

Supercritical Carbon Dioxide Extraction and Fractionation of Fennel Oil

Béla Simándi,* András Deák, and Erika Rónyai

Department of Chemical Engineering, Technical University of Budapest, H-1521 Budapest, Hungary

Gao Yanxiang

Department of Food Engineering, Tianjin Institute of Light Industry,
Tianjin, People's Republic of China 300222

Tibor Veress

Institute of Forensic Sciences, H-1930 Budapest, Hungary

Éva Lemberkovics and Mária Then

Institute of Pharmacognosy, Semmelweis University of Medicine, H-1085 Budapest, Hungary

Ágnes Sass-Kiss and Zsuzsa Vámos-Falusi

Central Food Research Institute, H-1022 Budapest, Hungary

Ground fennel seeds were extracted with supercritical carbon dioxide. Small-scale subsequent extractions of the same sample showed that the composition of volatile compounds was changed with the extension of extraction time and only principal volatile components (limonene, fenchone, methylchavicol, and anethole) were present in the last-extracted sample. Fennel oil was successfully fractionated into the essential oil rich and fatty oil rich products in pilot-scale apparatus using two separators in series. Designed experiments were carried out to map the effects of pressure and temperature in the first separator on the yields and compositions of the products. The minimum level of the total undesired components in both essential oil rich and fatty oil rich products appeared at a pressure of 80–84 bar and a temperature of 31–35 °C in the first separator. Supercritical CO₂ extraction of fennel seeds resulted in higher yield (10.0%) than steam distillation (3.0%), almost the same yield as hexane extraction (10.6%), and lower yield than alcohol extraction (15.4%). Analysis of the volatile compounds revealed the significant difference of the composition in distilled oil and oleoresins prepared by CO₂ and solvent extractions. Sensory evaluation showed that the CO₂ extraction product and distilled oil were more intense in odor and taste than alcohol and hexane extracts.

Keywords: *Supercritical fluid extraction; fractionation; Foeniculum vulgare Mill. (fennel); volatile oil; fatty oil*

INTRODUCTION

Supercritical fluid extraction (SFE) is an environmentally benign separation technique for the obtaining of flavoring compounds from plant materials, and it constitutes an industrial alternative to steam distillation and solvent extraction processes (McHugh and Krukoni, 1986; Stahl et al., 1987; Perrut, 1991; King and Bott, 1994; Brunner, 1994; Reverchon, 1997). SFE is a separation method that exploits the unique properties of gases above their critical values to extract soluble components from a raw material. Carbon dioxide (CO₂) is an ideal solvent for the extraction of natural products because it is nontoxic, nonexplosive, readily available, and easily removable from the products. Using SFE

instead of steam distillation or extraction with classical solvents (hexane, ethanol), which are the traditional spice-processing methods, has several advantages: the problem of toxic residual solvent in the products is eliminated; the lower temperatures involved lead to less deterioration of the thermally labile components in the extract; SFE is more selective than extraction with commonly used solvents, which extract unwanted components (e.g., tannins, chlorophylls, minerals) as well; and SFE with CO₂ retains the organoleptic characteristics of the starting spice materials (Moyler and Heath, 1988; Reverchon and Senatore, 1992; Simandi et al., 1993; Oszagyan et al., 1996). The extraction followed by the fractional separation of extracts in multiple-stage separators produces high-quality volatile oils (Stahl and Gerard, 1982; Simandi et al., 1993). Recently, this fractional separation technique has been refined by Reverchon and co-workers for selective precipitation of

* Author to whom correspondence should be addressed (fax +36 1 463 3197; e-mail simandi.vmt@chem.bme.hu).

cuticular waxes and volatile oils (Reverchon, 1992; Reverchon and Senatore, 1992; Reverchon et al., 1994).

Fennel (*Foeniculum vulgare* Mill., family Umbelliferae) is an annual, biennial, or perennial aromatic herb, depending on the variety, which has been known since antiquity in Europe and Asia Minor. The leaves, stalks, and seeds (fruits) of the plant are edible. The dried, aromatic fruits are widely employed in culinary preparations for flavoring bread and pastry, in candies, and in alcoholic liqueurs of French type, as well as in cosmetic and medicinal preparations (Farrell, 1985; Hänsel et al., 1993). Much work has recently been done on the yield and composition of both extracts and essential oils of fennel of several varieties and from several locations (Embong et al., 1977; Miura et al., 1986; Akgül and Bayrak, 1988; Katsiotis, 1988; Verghese, 1988; Arslan et al., 1989; Lawrence, 1989; Betts, 1992; Khan et al., 1992; Lawrence, 1992; Cavaliero et al., 1993; Piccaglia and Marotti, 1993; Wagner, 1993; Guillén and Manzano, 1994, 1996; Gupta et al., 1995; Venskutonis et al., 1996). From classification aspects *trans*-anethole and fenchone are the most important volatile components. In the essential oil of sweet fennel the fenchone content usually does not exceed 5%, whereas in the bitter types its content can be as high as 20%. In sweet fennel oil the anethole content reaches 84–90%, whereas its proportion in bitter fennel is ~61–70% (Lawrence, 1994; Bernath et al., 1996).

