Supercritical Fluid Extraction from Vetiver Roots: A Study of SFE Kinetics

Emeline Talansier[1, 2], Mara E.M. Braga[1], Paulo T.V. Rosa[1], Chantal Menut[2], M. Angela A. Meireles[1,*]

[1]**LA**boratory of **S**upercritical Technology: **E**xtraction, **F**ractionation, and **I**dentification of Vegetable Extracts (**LASEFI**). Department of Food Engineering (FEA) - Unicamp (State University of Campinas), P.O. Box 6177, 13083-970, Campinas, SP, Brazil. E-mail: meireles@fea.unicamp.br
[2]Laboratoire de Chimie Biomoléculaire, ENSCM, 8, Rue de l'Ecole Normale, 34296 Montpellier Cedex 5, France

The recovery of volatile oils from vegetal raw materials is an activity of great interest to the food, perfumes and cosmetic industries. The vetiver (*Vetiveria zizanioides*) oil is particularly appreciated in perfume and cosmetic products but may also be used in food industry as an aroma and as flavor agent in some beverage. The objective of this work, based on preliminary process parameters studies, is to improve the process – meaning both quantity and quality of the compounds – of vetiver extracts recovered by supercritical technology using carbon dioxide as supercritical fluid (SFE). Vetiver roots were purchased from a local producer in Brazil, dried, milled and classified. The extraction assays were carried out using the Spe-ed unit, unit I and II. The optimum extraction pressure was determined for the 40 °C isotherm, for pressures in the range of 100 - 300 bar. The determined optimum pressure was used to study the influence of percentage of ethanol as co-solvent (0, 5 and 10% [v/v]). The chemical composition of the extracts was determined by gas and thin layer chromatography (GC and TLC, respectively). Overall extraction curves (OEC) were constructed allowing the determination of the kinetic parameters. The results of the extraction done using 10% of ethanol [v/v] were statistically different from 0 and 5% for global yield and kinetics parameters t_{CER}, M_{CER} and R_{CER}. The TLC showed similar chemical profiles between SFE and hydrodistillation (HD), but there are some compounds that can be observed by TLC just during the t_{CER} period.

INTRODUCTION

The recovery of volatile oils from vegetal raw materials is an activity of great interest to the industry for the manufacturing of natural products for many purposes. The compounds from volatile oils can have sensorial properties that make them valorous for the manufacturing of perfumes, other cosmetic products and foods.

Vetiver (*Vetiveria zizanioides* (L.) Nash ex Small) is a plant of the *Gramineae* family widely spread in tropical regions of Asia, Africa, Oceania, and Central and South America. The volatile oil from vetiver roots is a viscous liquid, whose color varies from amber to dark brown, and whose odor has sweet, earthy, and woody notes. This oil is much appreciated by the perfume industry, where it is used as a fixative and as an odor contributor in bases, such as rose, chypre and in several masculine fragrances [1].

The extraction of essential oil components using supercritical fluids (SFE) has received much attention for several years, especially in food, pharmaceutical and cosmetics industries, since it is an alternative to conventional processes such as organic solvent

extraction and steam distillation [2]. Each of these processes can produce very high quality fragrance and flavour ingredients. However, elevated temperature (from distillation) and solvent usage (from organic solvent extraction) can detract from total quality [3].

Indeed, the use of supercritical fluid extraction (SFE) to obtain vegetable extracts can be of great interest to improve the quality of the product, since it is a clean and low temperature-required technology [1], [3].

In order to understand the behavior of the extraction processes, mathematical models are fitted to the experimental overall extraction curves. In general, two different approaches have been proposed for the mathematical modelling of the SFE of essential oils: (1) empirical equations as the Langmuir-like Isotherm (Naik et al. 1989) [4], (2) models based on differential mass balance integration. Among these models, the most studied are Crank [5], Tan&Liou [6], Goto et al. [7] and Sovová [8] models.

