DETERMINATION OF THE GLOBAL YIELD ISOTHERMS FOR THE SYSTEM ROSEMARY (*Rosmarinus officinalis*) + CO_2

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Rosemary (*Rosmarinus officinalis*) is an aromatic plant used to impart flavor to a variety of foods besides its medicinal applications. Rosemary or rosemary extracts are powerful antioxidants; among the pharmacological applications of rosemary infusions are the control of arterial blood pressure, spasmolytic effects on the gall bladder ducts and upper intestine. Rosemary SFE extracts have antioxidant activity as high as 90% compared to beta-carotene and linolenic acid; its minimum inhibitory concentration was as low as 3.9 μg/mL against *Mycobacterium tuberculosis* H37Rv. In the present work, the global yield isotherms for the systems Rosemary + CO_2 were determined at 30 and 40 ^oC; the pressure was in the interval of 100 to 300 bar. The assays were done using an SFE unit containing a fixed bed formed with 10 grams of rosemary particles (24 and 48 meshes, 1:1), humidity of 9.33 %, solvent flow rate of 8.3×10^{-5} kg/s; the assays were replicated. The compositions of the extracts were determined by gas chromatography (Shimadzu, G 17A, Kyoto, Japan) FID detector, and capillary column (DB-5, 30m × 0.25 m × 0.25 μm, J&W Scientific, USA); the external standard method was used to quantify the camphor and 1,8-cineole. The global yields varied from 1 to 5 %; the major compounds identified in the extracts were camphor and 1,8 cineole. Rosemary extracts were also obtained by hydrodistillation and hexane extraction.

INTRODUCTION

Rosemary (*Rosmarinus officinalis*) is an aromatic plant used to impart flavor to a variety of foods besides its medicinal applications. Rosemary is indigenous to Mediterranean and Portugal; It is also cultivated in Crimea, Central Asia, India, African Southeast, South Africa, Australia, United States, and South of Brazil [1].

With respect to the medical applications, the major product of rosemary is its volatile oil. Nonetheless, other product such as infusions, ethanolic extracts, etc are also employed. In the Germany pharmacology rosemary products are used to control arterial blood pressure, liver diseases, spasmolytic effects on the gall bladder ducts and upper intestine; the use in homeopathic medicine is mainly against sexual disorders [1].

The chemical compositions of rosemary extracts (volatile oil and oleoresin) vary due to climate factors during cultivation, part of the plant used (whole plant, leaves, etc.), humidity (dried or in natura), and extraction process. Reverchon & Sanatore [2] used GC–MS and retention index to compare the composition of the volatile oil (hydrodistillation) and the SFE extract. They observed that the amount of 1,8 cineole remained approximately constant varying from 20.6% for the volatile oil to 20.02% for the SFE extract; nonetheless, the

amount of camphor varied about 50% for the two processes: it was 10.26% for the volatile oil and 15.33% for the SFE extract. Laurence & Shu [3] compared the content of 1,8 cineole and camphor in the volatile oils obtained from rosemary, by SFE, from different countries: Spain, Morocco, Tunisia, Yugoslavia, and Portugal. The largest amount of 1,8 cineole was obtained for the rosemary from Morocco, which varied from 41.1 to 43%; the Yugoslavia rosemary possessed the largest amount of camphor that varied from 11.6 to 14.3%. Coelho *et al* [4] studied the effects of temperature (37 and 47^{o}C) and solvent density for the SFE process at pressures of 100 to 160 bar. The SFE extracts were analyzed by HRCG–MS and classified in three classes according to their molecular masses. The Sovová's model [5] was used to describe the mass transfer phenomena of the extraction process; the results indicated that the model is satisfactory to be used for process design of a specific type of extract. Señoráns *et al* [6] obtained the SFE extract using a pilot plant at several conditions of temperature and pressure (40-60 K, 300-350 bar); the SFE extracts were further divided into two fractions and analyzed by LC–MS. The fractions had chemical composition and antioxidant activity different from that of the volatile oil. Leal et al [7] have demonstrated that rosemary SFE extracts have antioxidant activity as high as 90% compared to beta-carotene and linolenic acid; its minimum inhibitory concentration was as low as 3.9 μg/mL against *Mycobacterium tuberculosis* H37Rv.

