ELSEVIER

Journal of Supercritical Fluids 15 (1999) 253–259

THE JOURNAL OF Supercritical Fluids

Extraction of essential oil and lipids from nutmeg by liquid carbon dioxide

Cinthia Bittencourt Spricigo *, Leonel Teixeira Pinto, Ariovaldo Bolzan, Adelamar Ferreira Novais

Departamento de Engenharia Química e Engenharia de Alimentos, Centro Tecnológico, Universidade Federal de Santa Catarina, CP 476, CEP 88040-900, Florianopólis, Santa Catarina, Brazil

Received 18 August 1998; received in revised form 12 February 1999; accepted 16 March 1999

Abstract

Nutmeg (*Myristica fragrans* Houttuyn) was extracted with liquid carbon dioxide at 90 bar and 23.0°C. The seed is rich in essential and fatty oils, with myristicin and myristic acid being the characteristic compounds in each group respectively. Coextraction of essential oil and fatty oil was observed, and extraction curves for each were obtained. Chromatographic analyses of essential and fatty oils were performed.

The effects of particle size and solvent flow rate in extraction yield and velocity were studied. It was demonstrated that extraction yield can be greatly improved by a reduction in particle size. The extraction rate could be enhanced during the second period of extraction via an increase in solvent flow rate.

Keywords: Essential oil; Extraction; Fatty oil; Liquid carbon dioxide; Nutmeg

1. Introduction

Extraction of fatty and essential oils from vegetable matrices is an important application of the solvent properties of dense gases. Performing an extraction operation at mild temperature conditions is especially desirable for essential oils, since these are high valued, thermolabile products, widely used in food, cosmetic and perfumery products.

Some investigators have studied the extraction of essential oils with liquid carbon dioxide. As reported by Moyler [1], essential oil extracts obtained at supercritical conditions of 200–300 bar, and 40–50°C, contained oleoresins and pigments from the matrices. However, extraction with liquid CO_2 at 50–80 bar and 0–10°C yielded cleaner extracts, which resembled the aroma of the original vegetable matrix better.

Ferreira et al.[2] have extracted black pepper essential oil with liquid CO_2 and achieved a recovery of 96 wt% from ground pepper at 63.5–76 bar and 14–20°C. Germer and Petenate [3] used liquid carbon dioxide to extract clove oil, the yield being 20 wt%.

Nutmeg is the seed of *Myristica fragrans* Houttuyn, a medium-size tree cultivated in tropical regions. Major producers of nutmeg are Indonesia

* Corresponding author. Tel.: +55-48-331-9448; fax: +55-48-331-9687.

E-mail address: cinthia@enq.ufsc.br (C.B. Spricigo)

0896-8446/99/$ – see front matter
PII: S0896-8446(99)00012-1

and Sri Lanka. The amount of essential oil found in the seed varies with origin, soil and climate. Giacometti [4] reported a wide range of essential oil content from 5 to 15 wt%. About 80% of the essential oil is composed of terpenes such as α, β and γ-pinene, sabinene, limonene and 4-terpineol. Other important components are safrol, elemicin, eugenol and myristicin, of which the last one is responsible for the characteristic aroma of nutmeg [5]. Nutmeg essential oil is used in bakery products, dehydrated soups, ice-cream, sauces and processed meat. It is also applied in medicinal drug, perfume and shampoo formulations. In aromatherapy, it is used as a stimulator and energizer [6].

Nutmeg is also characterized by a high fatty oil content. According to Gerhardt [7], extraction of nutmeg with diethyl ether can yield from 22.3 to 46.9 wt% of fatty material, rich in saturated low molecular weight lipids, especially trymiristin. The high fatty oil content in nutmeg plays an important role in the essential oil extraction with dense carbon dioxide.

Some investigators have observed the presence of high molecular weight compounds in carbon dioxide extracts of aromatic leaves and seeds. Sovová et al. [8] observed small quantities of fatty oil in the extraction of caraway seed oil by liquid CO_2. Reverchon et al. [9] observed the coextraction of cuticular waxes while extracting essential oils of herbs with supercritical carbon dioxide. The extracts were fractionated in collectors held at different temperature and pressure conditions, so that the extraction curves for the essential oil and for the cuticular waxes could be studied separately.