The objective of this work, based on preliminary process parameters studies, was to improve the process − meaning both quantity and quality of the compounds − of vetiver extracts recovered by supercritical technology using carbon dioxide as supercritical fluid (SFE).

I. MATERIALS AND METHOD

The vetiver roots (*Vetiveria zizanioides* (L.) Nash ex Small) was cultivated at São Paulo State - Brazil and it was milled and classified using an vibratory sieve system (Bertel, model 1868, Caieiras, Brazil), containing sieves of the Tyler series meshes 24, 32 and 48.

The Speed SFE system (Applied Separations, model 7071, Allentown, USA) with a 5mL extraction column was used to determine the global yield isotherm with pressures from 100 to 300 bar. For the determination of the OEC, the extraction assays were carried out using the equipment similarly to the ones described by França & Meireles [9] (Unit I, without cosolvent) and Pasquel et al. [10] (Unit II, with cosolvent). The raw material was packed inside of the extraction column via a nylon basket (mesh 80). The process pressure and outlet solvent flow rate were controled throughout the extraction by a micro-metering valve (Autoclave Engineering, model 10VRM 2812, USA).

The extracted samples from Brazilian Vetiver roots are analyzed by thin-layer and gas chromatography (TLC and GC, respectively). The TLC plates were revealed with an anisaldehyde solution used for the revelation of essential oil components or terpenes [11]. The GC results were normalized to the extract concentration and to the most important area obtained.

All the statistical analyses, including the effects of pressure on the global yield and influence of cosolvent percentage, were performed using an analysis of variance (ANOVA /MANOVA) by the Tukey HSD Test of Statistica 5.0 software.

The kinetic parameters (M_{CER} and t_{CER}) were computed using the procedures PROC REG and PROC NLIN of SAS®SYSTEM, for Windows, version 8e [12]. The mathematical models tested to fit the experimental extraction curves were: Crank [5], Tan and Liou [6], Goto et al [7], Sovová [8], and Naik et al [4]. The curves fits were done with the help of the software TECANALYSIS developed at LASEFI-DEA/ FEA-UNICAMP

II. RESULTS AND DISCUSSION

The global yield, X_0, is the amount of soluble material that can be extracted from a given vegetable matrix at a given pressure and temperature, expressed as the ratio of mass of soluble material to mass of solid substrate [13]. The influence of the process pressure on the extraction global yield is presented at Figure 1. There is a noticeable increase in the extraction

yield for pressures up to 200 bar − with a maximum value X_0 of 4.6% (dry basis, d.b.) obtained at 200 bar of pressure − and after that, the yield remains practically constant with pressure. Moreover, the yields obtained at 100, 150 and 200 bar are statistically different at 0.05 of confidence, whereas the ones obtained at 200, 250 and 300 are not. Considering that the solubility is directly linked to the temperature and to the pressure of the process, for a process temperature fixed at 40°C, the maximum solubility of the Vetiver extraction in the supercritical CO_2 occurs at a process pressure of 200 bar.
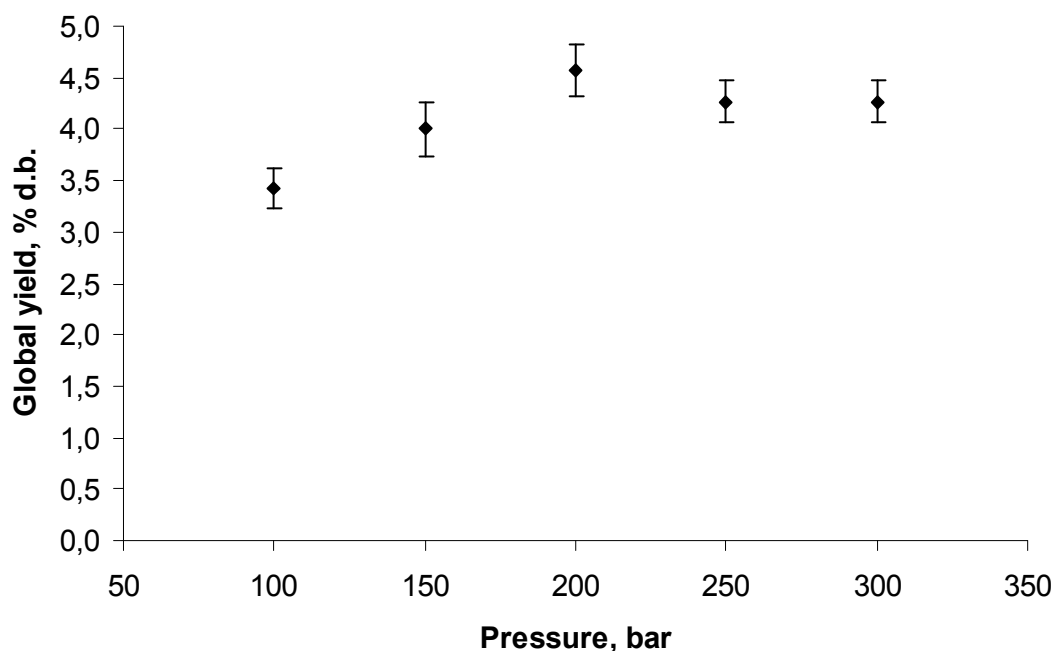
Figure 1: Global yield isotherm 40°C of the extract from Vetiver roots
using pressurized CO_2; CO_2 flow rate 9-12.10^{-5} kg/s.