The objectives of this work were to determine the global yields isotherms fore the system rosemary + CO_2 and to determine the chemical composition of the extracts and compare them to the composition of the volatile oil (hydrodistillation) and oleoresin (hexane extraction).

MATERIALS AND METHODS

Raw material characterization and preparation

Rosemary was cultivated under controlled conditions at Experimental Farm of Lageado (Plant Production Department, Agronomy Science College/UNESP, Botucatu, SP, Brazil). The rosemary leaves from the agronomic experiment (harvesting period) selected for this work were from the assays that produced the largest biomass, camphor and 1,8 cineole. The leaves from the different agronomic treatments were mixed together in order to obtain a homogeneous lot of raw material. The leaves were triturated using a knife mill (Tecnal, model TE 631/1, São Paulo, Brazil) at 21500 rpm for 10 seconds. The particle size distribution was determined with the aid of an agitator (Produtest, model 3580, São Paulo, Brazil) and sieves of the Tyler series (W.S. Tuler, USA) meshes – 18 to + 60. Particles of meshes 24, 32, and 48 were selected for the extraction assays; the triturated particles were packed under vacuum and kept in a domestic freezer (Brastemp, model Frostfree, São Paulo, Brazil) at approximately – 10 ^{o}C. The humidity was determined by the xylol (Ecibra, P.A.-ACS, São Paulo, Brazil) distillation method [8].

Extraction procedures: SFE, hydrodistillation and hexane extraction

The SFE assays were performed in the unit described by França and Meireles [9], which has a fixed bed extractor of 2.013×10^{-5} m^3 (inside diameter of 2.82×10^{-2} m and length of 0.032 m); carbon dioxide 99.8% pure was used (White Martins Gases Industriais, Campinas, Brazil).

The fixed bed was formed inside a nylon basket of mesh 80, using 10 g (± 0,2 g) of rosemary; the height of the fennel bed was 3.05×10^{-2} m and the apparent density of 521.2 kg/m^3; glass bed of mesh 60 were used to fill the rest of the extractor's cell. The extraction

cell was adapted into the extractor and the thermostatic bathes were turned on. It took about 2 hours for the system to reach thermal equilibrium. Once, the temperature of the thermostatic bath controlling the temperature of CO_2 pump head reached -10^oC the system was pressurized to the desired pressure and allowed to rest for 10 minutes to equilibrate the contents of the extractor cell. Afterwards the temperature of the micrometering valve was set at 110^oC. Capture columns of glass (8mm of diameter and height of 10 cm), containing the adsorbent Porapak-Q of meshes 80 to 100 (superficial area of 500–600 m^2/g, density of 0.34 g/cm^3, maximum temperature of 250^oC, Supelco, Milford, MA USA), were assembled just after the solvent outlet, to prevent loss of the low molecular weight components of the fennel extracts. The capture columns were exchanged as soon as they were saturated by the fennel extracts (visual analysis). The CO_2 flow rate was measured by a flow totalizer (± 0.02 L, LAO, model G-1, São Paulo, Brazil). The assays were done accordingly with a factorial design at pressures of 100 to 300 bar at every 50 bar for the temperatures of 30 and 40^oC, and an average solvent flow rate of 8.33×10^{-5} kg/s; the assays took approximately 3 hours and they were duplicated.

The volatile oil (essential) was obtained by hydrodistillation using the AOAC 962.17 method [10] using 0.10 kg of rosemary leaves of meshes 24, 32 and 48 and the extraction continued for 120 minutes.

The oleoresin (hexane extract) was obtained as follows: Fifteen grams of rosemary leaves of meshes 24 to 48 mesh and 80 mL of hexane (96%, Merck, São Paulo, Brazil) were placed inside a Soxhlet apparatus of 500mL and maintained under reflux for 3 hours. The hexane was removed using a rotatory evaporator (Laborota, model 4001, Viertrieb, Germany), with vacuum control (Heidolph Instruments GmbH, model Rotavac, Viertrieb, Germany).