As reported by Moyler [1], saturated low molecular weight lipids were extracted together with nutmeg essential oil using liquid carbon dioxide. According to that author, the presence of lipids is not an obstacle to the use of essential oils; on the contrary, it can even improve their solubility in some products.

The objectives of this work were to study the effects of particle diameter and solvent flow rate on the yield and velocity of nutmeg extraction by liquid CO_2, and to analyze the simultaneous extraction of fatty and essential oils.

2. Experimental

2.1. Equipment

The laboratory-scale extraction unit used in this work is represented schematically in Fig. 1. The extractor was a stainless steel jacketed vessel of 140 cm^3 and 2.1 cm internal diameter (ID) (Suprilab, Campinas, SP, Brazil), and its temperature was maintained by a thermostatic water bath (Model MQBTZ99-20, Microquímica, Florianópolis, SC, Brazil). Extraction pressure was maintained by means of a high pressure chromatographic pump (Model 3200 P/F, Thermo Separation Products, Fremont, CA, USA) filled with liquid carbon dioxide from a surge tank. The extracts were collected in a cold trap at the bottom of the extractor after expansion to atmospheric

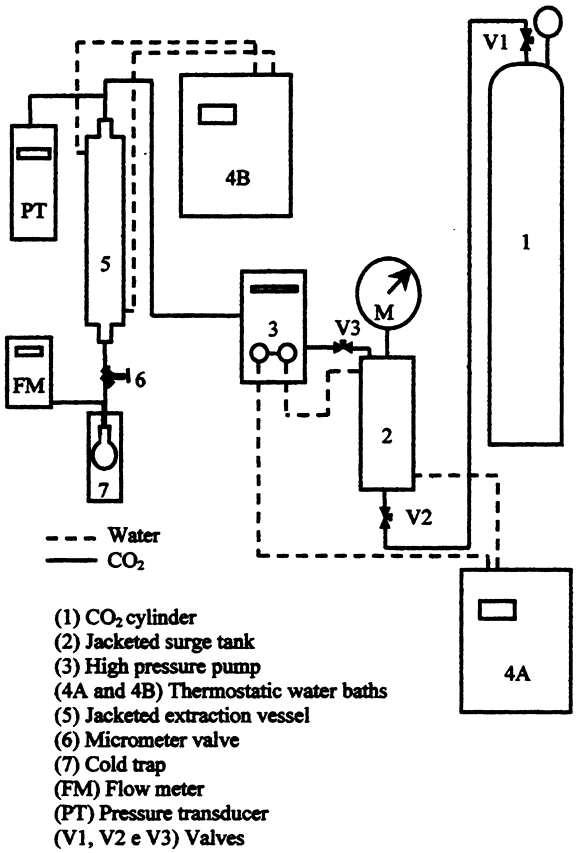

Fig. 1. Laboratory-scale extraction unit.

pressure in a heated metering valve. The mass of carbon dioxide used was measured by a calibrated flow meter (Model 821-1 Top Trak, Sierra Instruments, Monterey, CA, USA) and the extraction pressure was monitored with a pressure transducer (Model HT 201, Smar, Houston, TX, USA).

2.2. Procedure

Whole nutmegs from the same origin supplied by Bretzke Alimentos (Jaraguá do Sul, SC, Brazil) were ground with a domestic coffee grinder and sieved in the following particle size fractions: 10–14 mesh, 20–32 mesh and 42– 48mesh. Mean particle diameters, determined by microscopic analysis, were 1.454 mm, 0.678 mm and 0.300 mm respectively. Grinding was performed just before extraction, and within a time of 5 s in order to prevent heating of nutmeg. Ground nutmeg was placed at the bottom of the extractor, over a 200 mesh stainless steel screen, forming a bed 10 cm high. Nutmeg feed for each extraction was between 17 and 24 g. The void space up to the top of the extractor represented 75% of the total volume of the vessel, and was filled with 4 mm diameter glass beads. The extraction was carried out in semi-batch mode, with carbon dioxide flowing downwards continuously through the bed. The extractions were 180 min long and extract samples were collected every 5 min at the beginning of extraction, then at gradually longer intervals up to 60 min at the end of the experiment. In all experiments, extraction pressure and temperature were 90 ± 1 bar and 23.0 ± 0.3°C respectively. Solvent flow rates were 0.54 g/min and 0.90 g/min.