Furthermore, chromatographic analyses revealed that the SFE extracts have a similar behaviour. Thus, pressure does not have any influence on the chemical composition of the extracts. According to these results, 200 bar of pressure was selected as the optimum pressure, at a temperature of 40°C, and was selected for the following experiments.

Figure 2 presents the overall extraction curves of vetiver oil using ethanol as co-solvent (0, 5 and 10% (v/v)). Each presented curve is the result of the average of three individual experiments carried out at the same conditions. This Figure, in accordance to the kinetic parameters presented in Table2, shows that using a 10% of co-solvent improved the process, permitting to extract more in a shorter time (increase of the M_{CER} with the percentage of cosolvent) and with a higher global yield. Moreover, a statistical study performed on all the kinetic parameters ($M_{CER}, Y_{CER}, t_{CER}, R_{CER}$ and R_{total}) demonstrated that 0 and 10% EtOH runs were significantly different at 0.05 in relation with cosolvent percentage.
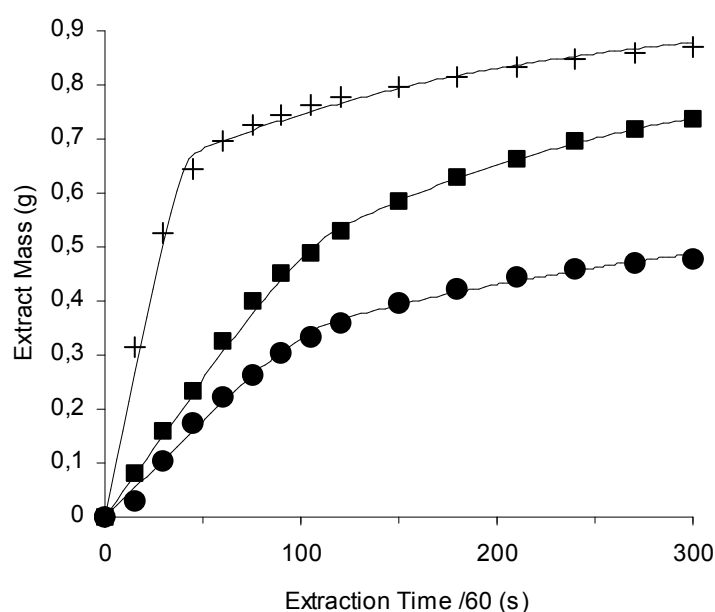
Figure 2: Overall Extraction curves of Vetiver roots at various cosolvent percentage at 200 bar and 40ºC and comparison of the experimental data with the Sovová model Q_{CO2} =3.74 (0%), 3.56 (5%),3.21 (10%) x10^{-5} kg CO_2 /s • Average 0% (Experimental data); ■ Average 5% (Experimental data); + Average 10% (Experimental data);— Sovová model

