Ind. Crops Prod. 33 (2011) p. 599–604.

[21] Sovová, H. Mathematical model for supercritical fluid extraction of natural products and extraction curve evaluation. The Journal of Supercritical Fluids 33 (2005) p. 35-52.