The percentage by weight of fatty oil present in the extract samples was determined after completion of the extraction experiment. A known mass taken from an extract sample was placed in an oven at 165°C. The essential oil was volatilized and the weight per cent of fatty oil in the sample was obtained gravimetrically. The procedure used took about 4 h, and was considered complete when mass variations were no longer observed.

Essential oil content in nutmeg was determined using steam distillation according to the method described by the American Spice Trade Association for determination of volatile oils (cited by Ferreira [10]). The amount of fatty material was determined with a Soxhlet apparatus by refluxing diethyl ether for 6 h through a sample of ground nutmeg, according to the method described by Instituto Adolfo Lutz [11].

2.3. Gas chromatographic (GC) analyses

The analysis of the essential oil was performed by the Institute of Technological Research — IPT (São Paulo, SP, Brazil) on a gas chromatograph (Model 14A, Shimadzu, Kyoto, Japan) with a capillary column (Carbowax 20, 25 m × 0.25 mm ID, 0.25 μm film thickness, Shimadzu, Kyoto, Japan). Injector and flame ionization detector (FID) temperatures were 250°C and 280°C respectively. The oven temperature program was: 50 to 70°C at a rate of 3°C/min and 70 to 200°C at a rate of 10°C/min. The carrier gas was He and sample volume injected was 0.2 μl. The analysis was replicated four times in order to guarantee reproducibility. Compound identification was performed by comparison with the retention time of standards.

GC analysis of fatty acid composition was performed by the Fat and Oil Laboratory of the State University of Campinas — UNICAMP (Campinas, SP, Brazil). The sample was injected after saponification of triglycerides and conversion of free fatty acids to their methyl esters. The gas chromatograph (Sigma 3B, Perkin Elmer, Norwalk, CT, USA) was equipped with a packed column (Silar 10C, 4 m × 1/8″ ID, HP, Palo Alto, CA, USA). The injector and FID detector temperatures were 225°C. The oven temperature was held at 140°C for 5 min, then programmed from 140 to 200°C at a rate of 2°C/min, and held at 200°C for 25 min. The carrier gas was N_2 and the sample volume injected was 1 μl. The analysis was replicated twice in order to guarantee reproducibility. Peak identification was based on comparison with the retention time of standards.

3. Results and discussion

The steam distillation of ground nutmeg yielded 6.9 wt% essential oil, and this value was taken as

the theoretical essential oil content of the seed. Extraction of nutmeg with diethyl ether, performed in a Soxhlet apparatus, yielded 46.5 wt% of fatty material. Although lipids are major components of the diethyl ether extract, some of the essential oil that is not lost during the solvent removal step of the Soxhlet extraction procedure is also present.

Fatty oil was coextracted during the extraction of nutmeg essential oil with liquid carbon dioxide at 90 bar and 23.0°C. Fig. 2 presents extraction curves for total extract, essential oil and fatty oil for a small particle size (0.300 mm) and demonstrates that the extraction of essential oil was preferred. The volatile fraction was higher in the extract samples collected at the beginning of the process (30.2 wt% fatty oil in the first extract sample), but gradually more fatty oil rather than essential oil was extracted (70 wt% fatty oil in the last extract sample). This extraction pattern changed for larger nutmeg particles (1.454 mm), as shown in Fig. 3. In this case, more fatty than essential oil was extracted, probably due to the fact that the essential oil is less accessible to the solvent in larger particles than in the smaller ones. At the beginning of this extraction, the extract samples collected were composed of 51 wt% fatty oil. At the end of the extraction, the amount of