Table 1: Kinetic parameters for the overall extraction curves (0, 5 and 10% of ethanol) of Vetiver roots at 200 bar and 40ºC, Q_{CO2} =3.74 (0%), 3.56 (5%),3.21 (10%) x10^{-5} kg CO_2 /s

EtOH percentage (%)	M_{CER}*10^7 (kgExtract/s)	t_{CER}/60 (s)	Q_{CO2} *10^5 (kg CO_2 /s)	Y_{CER} *10^3 (kg Extract/kg CO_2)	R_{CER} (% b.s.)	R_{TOTAL} (% b.s.)
0	0.60	99	3.74	1.59	1.69	2.99
5	0.86	98	3.56	2.40	2.47	4.18
10	2.93	38	3.21	9.14	3.11	4.69

The chromatographic analyses (CG and TLC) revealed that increasing co-solvent percentage the quantity of each one of the different compounds, which were obtained at the extracted fraction, was increasing (Figure 3). Consequently, this led to the increase of the total yield, as previously noticed. Moreover, they also revealed a specific behavior in the case of the extraction carried out in the pure supercritical CO_2. Some compounds are removed from the raw material faster than the others. So, during the kinetic, they are going to run out. The proportion of the compounds with a lower solubility is therefore increased with time. Thus, at the end of the extraction, they are proportionally more important than the first ones. The use of co-solvent permits to improve the solubility of the vetiver extract in the supercritical solvent. Moreover, the behavior given by TCL and GC analysis are similar. The single behaviour is a noticeable decreasing of the proportion of compounds along the extraction. At 10% of EtOH, the decrease is more visible, indeed, compounds are more quickly recuperated than for the 5% EtOH kinetic (Figure 3).
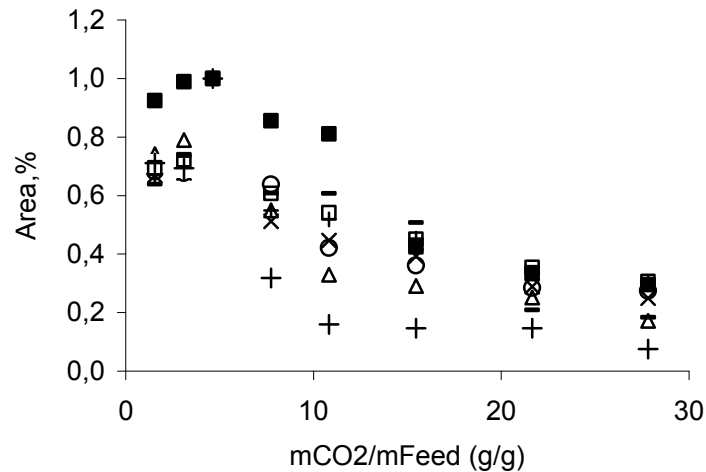
Figure 3. Chemical composition of the Vetiver extract obtained at 40 °C, 200 bar, 10% (v/v) of EtOH as cosolvent. **x** khusimol; **Δ** Isovalencenol; **+** Zizanoic Acid; **o** β-vetivone + n.i.V ; **-** n.i. VI; **+** α-vetivone; **■** n.i. VIII; ^{í} Volatile oil

The majority compounds obtained in vetiver volatile oil by GC were β-vetivone and α-vetivone; also these compounds were identified by Martinez et al [1] in Brazillian vetiver extracts. Kim et al [14] found these substances (β-vetivone and α-vetivone) in the crude volatile oil of vetiver with strong antioxidant activities.

