Brazilian Journal
of Chemical
Engineering

ISSN 0104-6632
Printed in Brazil
www.abeq.org.br/bjche

Vol. 23, No. 02, pp. 219 - 225, April - June, 2006

APPLICATION OF MOLECULAR SIEVES IN THE FRACTIONATION OF LEMONGRASS OIL FROM HIGH-PRESSURE CARBON DIOXIDE EXTRACTION

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(Received: October 20, 2004 ; Accepted: February 2, 2006)

Abstract - The aim of this work was to study the feasibility of simultaneous process of high-pressure extraction and fractionation of lemongrass essential oil using molecular sieves. For this purpose, a high-pressure laboratory-scale extraction unit coupled with a column with four different stationary phases for fractionation: ZSM5 zeolite, MCM-41 mesoporous material, alumina and silica was employed. Additionally, the effect of carbon dioxide extraction variables on the global yield and chemical composition of the essential oil was also studied in a temperature range of 293 to 313 K and a pressure range of 100 to 200 bar. The volatile organic compounds of the extracts were identified by a gas chromatograph coupled with a mass spectrometer detector (GC/MS). The results indicated that the extraction process variables and the stationary phase exerted an effect on both the extraction yield and the chemical composition of the extracts.

Keywords: High-pressure CO_2 extraction; Lemongrass; Molecular sieves; Fractionation.

INTRODUCTION

Lemongrass is a plant in the grass family that contains 1 to 2% essential oil on a dry basis (Carlson, 2001). The chemical composition of lemongrass essential oil can vary widely as a function of genetic diversity, habitat and agronomic treatment of the culture (Ferrua et al., 1994). Lemongrass essential oil is characterized by a high content of citral (composed of neral and geranial isomers), which is used as a raw material for the production of ionone, vitamin A and beta-carotene (Ferrua, 1994).

For the food, cosmetic and pharmaceutical industries, the extraction of essential oils from natural sources with supercritical fluids (SF) is a promising alternative to conventional processes, such as solvent extraction and steam distillation (Rizvi et al., 1986; Reverchon, 1992; McHugh and Krukonis, 1994; Rodrigues et al., 2003). This interest is due to the possibility of obtaining solvent-free extracts and the low temperatures employed, which is an attractive way to preserve the quality of thermo-sensitive products (Dugo et al., 1995; Zancan et al., 2002). Carbon dioxide is considered an appropriate solvent essential oil extraction from natural products, since it is readily available, nontoxic and non-inflammable, and has a low reactivity (Mohamed, 1997).

It is well know that SF properties can be tuned by manipulation of temperature and pressure, enabling the obtainment of essential oils with different

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*To whom correspondence should be addressed chemical compositions. Some works can be found in the literature regarding the use of supercritical fluids for fractionation of citrus essential oil (Sato et al., 1995; Stuart et al., 2001; Benvenuti et al., 2001). An alternative is to employ molecular sieves as adsorbents to concentrate the oil in some compounds or classes of compounds (Reverchon, 1997). The objective of this work was to study the extraction process using high-pressure carbon dioxide coupled with a simultaneous step of fractionation of the oil with different stationary phases. The effects of temperature and pressure and different stationary phases on the global yield, extraction kinetics and chemical composition of lemongrass essential oil are presented in this work.

EXPERIMENTAL SECTION

Material

Samples of lemongrass (*Cymbopogom citratus*) were collected in the city of Erechim, RS, and dried at room temperature for 24 hours. The material was classified by particle size (42 - 115 mesh) and stored in nitrogen atmosphere until the analyses. The CO_2 used in the experiments was 99.5% pure in the liquid phase (White-Martins S.A.).

Stationary Phases

Two molecular sieves, a microporous zeolite (ZSM-5) and a mesoporous material (MCM-41), alumina and silica particles were employed as stationary phases. The materials were characterized by X-ray diffraction and nitrogen adsorption.