An attempt to extract fennel oil using liquid CO₂ in a Soxhlet type apparatus has been described by Naik et al. (1989). Fractionated extraction was carried out by stepwise increase of the solvent power of the CO₂ in two stages. Volatile components were extracted at a pressure in the supercritical state (130 bar) and a temperature in the subcritical state (30 °C) in the first stage, and in the second stage the flavor components were recovered with supercritical solvent (300 bar, 40 °C) (Behr et al., 1984).

The essential oil or oleoresins from fennel seeds are important ingredients for flavoring cosmetics, pharmaceuticals, and food products; hence, the improvement of the quality of fennel oil is of economic importance. For this reason, the extraction of fennel oil using supercritical CO₂ could have an industrial impact.

Although the fennel essential oil and oleoresin have been produced for many years by using the conventional technologies, to our knowledge, they have not yet to be manufactured commercially by SFE. In this study, the following points will be covered to investigate the processing technology of fennel seeds: investigation of the supercritical CO₂ extraction of fennel oil in different scales; separation of fennel extract into the essential oil rich and fatty oil rich products by applying stagewise fractionation in two separators; optimization of the separation conditions to minimize the content of unwanted components in the products; comparison of yields and compositions of supercritical CO₂ extracts, solvent extracts, and distilled oil; and evaluation of the organoleptic characteristics of products prepared according to different methods.

MATERIALS AND METHODS

Solvents. For analytical SFE, CO₂ was of 99.99% purity (Union Carbide, Westerlo, Belgium), whereas for the laboratory extraction it was 95–96% (w/w) pure supplied by Linde (Repcelak, Hungary). The *n*-hexane used for the dissolution of the extracted and sorbed material was of Lichrosolv grade

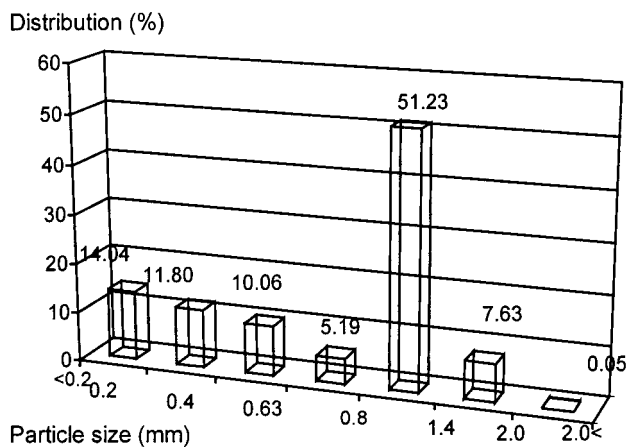


Figure 1. Particle size distribution of the ground fennel.

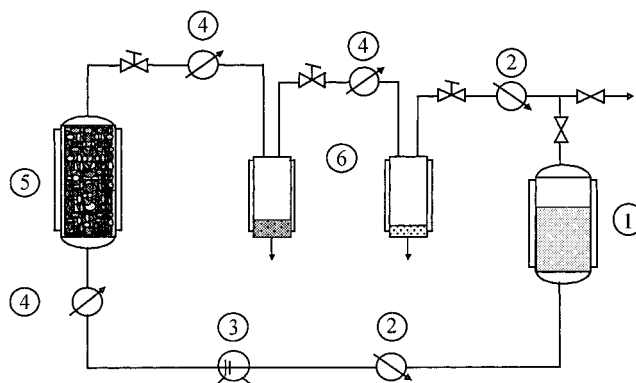


Figure 2. Flow diagram of the extraction system: 1, CO₂ storage vessel; 2, cooler; 3, high-pressure pump; 4, heater; 5, extractor; 6, separators.

(Merck, Darmstadt, Germany). For the conventional extractions, hexane was of analytical grade produced in Hungary and ethyl alcohol was of food grade 96% (v/v) pure.

Fennel Seeds. The fennel seeds used were purchased from a local market in Hungary. The moisture content of the raw material was 10.8% (w/w). The seeds were ground to specific particle size before the extractions. A typical particle size distribution is shown in Figure 1.

Equipment and Extraction. Analytical SFE Equipment. SFE experiments were performed on a Hewlett-Packard (Avondale, PA) model 7680T supercritical fluid extractor controlled by a Hewlett-Packard Vectra 386/16N personal computer. For the extraction, 7 mL thimbles were used as extractor chambers. For analyte trapping, a Hypersil ODS octadecyl silica (dp 30–40 μm) (Shandon Scientific, Runcorn, U.K.) packed column was used. The sample was consecutively extracted for three times at the given extraction conditions (pressure = 81 bar, chamber temperature = 40 °C, flow rate = 2.0 mL/min, time of static extraction = 0.5 min, time of dynamic extraction = 22.7 min), and extract trapping conditions (nozzle temperature = 45 °C, trap temperature = 0 °C).

From the ground and homogenized fennel seeds 0.8 g was weighed into a 5 cm × 3 cm sized filter paper. The material to be extracted was wrapped into the paper to hinder the plugging of the frits of the extractor thimble with small particles.