As can be observed in the Figure 2, the Sovová model is the one that best represented the experimental results, 10 % of cosolvent in particular. Indeed, due to the parameters taken into account in this model [8], it can be used to predict the overall extraction curve in other experimental conditions such as bed characteristics (bed porosity, for instance) and in scale up studies (taken into account the diameter of the column and the bed height), once the mass transport coefficient (k) can be estimated.

The fitted parameters of the tested models and the mean square deviations (MSD) obtained for each model are presented in Table 3.

Table 3: Parameters adjusted for SFE of extracts from Vetiver roots with several models and mean square deviation of experimental data fitting

Model		Co-solvent Percentage		
		0	5	10
Crank (1975)	$Dx10^{12}$ (m^2/s)	0.07	0.08	0.22
	MSD	0.0269	0.0604	0.0281
Tan & Liou (1989)	Kd (min^{-1})	0.0069	0.0077	0.0140
	MSD	0.0081	0.0049	0.1931
Goto et al. (1993)	Φ	0.2094	0.1960	1.9635
	K	0.854	0.735	0.774
	MSD	**0.0002**	**0.0024**	0.0192
Sovova (1994)	t_{CER} (min)	19.931	18.733	7.2666
	k (-)	0.039	0.063	0.059
	MSD	**0.0013**	**0.0015**	**0.0045**
Naik et al. (1989)	k (min)	88.090	75.118	23.836
	MSD	0.0099	0.0352	**0.0046**

CONCLUSIONS

The use of ethanol as co-solvent permitted an increase in the total yield of vetiver oil obtained at 200 bar and 40ºC. The total yield obtained using 10% of ethanol was 57% larger than the one obtained without co-solvent. In addition, the kinetic parameters (M_{CER} and t_{CER}) were also improved by the use of co-solvent. The results of the TLC and the GC showed that the quality of the extract was not altered by the use of co-solvent. The Sovová model was the one that best represented the overall extraction curves.

ACKNOWLEDGMENTS

REFERENCES:

[1] MARTINEZ, J., ROSA, P. T.V., MENUT, C., LEYDET, A., BRAT, P., PALLET, D., MEIRELES, M.A.M. Journal of Agricultural and food chemistry, Vol.52, **2004,** p.6578

[2] FERREIRA, S.R.S., NIKOLOV, Z.L., DORAISWAMY, L.K., MEIRELES, M.A.M., ETENATE, A.J. Journal of Supercritical Fluids, Vol.14, **1999,** p.235

[3] PELLERIN, P., Perfum Flavor, Vol.16, **1991**, p.37

[4] NAIK, S.N., LENTZ, H. Fluid Phase Equilibria, Vol.49, **1989,** p.115

[5] CRANK, J. Clarendon Press, Oxford, **1975**

[6] TAN, C., LIOU, D. AICHE J., Vol.35, **1989**, p.1029

[7] GOTO, M., SATO, M., HIROSE, T. Journal Of Chemical Engineering Of Japan, Vol.26, **1993**, p.401

[8] SOVOVÁ, H. Chemical Engineering Science, Vol.49, **1994**, p.409

[9] FRANÇA, L.F., MEIRELES, M.A.A. Journal of Supercritical Fluids, Vol.18, **2000**, p.35

[10] PASQUEL, A, MEIRELES, M.A.A., MARQUES, M.O.M., PETENATE, A.J. Brazilian Journal Of Chemical Engineering. Vol.17, **2000,** 16p

[11] JORK. H, FUNK, W., FISCHER, W., WIMMER, H. Vol 1a, VCH, **1990**, p.196, 464p.

[12] FREUND, R.J., LITTELL, R.C. SAS Institute Inc., Cary, NC, **1995**, 211p

[13] MEIRELES, M.A.A. Current Opinion in Solid State and Materials Science, Vol.7, **2003,** p.321

[14] KIM, H., CHEN, P., WANG, X., CHUNG, H.Y., JIN, Z. Journal of Agricultural and Food Chemistry. Vol. 53, **2005**, p.7691