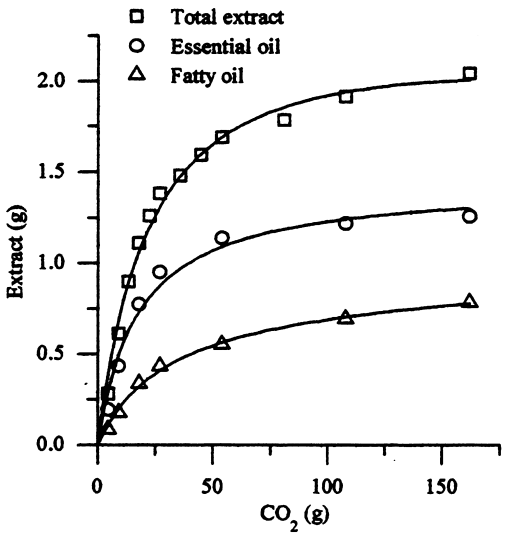

Fig. 2. Extraction curves for total extract, essential oil and fatty oil. Mean particle size: 0.300 mm; solvent flow rate: 500 cm^3/min (measured at 1 bar, 25°C).

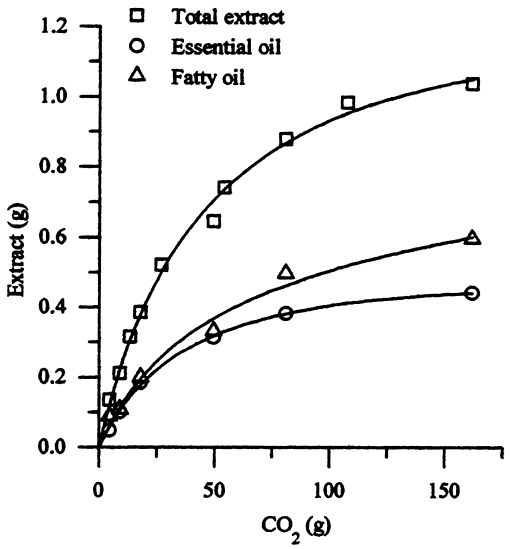

Fig. 3. Extraction curves for total extract, essential oil and fatty oil. Mean particle size: 1.454 mm; solvent flow rate: 500 cm^3/min (measured at 1 bar, 25°C).

essential oil decreased even more, and 63 wt% of the last extract sample was fatty oil. The total amount of extract collected after 180 min of extraction contained from 36.4 to 57.4 wt% fatty oil for all the parameters studied. Moyler [1] reported 16 wt% yield in the extraction of nutmeg with liquid carbon dioxide, and the extract contained 40 wt% low molecular weight saturated lipids. In fact, liquid CO_2, in the density range 0.8–1.1 g/cm^3, exhibits low extraction selectivity compared with supercritical CO_2 at lower densities (0.25–0.50 g/cm^3), being able to extract nonvolatile compounds present in vegetable matrices [12].

Fig. 4 shows the GC analysis of the total essential oil fraction obtained during 180 min of liquid carbon dioxide extraction. The identification of the major peaks is shown in Table 1. From these results it can be observed that major components of the essential oil are α-pinene, β-pinene, sabinene and myristicin, the characteristic aromatic component of nutmeg. The fatty acid analysis of the nonvolatile fraction coextracted with the essential oil after 180 min of extraction is presented in Fig. 5, with identification of some peaks presented in Table 2. From these results, the high quantity of myristic acid, as well as other low molecular