Chemical composition of the extracts

The chemical composition of the extracts (essential oil and part of the oleoresin) was determined by GC-MS (Shimadzu, model QP-5000, Kyoto, Japan) equipped with a capillary column of fused silica (DB-5; 30 m x 0.25 mm x 0.25 μm, J&W Scientific, USA). The electron impact technique (70 eV) was used. The carrier gas (1.7 mL/min) was helium (99.9% purity, White Martins Gases Industriais, Campinas, Brazil). The rosemary extracts (0.005 grams) were diluted in 1 mL of ethyl acetate (EM Science, chromatographic grade, lot 36079631, Darmstadt, Germany); 1 μL of sample was injected and the split ratio was 1:30. The temperature programming was 50°C (5 min), 50-180, 5 °C/min, 180-280, 10°C/min. The injector and detector temperatures were 180 and 280ºC, respectively. The identification of the chemical constituents was based on: (i) comparison of the substance mass spectrum with the GC-MS system data bank (Wiley 139 Library); (ii) comparison of the mass spectra with the data in literature [11]; and (iii) retention index [12].

The quantification of the substances was done by gas chromatography (GC) (Shimadzu, GC 17A, Kyoto, Japan) equipped with a capillary column DB-5 (30 m x 0.25 mm x 0.25 μm, J&W Scientific, Folsom, USA), flame ionization detector (FID) and split injector; using the GC-MS conditions. The quantitative analysis of the extract employed the external standard method [13]. The quantification was done using a standard solution for two major substances identified in the extracts: camphor (Sigma, lot: 73H3697, Steinheim, Germany), 1,8 cineole (Aldorich, lot: 2817K, Steinheim, Germany).

Thin Layer Chromatography (TLC)

This technique was used to verify the presence of substances not detectable in the gas chromatography of the rosemary extract, such as wax. The TLC analysis was carried out in

silica plates (60-PF254, Merck 20×20 cm, 0.25 mm of height, lot 940378601, Germany), the mobile phase was composed by 60% of hexane (Merck, analytical grade, lot HX0290-44, Germany) and 40% of ethyl acetate (Merck, analytical grade, lot K225488323, Germany). The plates were reveled using an anisaldehyde solution (100 mL of glacial acetic acid, 2 mL of sulfuric acid, and 1 mL of anisaldehyde) followed by heating at 100°C until the visualization of the substances.

RESULTS AND DISCUSSIONS

The process in which one solute molecule became part of the supercritical phase can be described as: vaporization, as the molecule goes from a condensed phase to a more expanded one or solubilization if there is any interaction between the solute and the solvent. This vaporization-solubilization combined characterizes the intermediate nature of the supercritical state [14]. The solubilization power of supercritical CO_2 depends on its density. In the supercritical region, the density strongly increases with pressure, at constant temperature, and decrease with temperature, at constant pressure. Although the CO_2 density is known at a given temperature and pressure, it is necessary to know the Rosemary extract + CO_2 interaction to determine the system density. This interaction can be described in terms of the solubility of rosemary extract in CO_2 or by the determination of the global yield of the supercritical extraction.

Figure 1(a) shows the variation of the global yield for the CO_2 + rosemary system as a function of pressure and two temperatures. For constant temperature, raising the pressure from 100 to 177 bar is accompanied by the increase in the solvent density and, consequently, to the increase in the solubility of the rosemary extract. This can be explained by the decrease in the mean distance between molecules, increasing the specific interaction between solute and solvent [14]. For pressures larger than 200 bar, the increase in pressure cause a moderate increase in density and, consequently, a moderate increase in solubility. There are two main effects of the temperature on solubility: i) solute vapor pressure, ii) solvent density. For pressures below 177 bar, the CO_2 density strongly decreases as the temperature increases. As consequence, the solubility of rosemary extract decreases and the retrograde phenomena is observed. For pressures close to 177 bar, the two effects of temperature on the solubility have approximately the same importance. For pressures larger than 177 bar, the increase in the solute's vapor pressure with temperature is more important than the solvent density decrease.