Apparatus and Extraction Procedure

The experiments were performed in a laboratory-scale unit, which basically consists of a CO_2 reservoir, two thermostatic baths, a syringe pump (Isco Inc., Lincoln, Nebraska, USA, Model 500D) and an extractor with an internal volume of approximately 100 mL. In each experiment the extractor was loaded with approximately 24 g of the sample. The CO_2 was pumped into the bed of lemongrass, which was supported by two 300-mesh wire disks at both ends, at a constant flow rate of 1 $gmin^{-1}$. After a pre-established period (20 minutes), extraction was interrupted for measurement of the extract mass. The experiments were carried out in approximately 240 minutes, isothermally at constant pressure. Runs were duplicated for all conditions.

To study the effect of the different stationary phases, the experiments were conducted with a high-pressure column at a fixed temperature of 303 K and a fixed pressure of 150 bar. The high-pressure column was placed just after the extractor exit before the expansion (micrometering) valve, and the essential oil flowing through the column was collected in a glass flask. All experiments with a coupled high-pressure column were conducted in 20 minutes.

Characterization of the Extract

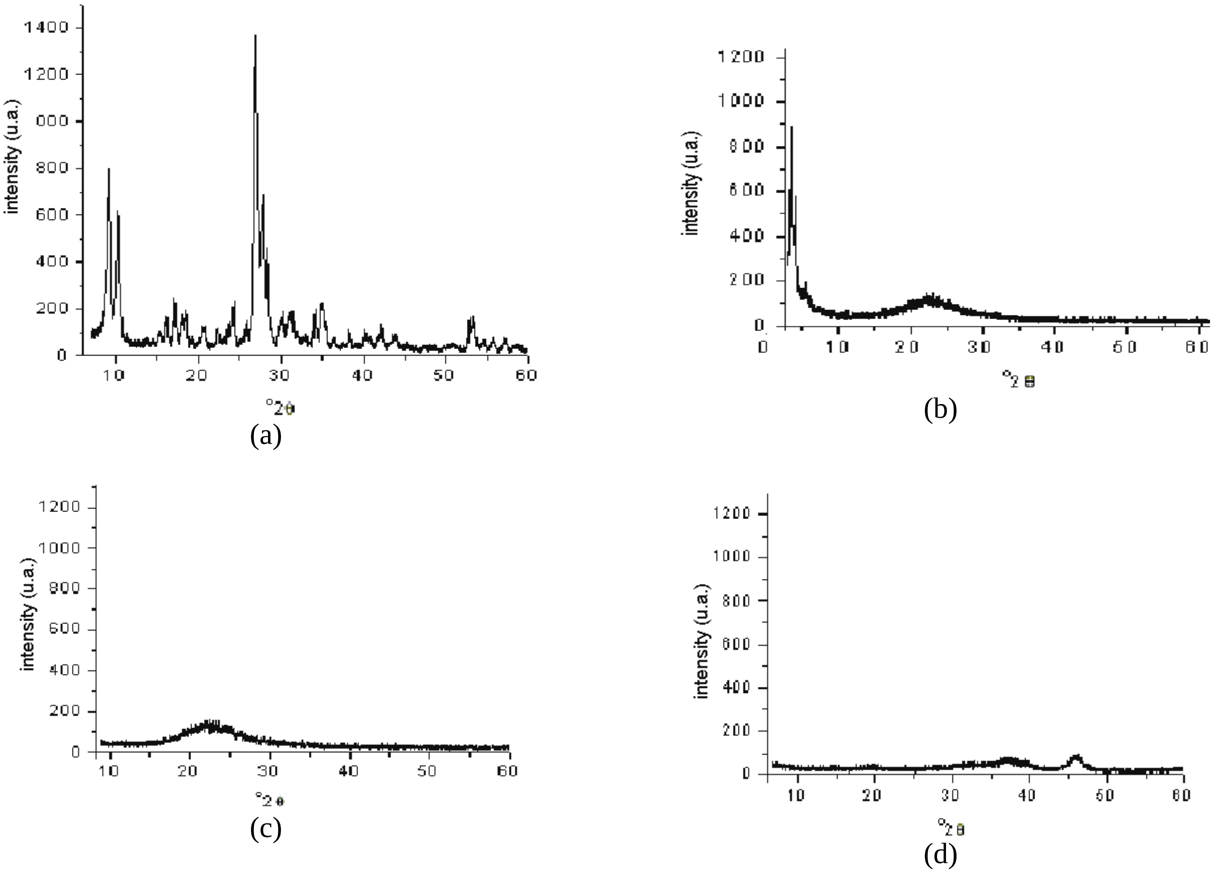
The chemical composition of the extracts was analyzed by a gas chromatograph coupled with a mass spectrometer detector, GC/MS (Shimadzu, Japan, Model QP 5090A), using a capillary column DB5 (30 m, 0.25 mm, 25 μm). The column temperature gradient was as follows: 333 K / 2.5 min, 3 $Kmin^{-1}$ to 423 K, 5 $Kmin^{-1}$ to 523 K, 10 $Kmin^{-1}$ to 563 K / 10 mim. Helium was used as carrier gas and the injector and detector temperatures were maintained at 553 K and 583 K, respectively. The sample (1 μl of 20000 mg/L in CH_2Cl_2) components were identified by matching their mass spectra with those from the library database and by comparison of retention times with standards. In all samples an internal standard (biphenyl 50 mg/L) was added and the content of each compound was calculated by the ratio of the compound peak area to the internal standard peak area. All analyses were replicated at least three times.

