EXTRACTION OF VOLATILE OIL FROM *Croton zehntneri* PAX ET HOFF WITH PRESSURIZED CO$_2$

Elisa M. B. D. Sousa[1], Osvaldo Chiavone-Filho[1], Tyrone Domingos[1], Marcia O. M. Marques[2], M. Angela A Meireles[3],*

[1]Universidade Federal do Rio Grande do Norte (UFRN), Depart. of Chem. Eng, 59072-970 Natal, RN, Brazil; [2]LPN – Laboratório de Produtos Naturais, IAC, Cx. P. 28, 13001-970 Campinas, São Paulo, Brazil; [3]LASEFI - DEA / FEA (College of Food Eng)– UNICAMP, Cx. P. 6121, 13083-970 Campinas, São Paulo, Brazil

meireles@fea.unicamp.br; phone: 55 19 37884033; fax: 55 19 37884027

Croton zehntneri Pax et Hoff is indigenous to the Northeastern of Brazil; its popular name is "canela de Cunhã" (cinnamon of Cunhã). The specie is rich in anethole and is largely used in cooking to impart flavor to a variety of foods; the infusion of its leaves is used in folk medicine to treat manifestation that involve from a simple indigestion up to cases of insomnia. In this work the volatile oil from *C. zehntneri* was extracted using pressurized CO$_2$. Both kinetic and solubility experiments were performed using a fixed bed extractor. The composition of the volatile oil was determined using gas chromatography coupled to mass spectrometry. At 15 ^{o}C and 66.7 bar the solubility for the system *C. zehntneri* + CO$_2$ was 28.3×10^{-3} kg/kg and the global yield was 3.4% (mass). The volatile oil contained predominantly (E)–anethole (up to 88.9 %, area) and small amounts of sabinene, myrcene, 1,8-cineole, (E)-beta-ocimene, alpha-terpineol, methyl chavicol, (Z)–anethole, delta-elemene, beta-elemene, trans-caryophyllene, germacrene D, alpha muurolene, beta-bisabolene.

Keywords: High pressure, Extraction, *Croton zehntneri* Pax et Hoff, Solubility, Modeling

INTRODUCTION

Croton zehntneri Pax et Hoff is indigenous to the Northeastern of Brazil; its popular name is "canela de Cunhã" (cinnamon of Cunhã). Among the Euphorbiaceae family the *Croton* gender is the most widely spread in the Brazilian Northeast. The *C zehntneri* grows as part of the local vegetation called caatinga. It is a delicate bush, of fine branches, dark brown color, small and oval leaves, presenting leaves with starry hair, and strong scent of anisette [1]. The specie is rich in anethole [2] and is largely used in cooking to impart flavor to a variety of foods [1]; the infusion of its leaves is used in folk medicine to treat manifestation that involve from a simple indigestion up to cases of insomnia. Several pharmacological applications have been reported in literature: Giorgio et al [3] studied the effects of the aqueous extracts of *C. zehntneri* on the behavior of laboratory rodents. Batatinha et al [4] demonstrated the effects of the volatile oil in the behavior of rats and mice. Recently, Oliveira et al [5] showed the antinociceptive effects of the essential oil of *C. zehntneri* in mice. Some of these properties are a result of the combined action of anethole and methyl chavicol (estragole), the major compounds of *C. zehntneri* extracts.

In this work the solubility for the system *C. zehntneri* + CO$_2$ was measured at 66.7 bar and temperatures of 10, 15, 20, 25, and 28 ^{o}C; kinetic data was obtained at 15 ^{o}C and 66.7 at two solvent flow rates.

Mise en forme : Puces et numéros

Commentaire : Nociceptive = painful

MATERIALS AND METHODS

Raw material characterization and preparation

The *C. zehntneri* leaves were from the municipality Serra do Ibiapaba (State of Ceará, Brazil) located in the Brazilian Northeastern. The leaves were dried naturally in the shade. The dried raw material was conditioned in plastic bags under vacuum and stored in a domestic freezer (Consul, model 280, Brazil). The humidity of the *C. zehntneri* leaves was determined by the xylol distillation method [6], recommended for odoriferous plants. Before each experimental run the material was triturated in a domestic food processor (Arno, model PRO, Brazil) for 15 seconds. The particle size distribution of the solid was determined using an agitator (Produtest, model 3614, Brazil) for 15 minutes (rheostat at position 10) and sieves of the Tyler series meshes 20, 28, 35, and 48.