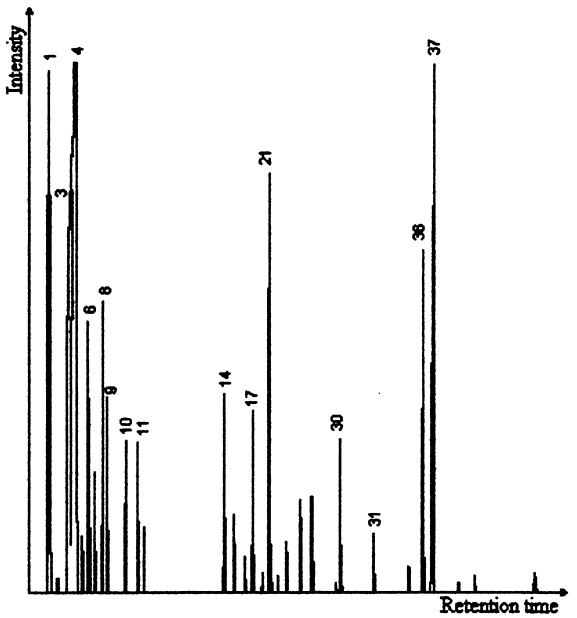

Fig. 4. GC trace of nutmeg essential oil obtained with liquid CO_2 at 90 bar and 23.0°C.

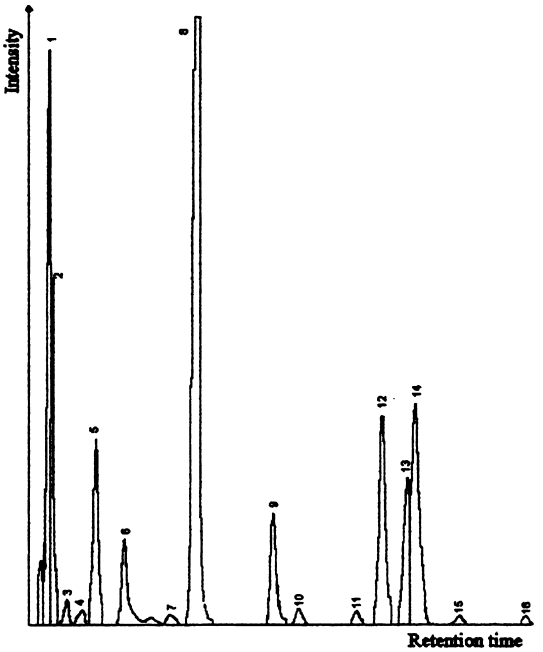

Fig. 5. GC trace of fatty acids of the nutmeg extract obtained with liquid CO_2 at 90 bar and 23.0°C.

Table 1
GC analysis of nutmeg essential oil obtained by liquid CO_2 at 90 bar and 23.0°C — identification of compounds

Peak number	Retention time (min)	Area (%)	Identification
1	2.880	11.0520	α-pinene
3	3.898	10.1872	β-pinene
4	4.175	36.6348	sabinene
6	4.763	3.1476	myrcene
8	5.470	3.7710	limonene
9	5.655	2.2727	β-phelandrene
10	6.545	1.8826	γ-terpinene
11	7.097	1.6559	cymene
14	11.287	1.8101	1-methyl-4(1-methylethyl)2-cyclohexen-1-ol (cis)
17	12.658	1.7945	1-methyl-4(1-methylethyl)2-cyclohexen-1-ol (trans)
21	13.455	3.6766	terpinen-4-ol
30	16.907	1.0075	safrol
31	18.512	0.3965	methyleugenol
36	20.907	3.2933	elemicin
37	21.393	6.9835	myristicin

weight saturated fatty acids present in nutmeg fatty oil can be observed.

The influences of particle size and solvent flow rate on extraction yield and velocity were analyzed for the essential oil extraction curves. Fig. 6 shows essential oil extraction curves obtained for three different particle sizes at a constant solvent flow rate of 500 cm^3/min (measured at 1 bar, 25.0°C). From Fig. 6, a reduction in both extraction rate and yield with the increase in particle size can be observed. The grinding process increases the surface area and may disrupt the cell walls, reducing mass transfer resistance, leaving the essential oil more accessible to the solvent and, consequently,

Table 2
Identification of compounds related to the GC analysis of fatty acids of the nutmeg extract obtained by liquid CO_2 at 90 bar and 23.0°C

