DETERMINATION OF THE GLOBAL YIELDS FOR THE SYSTEM FENNEL (*Foeniculum vulgare*) + CO2

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The objectives of the present work were to determine the global yield isotherms for the system fennel + CO2 at temperatures of 30 and 40 ^{o}C; 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 fennel particles (24, 32 and 48 meshes), humidity of 7.6 %, solvent flow rate of 8.3×10-5 kg/s; the assays were replicated. The compositions of the extracts were determined by gas chromatography (GC) (Shimadzu, GC 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 trans-anethole and fenchone. The global yields varied from 3 to 12 %; the major compounds identified in the extracts were trans-anethole and fenchone. Fennel extracts were also obtained by hydrodistillation and ethanol extraction.

Keywords: Anethole, fenchone, *Foeniculum vulgare*, SFE

INTRODUCTION

Fennel (*Foeniculum vulgare*) is a plant of the Apiaceae (Umbelliferae) family that is worldwide used as infusion or tea against flatulence. Popularly its essential oil is confused with the anise oil (*Pimpinella anisum*) and has a variety of applications in the food and pharmaceutical industries [1]. Fennel (*Foeniculum vulgare*) infusion is worldwide domestically used to stimulate the gastrointestinal motility. In higher concentration can be antispasmodic; anethole and fenchone the major constituents of its essential oil have shown secretolytic action on the respiratory tract. Fennel is a shrub that measures 80 to 150 cm of height and has a pungent aroma. It is indigenous to Mediterranean and is cultivated in England, Germany, Tyrol, China, Vietnam and South America [1]. The primary source of anethole is anise (*Pimpinella anisum*) nonetheless, in Brazil fennel has being the preferred source, instead, due to agricultural difficulties associated with anise cultivation.

The major compounds of fennel volatile (essential) oil are trans-anethole (50 to 70%), fenchone (12 to 33%), methyl chavicol (estragole) (2 to 5%); α-pinene, camphene, p-cymene, myrcene, limonene, α- and β-phellandrene, γ-terpinene, terpineol, cis-ocimene e γ-fenchone [1]. Because of the large amount of anethole the fennel oil can be used in the synthesis of chloral and pentobarbital [2]. The content of anethole in the oil varies with both the part of the plant used and its maturity [3]. The worldwide production of anethole is 1000 ton per year; currently China and the Vietnam the main producers [4].

In the medical area, the main product of fennel is its essential oil because of its functional properties such as anti-inflammatory, antispasmodic, carminative, diuretic,

expectorant, laxative, analgesic, stimulant of gastrointestinal mobility, etc [5]. The infusion is used to treat indigestion, abdominal distention, etc [6]. The extracts of the fennel seeds are used in the treatments of the bile and nervous disturbances [7]. Also it can be used for throat pains, in the combat of conjunctivitis and for the treatment of bronchitis and other pathologies of the respiratory tract, besides being preventively used for the control of illnesses in the chest, lung, kidneys and etc [6].

Fennel seeds are largely used to impart flavor to a number of foods such as soups, sauces, pickles, bread, cakes, and so on [8]. Generally, formulations containing fennel or fennel products do not promote adverse reactions; nonetheless, some allergic reactions have being reported [1]. The modern therapeutic uses of fennel in Germany and United States originated from the Greek traditional medicine as practiced by Hypocrites and Discorides [7]; it is widely used in the traditional medicine of Saudi Arabia as diuretic [9]. Fennel has being introduced in many other traditional medicines such as of China and Japan [10].

For these reasons, it can be observed lately a huge [4] increase in the worldwide production of fennel, supported also by the fact that the traditional medicine is incorporating some of the chemical species generally used in homeopathy [11]. In Brazil, the primary source of anethole is anise, nonetheless, due to agricultural constraints this species is scarcely cultivated [2].

Because of the growing attention given by consumer to both high quality products and environmentally friendly technologies the SFE process has imposed itself as a preferably process to obtain vegetable extracts. Thus, the objectives of this work were to obtain the global yield isotherms for the system fennel + CO_2 and characterize the extracts with respect to the chemical composition.