RESULTS AND DISCUSSION

Characterization of the Stationary Phases

In Figure 1 the diffractograms of the materials, where it can be noted that the ZSM-5 zeolite is a crystalline material, are presented. The MCM-41 had the amorphous characteristic of a 2θ angle greater than 5^o, and at lower angles it showed peaks representing the pore ordination. The silica and alumina materials had diffractograms characteristic of amorphous materials.

In Figure 2 the nitrogen adsorption isotherms for the materials used as stationary phases are presented. It can be observed that the ZSM-5 zeolite had a typical isotherm of microporous material. For the MCM-41 material one can observe an increase in the nitrogen adsorption in P/Po=0.40, as the mesopores of the material are full. The silica and alumina materials had type IV isotherms, typical of materials with a wider pore range. A summary of surface area and pore size distribution is presented in Table 1.



(a)

(b)

(c)

(d)

Figure 1: X-ray diffractograms for the stationary phases employed in this work. (a) ZSM-5 zeolite, (b) MCM-41 mesoporous material, (c) silica, (d) alumina.

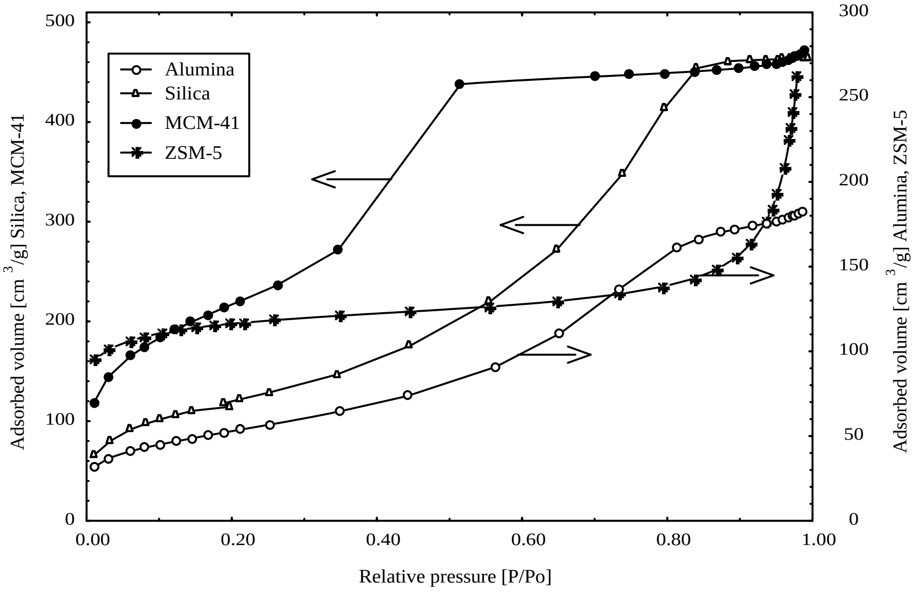


Figure 2: Nitrogen adsorption isotherms for the stationary phases employed in this work.