Peak number	Retention time (min)	Area (%)	Identification[a]
1	3.266	10.76	C8:0
2	3.516	5.36	C10:0
3	4.650	0.62	NI
4	5.900	0.30	NI
5	6.850	6.60	NI
6	8.966	2.99	C12:0
7	12.250	0.26	NI
8	14.050	46.47	C14:0
9	19.933	3.94	C16:0
10	21.800	0.53	C16:1
11	25.983	0.51	C18:0
12	27.783	7.58	C18:1
13	29.650	4.14	NI
14	30.216	9.15	C18:2
15	33.766	0.16	C18:3
16	38.200	0.35	NI

[a] NI: not identified.

increasing the extraction rate. The observed reduction in extraction yield with increasing particle size indicates that liquid carbon dioxide is not able to reach all of the oil inside the cells, extracting only the exposed oil. Snyder et al. [13] obtained similar results for the extractability of oil from soybeans with supercritical CO_2. They concluded that oil was not transported through the unbroken cell walls, and only surface oil was removed.

Fig. 7 presents extraction curves obtained at different flow rates and two different particle sizes. The extraction curves exhibit a first linear period, characterized by a constant extraction rate, and a

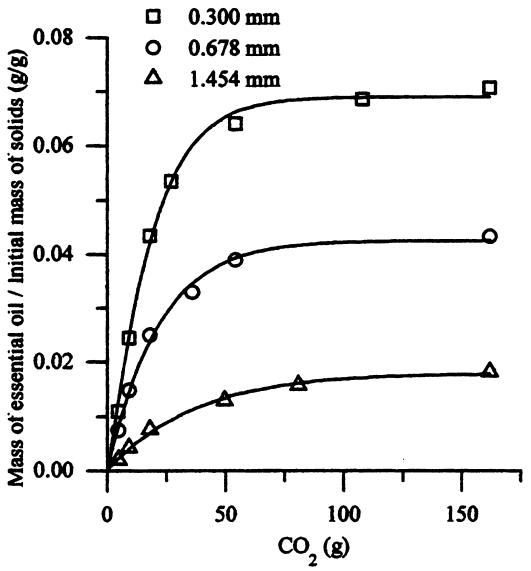

Fig. 6. Essential oil extraction curves for different particle sizes. Solvent flow rate: 500 cm^3/min (measured at 1 bar, 25°C).

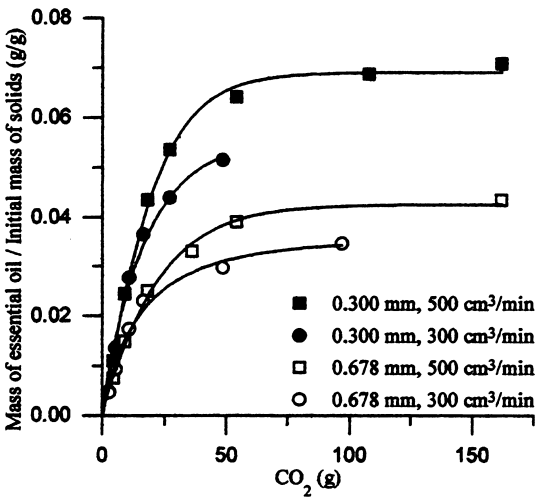

Fig. 7. Comparison of essential oil extraction curves for different particle sizes and solvent flow rates (measured at 1 bar, 25°C).

second period, when the extraction rate decreases due to the depletion of essential oil in the solid phase. The concentration of extract in the solvent during the first period of extraction is dominated by the mass transfer resistance in the fluid phase [14]. The curve slopes during the constant rate extraction period were 0.046 g essential oil per gram of CO_2 for the 0.300 mm particles and 0.032 g essential oil per gram of CO_2 for the 0.678 mm particles. These slopes did not change with solvent flow rate, indicating that different equilibrium concentrations were reached for each particle size. The equilibrium concentration depends on the quantity of solute available in the solid–fluid interface and on interactive forces within the solid substrate, and its value may be lower from solubility values obtained for pure extract components [14]. During the second period of extraction, more essential oil per unit mass of solvent is extracted with an increase in solvent flow rate for the same particle size (Fig. 7). This is probably due to the fact that an increase in solvent flow rate allows the development of a larger concentration gradient between the solid and the fluid phases, thereby increasing solvent loading.