Characterization of the particles and fixed bed

The true density of the *C. zehntneri* particles was measured by helium pycnometry at the Analytical Facilities of the Chemistry Institute – IQ/Unicamp. The apparent bed density was calculated using the mass of solid loaded into the fixed bed cell and the cell's volume. The total porosity of the bed plus particles was determined using the true and the apparent densities.

SFE unit and experimental procedure

The assays were performed using the SFE unit described by Sousa et al [7]. For each load about 120 g of triturated *C. zehntneri* were used to for the fixed bed; the particle distribution was 25% (mass) particles of meshes – 35/ +48, 50% (mass) particles of meshes – 20 to + 35, and 25% (mass) particles of meshes – 28/ +35. The solid particles were placed inside of the extraction cell with the aid of a funnel and compressed with the help of a stem in order to achieve a complete and uniform accommodation of the bed. Once the extraction cell was assembled into the SFE unit the system was allowed to reach the desired temperature and pressure. Then, the downstream valve was opened and the extraction began. An adsorbent column was fitted just after the separation flask to prevent the losses of more volatile substances. Extract samples were collected at pre-selected time intervals in order to build the overall extraction curves.

The solubility for the system *C. zehntneri* + CO_2 was measured using the dynamic method [8]; in order to identify the solvent flow rate at which saturation occurred assays were performed at 0.35 to 1.26±0.05 g/min.

Characterization of the extract: chemical composition

The chemical composition of the *C. zehntneri* was determined using a gas chromatographer coupled to a mass spectrometer system (CG-MS, Shimadzu, model QP 5000, Japan) equipped with a capillary column DB-5 (30m $\times$ 0.25 mm $\times$ 0.25μm). The carrier gas was helium (99.99% purity, White Martins Gases Industriais, 1.0 ML/min.). The injector and detector temperatures were 220 ^{o}C and 240 ^{o}C, respectively. The column temperature was programmed from 60 ^{o}C to 240 ^{o}C, at 3 ^{o}C/min. The sample injected consisted of 1μL of extract diluted in ethyl acetate (0.005 g of extract diluted in 1 mL of ethyl acetate, HPLC grade, Merck, lot 3607913, Brazil) and the split ratio was 1/30. The identification of the substances was based on (i) comparison of substance mass spectrum with GC-MS system data bank (Nist 62 Library), (ii) comparison of mass spectra with data in literature [9], and (iii) retention index [10].

Calculation of kinetic parameters

Using the experimental data, the overall extraction curves were fitted to a spline using two straight lines. The first line was identified with the constant extraction rate period (CER). The rate of mass transfer for the CER period (M_{CER}) as well as the time corresponding to the interception of the two lines (t_{CER}) was computed from the spline. The spline was fitted using multiple-regression analysis (STATISTICA 5.0). MS Excel 97 was used to determine the interception of the two lines. The mass ratio of solute at the extraction cell outlet was obtained by dividing M_{CER} by the mean solvent flow rate for the CER period.