Table 1: Surface area and pore size distribution of the stationary phases employed in this work.

Material	Total area (BET) [m^2g^{-1}]	Micropore area [m^2g^{-1}]	Pore distribution [Å]
ZSM-5	391.2	339.8	< 10 Å
MCM-41	788.4	45.9	32.8 Å
Silica	434.7	-	20 - 180 Å
Alumina	190.6	12.1	20 - 130 Å

Global Yield

In this work, global yield was defined as the amount of essential oil obtained (g) by feeding 100 g of dry raw material into the extractor. In Table 2 the results for global yield of the extractions of lemongrass essential oil with carbon dioxide at high pressures are presented. It can be observed from this table that global yields up to 2.97 were obtained. In Figure 3 the effects of the extraction variables on the kinetics of extraction of lemongrass essential oil with carbon dioxide at high pressures are presented. It can be observed in this figure that at the highest pressure (200 bar), a slight increase in global yield occurred due to higher solvent power than that obtained in the runs at 100 bar. The effect of solvent density can be better verified when one compares runs 1 and 2 and also runs 4 and 5 in Table 2. In the experimental range under study, temperature also had a positive effect, where global yield increased with an increase in temperature at constant density (runs 1, 3 and 5 in Table 2). This can be attributed to the increase in vapor pressure of the essential oil compounds and to favorable changes in the transport properties of the solvent.

In Table 2 the results for extraction yield with the coupling of the high-pressure column are presented. Extraction yield was calculated based on the essential oil that passed through the stationary phase after 20 minutes of extraction. It should be observed in this table that the extraction yield was lower when a stationary phase was employed as a consequence of compound adsorption in the solid phase.

Table 2: Global yield of lemongrass essential oil obtained from the process of extraction coupled with fractionation with different stationary phases.

Run	Stationary phase	T [K]	P [bar]	Density* [gcm^{-3}]	Global yield ($g_{extract}$ / $100g_{raw\ material}$)
1	-	293	100	0.854	2.61 ± 0.03
2	-	293	200	0.938	2.86 ± 0.02
3	-	303	150	0.848	2.74 ± 0.03
4	-	313	100	0.616	2.10 ± 0.05
5	-	313	200	0.841	2.97 ± 0.04
6	-	303	150	0.848	1.08 ± 0.09
7	Sílica	303	150	0.848	0.97 ± 0.01
8	Alumina	303	150	0.848	0.93 ± 0.07
9	MCM-41	303	150	0.848	0.74 ± 0.03
10	ZSM-5	303	150	0.848	0.48 ± 0.03

*Angus et al. (1976).
Runs 6 to 10 were accomplished in 20 minutes.

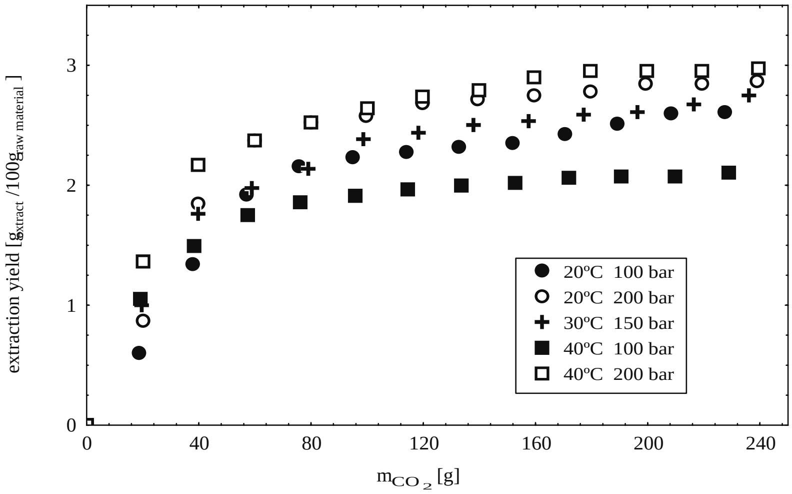


Figure 3: Kinetics of lemongrass extraction with dense carbon dioxide.