MATERIALS AND METHODS

Raw material characterization and preparation

Fennel was cultivated under controlled conditions at the Experimental Farm of Lageado (Plant Production Department, Agronomy Science College/UNESP, Botucatu, SP, Brazil) [12]. For this work, were selected fennel seeds from the agronomic experiment (harvesting period) that produced the largest biomass, and higher contents of trans-anethole and fenchone. The seeds from the different agronomic treatments were mixed together in order to obtain a homogeneous lot of raw material. The seeds 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 (standard testing sieve, series Tyler) of mesh sizes – 18 to + 60. Mesh sizes –24 to + 48 were selected for the assays. Tthe 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.) distillation method [13].

Extraction procedures: SFE, hydrodistillation and ethanol extraction

The SFE assays were performed in the unit described by França and Meireles [14], 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).

The fixed bed was formed inside a nylon basket of mesh 80, using 10 g (± 0,2 g) of fennel; the height of the fennel bed was 3.2×10^{-2} m and the apparent density of 496.8 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°C 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°C. 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°C, 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°C, 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 [15] using 0.10 kg of fennel seeds of meshes 24 to 48 and the extraction continued for 120 minutes.

The oleoresin (ethanol extract) was obtained as follows: Fifteen grams of fennel seeds of meshes 24 to 48 and 80 mL of ethanol (96%, Merck) were placed inside a Soxhlet apparatus of 500mL and maintained under reflux for 3 hours. The ethanol 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 done by GC-MS (Shimadzu, model QP-5000, 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%, White Martins Gases Industriais). The fennel extracts (0.005 grams) were diluted in 1mL of ethyl acetate (chromatographic grade, EM Science, lot 36079631); 1 μL of sample was injected and the sample 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 [16]; and (iii) retention index [17].

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 × 0.25 mm × 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 [18]. The quantification was done using a standard solution for each substance identified in the extract: anethole (P. A., Aldrich, lot 06605HR), fenchone (P. A., Aldrich, lot 04416TS).

Thin Layer Chromatography (TLC)

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

silica plates (60-PF254, Merck 20×20 cm, 0.25 mm of height, lot 940378601), the mobile phase was composed by 60% of hexane (Merck, analytical grade, lot HX0290-44) and 40% of ethyl acetate (Merck, analytical grade, lot K225488323). 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 DISUSSIONS

The yields of hydrodistillation and ethanol extraction were 2.4 and 16%, respectively. The variation of the SFE global yield of fennel extract as a function of extraction pressure and temperature is presented in Figure 1. One can observe in this figure that for pressures larger than 150 bar the influence of temperature is marginal. At pressure of 100 bar, the lower temperature leads to a larger global yield. This effect is also observed when the global yield is plotted as a function of the solvent density (Figure 1b).

The effect of pressure and temperature on the global yield is similar to the effect of these parameters on the solubility of extract in supercritical CO_2. There are two effects of temperature on solubility: i) the vapor pressure of the solute and ii) the solvent density. For pressures lower than 150 bar the CO_2 density is strongly influenced by temperature, thus, the effect of the increase in the solute's vapor pressure is less important than the decrease in the solvent density. As a consequence, there is a decrease of the solute's solubility, in a phenomenon known as retrograde condensation. For pressures close to 150 bar, both effects are important. For pressures larger than 150 bar, the increase of vapor pressure is more important than the decrease of the solvent's density.

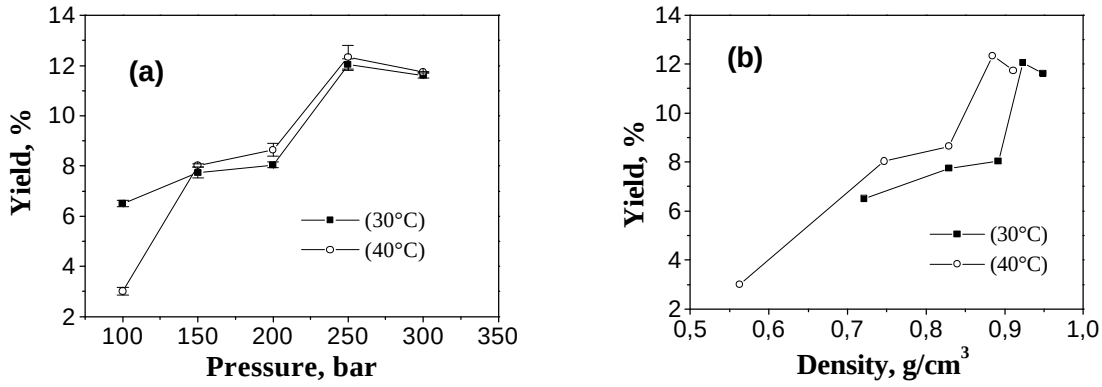

Figure 1: Global yields isotherms for the system fennel + CO_2.