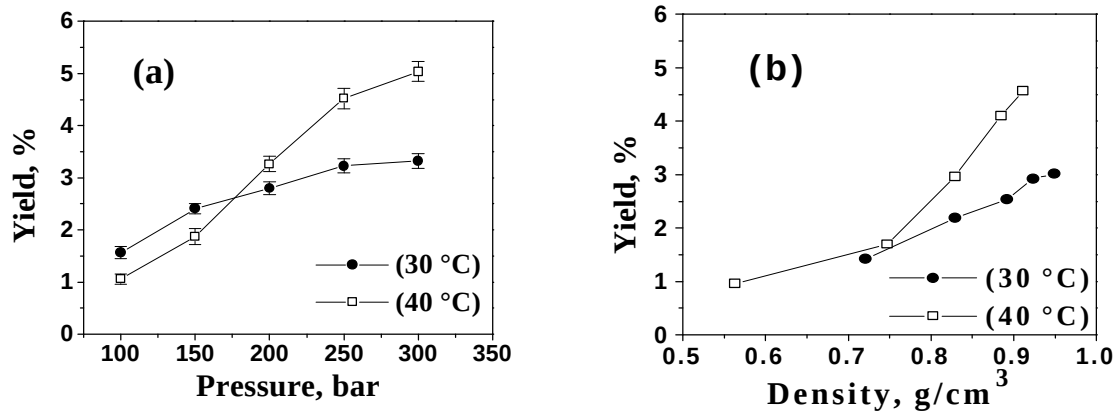

Figure 1 -Global yield isotherms for the system rosemary + CO_2.

The yields of hydrodistillation and organic solvent (hexane) extraction were 1.84 and 8.62%, respectively

Chemical composition of the extracts

The identification and quantification of the main compounds in the rosemary extract (camphor and 1,8 cineole) were assessed by gas chromatography using the external standard method as previously described. Table 1 presents the yield of camphor and 1,8 cineole in the extracts obtained by hydrodistillation, hexane extraction, and supercritical CO_2 extraction.

Table 1 - Chemical composition of rosemary extract

Compounds	% (mass of solute /mass of dry raw material) × 100					
	Hydrodistillation	Hexane Extraction	Supercritical Extraction			
			30 °C		40 °C	
			100 bar	300 bar	100 bar	300 bar
Camphor	1.22	1.07	0.025	0.6	0.26	0.44
1,8 Cineole	0.23	0.18	0.0011	0.043	0.012	0.029

The TLC plate of the rosemary extracts can be seen in Figure 2. The 1,8 cineole is presented in all extracts. In the SFE and hexane extracts (A, B, and D) there is a larger amount of compound (larger number of bands). The extract produced by hydrodistilation was the one with the lower number of compounds. The camphor standard produced a white spot in the TLC plate probably due to its volatilization during the staining process.

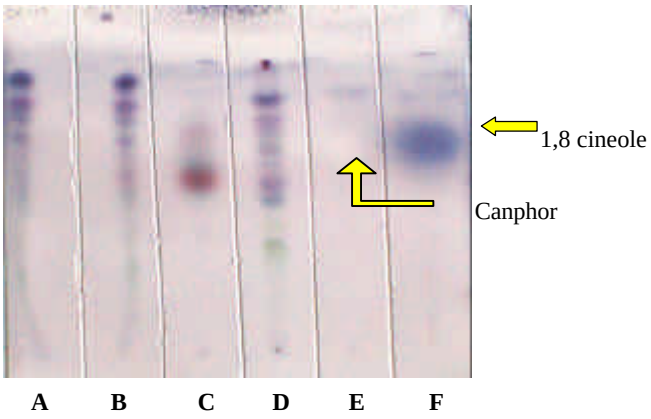

Figure 2 - TLC analysis of the *Rosmarinus officinalis* extracts: (A) SFE at 100 bar/ 30°C, (B) SFE at 300 bar/30°C, (C) Hydrodistillation, (D) Hexane extraction, (E) Camphor standard, (F) 1,8 Cineole standard.

CONCLUSION

The largest yield for the SFE process was obtained at 300 bar and 40°C. In spite of that, larger yields of 1,8 cineole and camphor were observed at 300 bar and 30°C. In the extract produced by hydrodistillation was detected the largest yield of both camphor and 1,8 cineole.

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