RESULTS AND DISCUSSION

The humidity of the *C. zehntneri* leaves was 13.2± 0.1% (wet basis); the bed apparent density was 542.8 kg/m^3 and the particle true density was 1320± kg/m^3 resulting in a porosity of 0.59. The overall extraction curves had the typical shape with 3 distinctive regions: (i) Constant extraction rate period (CER), (ii) Falling extraction rate period, and (iii) Diffusion controlled extraction rate period (DC). The saturation solvent flow rate varied from 1.0 to 1.32×10^{-5} kg/s. Figure 1 shows the solubility (Y^*) and the global yield (X_0), expressed as mass of extract divided by mass of dry feed, for the system C. zehntneri + CO_2 for the isobar of 66.7 bar. It is interesting to observe that the maximum in solubility occurred at 15 ^{o}C while the maximum yield occurred at 20 ^{o}C, instead. This indicates that a region of retrograde behavior should be observed at approximately 66.7 bar at either the 15 or the 20 ^{o}C isotherms.
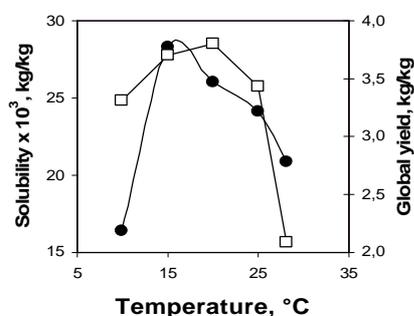

Figure 1: Solubility and global yield for the 66.7 bar isobar: solubility (·) and global yield (•).

Table 1 shows the composition of the *C. zehntneri* SFE extract obtained at 66.7 bar and 15 ^{o}C. The major component of the *C. zehntneri* extracts was (E)–anethole reaching up to 89.66% for certain operating conditions. The content of methyl chavicol (estragole) that has been reported [11] as the major component of *C. zehntneri* is well bellow the content of (E)-anethole and alpha-muurolene.

Tables 2 to 4 show the effects of extraction time intervals, solvent flow rate, and temperature in the content of the major compounds of *C. zehntneri* SFE extracts.

Table 2 shows that the composition of the SFE extract was approximately constant during the CER period, which is a condition required in order to use the dynamic method to measure solubility. It was determined that approximately 5.5% of the extract was retained in the capture column and the content of (E)-anethole was 82.02%; a value that is approximately the as that in the samples retained in the collector vessel. Thus, no further separation was observed after the collector vessel.

A comparison of the data in Table 1 with that in Tables 2 to 4 shows that GC-FID

system is more sensitive to (E)-anethole than the GC-MS system: The difference in the content of (E)-anethole obtained by the two methods is roughly 11%. Thus, the use of GC-MS data in process design is not appropriate.

The contents of (Z)-anethole and methyl chavicol in the capture column were 1.06% and 2.30%, which are approximately equal their contents in the collector's flask sample. This indicates that the collector flask has behaved as a flash separator, thus, further fractionating the SFE extract.

Table 1: Components identified by GC-MS in the *Croton zehntneri* Pax et Hoff. Extract obtained at 15 ^{o}C, 66.7 bar at the saturation flow rate; the process ran for 4 hours.

Substance	Composition (area %)
Sabinene	0.06
Myrcene	1.02
1,8–cineole	0.78
(E) – beta-ocimene	0.23
Alpha–terpineol	0.21
Methyl chavicol (estragole)	2.52
(Z) – anethole	1.37
(E) – anethole	73.60
Delta–elemene	0.19
Beta–elemene	0.33
Trans–caryophyllene	1.46
Germacrene D	2.11
Alfa–muurolene	7.81
Beta–bisabolene	0.32
Non identified	7.99

Table 2: Effect of the extraction time interval in the content of the major components of the SFE extracts obtained at 66.7 bar, 15 ^{o}C, 1.03 ´ 10^{-5} kg/s (saturation flow rate) and t_{CER} = 152 min; data from de GC-FID system.

	Composition (area %)				
	Extraction time interval (min.)				
Substance	0 – 50	50 – 100	100 – 150	150 – 260	260 – 300
(E) – Anethole	88.83	85.03	88.51	89.66	86.23
Alpha-muurolene	0.46	1.41	0.97	0.37	--
Methyl chavicol	2.09	2.09	2.32	1.85	0.64

Table 3 shows that the solvent flow rate marginally affected the composition of the SFE extracts. The effects of temperature on the composition of the SFE extract for the CER period are in Table 4, again, the effect was negligible and a similar behavior was observed for the pressure.