4. Conclusions

The extract of nutmeg obtained with liquid carbon dioxide at 90 bar and 23.0°C was composed of essential and fatty oils. Fatty oil fraction made up 36.4 to 57.4 wt% of extracts and contained a high amount of low molecular weight saturated fatty acids, especially myristic acid. Extraction rate and yield were strongly enhanced with a decrease in mean particle size (from 1.454 to 0.300 mm). The lower extraction yield obtained for larger particle sizes indicates that liquid carbon dioxide is not able to reach all of the oil inside the cells, extracting only the exposed oil. An increase in solvent flow rate improved the extraction velocity during the second period of the extraction, probably due to the establishment of larger concentration gradients between the solid and the fluid phases.

Acknowledgements

This work was supported by CAPES and CNPq, Brasília, Brazil). The authors wish to thank Bretzke Alimentos for supplying the nutmeg.

References

[1] D.A. Moyler, Extraction of flavours and fragrances with compressed CO_2, in: M.B. King, T.R. Bott (Eds.), Extraction of Natural Products Using Near-Critical Solvents, Chapman & Hall, London, 1993, pp. 140–183.
[2] S.R.S. Ferreira, M.A.A. Meireles, F.A. Cabral, Extraction of essential oil of black pepper with liquid carbon dioxide, J. Food Eng. 20 (1993) 121.
[3] S.P.M. Germer, M.A.A.M. Petenate, Extraction of clove essential oil with liquid carbon dioxide, Brazilian Chemical Engineering Institute, Proceedings of the 8th Brazilian Congress of Chemical Engineering, vol. 2 (1988) 541.
[4] D.C. Giacometti, Ervas Condimentares e Especiarias, Nobel, São Paulo, 1989, p. 113.
[5] K. Bauer, Common Fragrance and Flavor Materials: Preparation, Properties and Uses, VHC, Weinhein, 1985, p. 159.
[6] C.B. Spricigo, Extração de óleo essencial de noz moscada com dióxido de carbono a altas pressões, M.Sc. Thesis, Universidade Federal de Santa Catarina, Brasil, 1998, p. 17.
[7] U. Gerhardt, Especias y Condimentos, Acribia, Zaragoza, 1975, p. 62.
[8] H. Sovová, R. Komers, J. Kucera, J. Jez, Supercritical carbon dioxide extraction of caraway essential oil, Chem. Eng. Sci. 49 (1994) 2499.
[9] E. Reverchon, G. Donsi, L.S. Osséo, Modeling of supercritical fluid extraction from herbaceous matrices, Ind. Eng. Chem. Res. 32 (1993) 2721.
[10] S.R.S. Ferreira, Cinética de transferência de massa na extração supercrítica de óleo essencial de pimenta-do-reino, Ph.D. Thesis, Universidade Estadual de Campinas, Brasil, 1996, p. 51.
[11] Instituto Adolfo Lutz, Normas Analíticas do Instituto Adolfo Lutz, 3rd edition, vol. 1, Instituto Adolfo Lutz, São Paulo, 1985, pp. 42–43.
[12] E. Reverchon, Supercritical fluid extraction and fractionation of essential oils and related products, J. Supercrit. Fluids 10 (1997) 1.
[13] J.M. Snyder, J.P. Friedrich, D.D. Christianson, Effect of moisture and particle size on the extractability of oil from seeds with supercritical CO_2, J. Am. Oil Chem. Soc. 61 (1984) 1851.
[14] G. Brunner, Gas Extraction: an Introduction to Fundamentals of Supercritical Fluids and the Applications to Separation Processes, Steinkopff, Darmstadt, 1994, pp. 188–196.