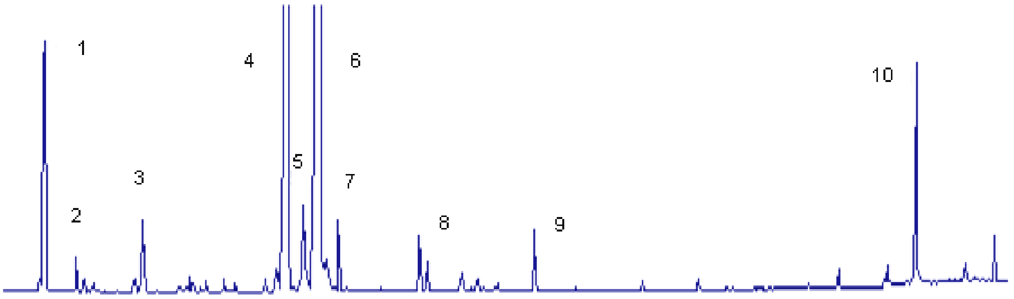
Characterization of the Extract

Figure 4 is a typical TIC chromatogram of lemongrass essential oil obtained with dense carbon dioxide. The labeled compounds are presented in Table 3, which shows the composition of the lemongrass essential oil when no stationary phase was employed.

Results reported in Table 3 are in fact average values of at least three replicates and the concentrations were calculated by the ratio of the peak compound area to the peak internal standard area. In Table 3, peaks 4 and 6 (neral and geranial isomers, respectively) correspond to the citral compound, which comprised around 85% of the essential oil. It was not possible to safely identify peak 10, but it is a heavier compound like a triterpene or wax found in the oil. Also, one should note from this table that the chemical profile for the extracts was affected by the extraction variables, where in general low densities and temperatures of around 313 K increased the concentration of oxygenated compounds.

In Table 4 average values of the concentrations of compounds of the lemongrass essential oil that passed through the high-pressure column are presented. The first aspect that should be noted in this table is the concentrations of the compounds compared to those in Table 3. The values of the volatile compounds found in the essential oil obtained in 20 minutes of extraction (Table 4) were much higher than those obtained in the whole essential oil extracted in 240 minutes (Table 3). This aspect indicates that more volatile compounds were preferably extracted at the beginning.

It can be also observed in Table 4 that the concentrations of compounds in the essential oil depended on the phase used. In general, the volatile compounds, mainly the lightest compounds, were more concentrated when silica and alumina were used as stationary phases, probably due to the wide range of pore sizes, which did not retain or adsorb the compounds studied. Some heavy compounds or classes of compound not identified by the gas chromatography technique employed (like triterpenes and waxes) may have been adsorbed, increasing the concentration of volatile compounds. On the other hand, the micro and mesoporous materials (ZSM-5 and MCM-41) could adsorb or retain a wide range of compounds, and as a consequence, did not concentrate the lemongrass oil in some classes of compounds.



1. Mircene,
2. Limonene,
3. Linalool,
4. Neral,
5. Geraniol,
6. Geranial,
7. Undecanone,
8. Geranil acetate,
9. Tridecanone,
01 not identified (NI).

Figure 4: Typical chromatogram of lemongrass essential oil obtained by dense CO_2 extraction.

Table 3: Major compounds of lemongrass essential oil extracted with dense carbon dioxide. Concentrations were calculated by the ratio of the peak compound area to the internal standard (100 ppm of biphenyl) peak area.