Modeling of the overall extraction curves

The mathematical description of the overall extraction curves is an important tool for scale up of process. Several models are available in the literature to describe the overall extraction curves. Among them the Sovová's [12] model is chosen due to its physical description of the process. Table 5 shows the parameters used to calculate the overall

extraction curves and Figure 2 compares experimental and calculated overall extraction curves.

Table 3: Effect of solvent flow rate in the content of the major components of the SFE extracts obtained at 66.7 bar and 15 °C during the CER period; data from GC-FID system.

Substance	Composition (area %)				
	5.83×10^{-5} kg/s	1.03×10^{-5} kg/s	1.57×10^{-5} kg/s	2.05×10^{-5} kg/s	2.13×10^{-5} kg/s
(E) – Anethole	82.11	88.51	83.52	80.72	82.47
Alpha-muurolene	1.39	0.96	1.42	1.55	1.33
Methyl chavicol	2.37	2.32	2.56	2.17	2.38

Table 4: Effect of temperature in the content of the major components of the SFE extracts obtained at 66.7 bar and at the saturation flow rate. data from GC-FID system.

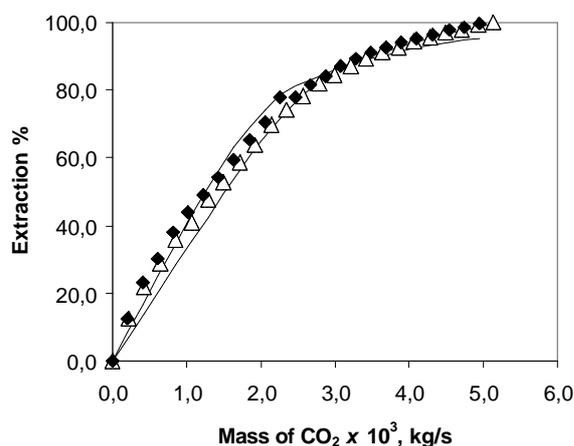| Substance	Composition (area %)				
	10 °C	15 °C	20 °C	25 °C	28 °C
	1.3×10^{-5} kg/s	1.03×10^{-5} kg/s	1.18×10^{-5} kg/s	1.32×10^{-5} kg/s	1.32×10^{-5} kg/s
(E) – Anethole	87.09	88.51	87.69	87.19	82.52
Alpha-muurolene	0.15	0.96	0.97	0.74	1.32
Methyl chavicol	2.04	2.32	2.05	1.13	2.29

Figure 2: Comparison between experimental and calculated overall extraction curves: 66.7 bar, 15 °C; 2.05 ´ 10^{-5} kg/s (◆) 2.14 ´ 10^{-5} kg/s (△).

Table 5: Parameters of the Sovová's model for OEC obtained at 66.7 bar and 15 °C[*]

Q_{CO2}, kg/s	Z	X_P/X_0, kg/kg	$k_Ya \ 10^4$, s^{-1}	$k_Xa \times 10^5$, s^{-1}	$t_{CER}/60$, s	$t_{FER}/60$, s
2.05	2.6429	69.2	2.9877	8.6171	29.0	119
2.13	1.0827	72.4	1.2760	5.9665	68.6	151

[*]Q_{CO2} is the solvent flow rate; Z: parameter of the Sovová model for the CER period; X_p: mass ratio of solute in ruptured cells; X_o: mass ratio of solute in the cellulosic structure (global yield); k_{Ya}: fluid-phase mass transfer coefficient; k_{Xa}: solid-phase mass transfer coefficient; t_{CER}: duration of the CER period; t_{FER}: end of the falling rate period.

CONCLUSIONS

The overall extraction curves for the system *C. zehntneri* + CO_2 have the typical shape consistent of the CER, FER and DC periods. At 66.7 bar the solubility has a maximum at 15 ^{o}C; for the global yield the maximum was detected at 20 ^{o}C. The major compounds presents in the SFE extracts were (E)-anethole and alpha–muurolene (menthol); estragole (methyl chavicol) was also detected but is smaller quantities. The Sovová's model described well the overall extraction curves.

ACKNOWLEDGMENTS

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