Chemical composition of the extracts

The identification and quantification of the main compounds of fennel extract (trans-anethole and fenchone) were assessed by gas chromatography, using the external standard method previously described. Table 1 presents the yield of fenchone and trans-anethole from the fennel (*Foeniculum vulgare*) extracts obtained by hydrodistillation, ethanol extraction, and supercritical fluid extraction.

The TLC analysis of the fennel extracts is presented in Figure 2. Fenchone and trans-anethole were detected in all extracts. In the SFE and ethanol extracts (strips A, B, and D) there are a larger amount of high molecular mass compounds, represented by the bands on the

top of the plate. In the extract obtained by hydrodistillation (strip C) one can observe only the presence of the essential oil.

Table 1: Chemical composition of fennel extract

Compounds	% (mass of solute/mass of dry raw material) × 100					
	Hydrodistillation	Ethanol Extraction	Supercritical Extraction			
			30°C		40ºC	
			100 bar	250 bar	100 bar	250 bar
Fenchone	0.17	0.08	0.034	0.063	0.075	0.056
trans-Anethole	1.82	1.39	1.19	1.76	0.99	1.66

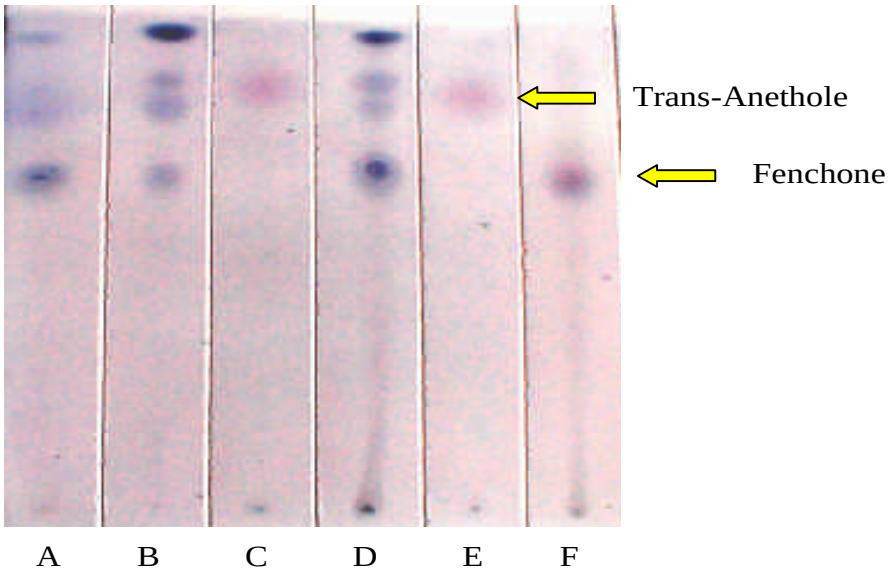

Figure 2 – TLC analysis of *Foeniculum vulgare* extracts: (A) SFE at100 bar/ 40°C; (B) SFE at 250 bar/30°C; (C) hydrodistillation; (D) ethanol extraction; (E) trans-anethole standard; (F) fenchone standard.

CONCLUSIONS

The maximum global yield of fennel extract was obtained at 250 bar and 30 °C. In spite of that, the maximum yield of trans-anethole and fenchone was attained at 100 bar and 40°C. The extract produced by hydrodistillation presented the highest yield of both trans-anethole and fenchone. Furthermore, this extract presented the lower amount of a high molecular mass compounds.

ACKNOWLEDGEMENTS

The authors are also grateful to FAPESP (1999/01962-1) for the financial support. The present work is part of the doctoral thesis of Lucinewton S de Moura who wants to thank FAPESP (00/04972-7) for the Ph.D. assistantship.

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