Compound	Run 1‡	Run 2	Run 3	Run 4	Run 5
Mircene	3.6 ± 0.3	0.8 ± 0.1	3.0 ± 0.2	3.1 ± 0.3	2.3 ± 0.2
Limonene	0.4 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
Linalool	1.7 ± 0.1	0.9 ± 0.4	1.8 ± 0.2	2.4 ± 0.2	1.8 ± 0.1
Neral	47.1 ± 0.4	22.6 ± 1.3	45.2 ± 3.0	63.7 ± 4.0	43.5 ± 0.7
Geraniol	3.8 ± 0.1	1.9 ± 0.1	2.7 ± 0.4	3.8 ± 0.2	3.5 ± 0.1
Geranial	80.6 ± 0.6	46.2 ± 1.6	73.8 ± 4.4	106.1 ± 6.3	71.8 ± 1.3
Undecanone	1.3 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.8 ± 0.1	1.2 ± 0.1
Geranil acetate	0.8 ± 0.1	0.3 ± 0.1	0.8 ± 0.1	1.1 ± 001	0.7 ± 0.1
Tridecanone	1.5 ± 0.1	1.1 ± 0.1	1.5 ± 0.1	2.1 ± 0.2	1.4 ± 0.1
NI	3.1 ± 0.3	2.5 ± 0.3	3.0 ± 0.1	4.6 ± 0.4	3.2 ± 0.1

‡The extraction conditions for runs 1 to 5 are labeled in Table 2.

Table 4: Effects of the stationary phases on the chemical profile for the classes of compounds in the lemongrass essential oil. Concentrations were calculated by the ratio of the peak compound area to the internal standard (100 ppm of biphenyl) peak area.

Compound	Stationary phase in the high-pressure column				
	no phase(run 6‡)	silica(run 7)	alumina(run 8)	MCM-41(run 9)	ZSM-5(run 10)
Mircene	51.1 ± 2.5	59.2 ± 3.4	60.1 ± 11.6	33.3 ± 5.7	24.4 ± 1.4
Limonene	1.8 ± 0.1	1.9 ± 0.1	2.1 ± 0.1	5.0 ± 0.4	0.5 ± 0.1
Linalool	3.0 ± 0.9	6.9 ± 0.4	8.6 ± 1.0	1.0 ± 0.1	5.5 ± 0.8
Neral	226.0 ± 5.3	290.6 ± 13.7	303.1 ± 35.4	217.7 ± 16.9	197.3 ± 30.3
Geraniol	4.3 ± 0.7	6.6 ± 0.9	6.7 ± 0.8	5.2 ± 0.5	4.1 ± 0.6
Geranial	387.8 ± 7.0	498.9 ± 24.4	523.4 ± 60.9	379.8 ± 29.8	351.0 ± 52.9
Undecanone	12.9 ± 0.3	17.0 ± 0.9	16.9 ± 2.0	12.4 ± 0.9	11.0 ± 1.3
Geranil acetate	1.3 ± 0.4	1.6 ± 0.1	1.6 ± 0.2	1.3 ± 0.1	1.2 ± 0.1
Tridecanone	8.4 ± 1.0	9.9 ± 0.6	10.8 ± 1.1	8.5 ± 0.7	5.5 ± 0.6
NI	13.9 ± 1.3	17.4 ± 1.3	18.2 ± 1.9	14.4 ± 2.0	18.0 ± 0.3

‡The extraction conditions for runs 6 to 10 are labeled in Table 2.

CONCLUSIONS

The results indicate that the extraction variables (temperature and pressure) did not have a remarkable effect on the global yield of extraction or on the chemical profile of lemongrass essential oil obtained by extraction with carbon dioxide at high pressures. Chemical analyses permitted identification of two major compounds in the extracts (representing about 85% of the total essential oil): neral and geranial. Results also indicated that the use of a stationary phase in a simultaneous extraction-fractionation step might be an appropriate means of concentrating the extracts from the supercritical fluid extraction process. Furthermore, the selection of an appropriate stationary phase may produce extracts with specific characteristics.

ACKNOWLEDGEMENTS

The authors thank FAPERGS, Secretaria de Ciência e Tecnologia/RS, URI-Campus de Erechim and AMEX for their financial support of this work.

The authors would also like to express their gratitude to NUCAT/UFRJ and the Diffractometry Laboratory at the Geoscience Institute/UFRGS for the helpful discussions concerning characterization of the stationary phases.

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