Pilot SFE Equipment. A schematic flow diagram of the pilot scale SFE apparatus produced in Hungary is shown in Figure 2. Liquid CO₂ is supplied from a gas cylinder and passed into the inner storage vessel. It is then compressed to a desired pressure by means of the pump (LEWA, type EH-1) and heated to a specified extraction temperature to be brought into supercritical state before it passes into the extraction vessel filled with the raw material. The compressed CO₂ is then allowed to flow upward through the vertically mounted bed,

and the essential oil and other lipophilic substances are extracted. The solution leaves the extractor and through the pressure reducing valve flows into the first separator. The fatty oil rich product settles to the bottom and can be collected and weighed. The remaining solution is passed into the second separator, where the CO₂ is evaporated and the essential oil rich product is recovered. The automatic meter (MicroMotion, model DS006s) measures the total weight of CO₂ consumed. By opening the appropriate valves CO₂ can be recycled to the storage vessel. Pressure is set by semiautomatic back pressure valve with an accuracies of ± 3 bar in the extractor and ± 1 bar in separators, and the temperature is adjusted by thermostats with an accuracy of ± 1 °C. The flow rate of the fluid is regulated by adjusting the length of the pump stroke.

About 750 g of the ground fennel seed was weighed accurately and filled into the 5 L extractor. The desired temperature and pressure were adjusted, and then CO₂ feed was started. The accumulated products were removed and weighed at certain time intervals.

General Procedures. Standard methods were used for the determination of moisture, oleoresin (hexane and alcohol Soxhlet extraction), and essential oil (steam distillation) content of the raw and residual plant materials. Volatile oil contents of the fractionated extracts were also determined by steam distillation. The particle size distribution of the starting material was determined by passing the ground material through sieves of various mesh sizes and weighing the fraction taken from each tray.

Gas Chromatographic/Mass Spectrometric Analysis (GC/MS) of Volatile Components. Sample Preparation. For gas chromatographic analysis 50 μ L aliquots of the extracts were diluted with 1500 μ L of *n*-hexane. From each reference compound a 25 μ L volume was mixed with 1500 μ L of *n*-hexane.

For GC/MS analysis a Hewlett-Packard 5890 series II gas chromatograph equipped with a 7673 autosampler and a 5989A MS engine mass spectrometer were used. GC conditions were as follows: carrier gas, helium; flow rate, 1.1 mL/min; column, Hp-5 MS fused silica capillary column (film thickness = 0.25 μ m), 15 m \times 0.25 mm i.d.; injection temperature, 250 °C; oven temperature program, 40 °C (2 min), 15 °C/min to 80 °C (1 min), 5 °C/min to 130 °C; MS interface temperature, 280 °C; injection, 1 μ L of *n*-hexane solution in split mode (1:60); MS condition, electron impact (EI) mass spectra were recorded. The ion source temperature was 200 °C, the temperature of the quadrupole mass filter was 100 °C, and *m/z* range of 40–400 was scanned. The constituents of the extracts were identified by comparing their retention times with those of standards and by searching their mass spectra in a mass spectral database (Wiley 138 K). This method is referred to as GC-1 in the following sections.

GC Investigation of Fennel Extracts. For the qualitative and quantitative analysis of fennel volatiles a gas chromatograph (Shimadzu GC-A14, with FID detector, and Shimadzu C-R6A chromatographic integrator) was used under the following GC conditions: column, 25 m \times 0.25 mm i.d. \times 0.25 μ m film thickness; CW-20M stationary phase; oven temperature program, 110 °C (5 min), 6 °C/min, 200 °C (15 min); injector temperature, 220 °C; detector temperature, 250 °C; carrier gas, helium 1 mL/min. Hereafter it is referred to as the GC-2 method.

Analysis of Fatty Oil Composition. To 7 mg of fennel extract was added 250 μ L of 0.5 M KOH in methanol. After 10 min of boiling, 200 μ L of 14% BF₃ in methanol was added to the reaction mixture and boiling was continued for 5 min. Heptane (250 μ L) was added to this mixture and warmed again for 2 min. Saturated sodium chloride solution was added to the fatty acid methyl ester mixture, kept in a refrigerator for 15 min. The heptane phase was taken over dried sodium sulfate (Toth-Markus and Sass-Kiss, 1993).

The GC analysis was performed with a Hewlett-Packard 5720A apparatus with an FID detector. The column used was a boriumsilicat capillary with inner diameter of 0.75 mm, length of 30 m, and Supelcowax 10 bonded phase in 1 μ m thickness. The temperature program was 160 °C, 4 °C/min to

Table 1. Volatile Components of Subsequent Fennel Seed Extracts Obtained by Analytical Scale SFE (GC-1 Method)

compound	retention time (min)	composition (% of peak area)		
		1	2	3
α -pinene	4.427	3.12	1.31	tr ^a
camphene	4.613	0.43	tr	tr
β -pinene	4.983	0.65	0.77	tr
β -myrcene	5.214	2.10	tr	tr
α -phellandrene	5.405	0.97	tr	tr
limonene	5.821	4.86	2.71	1.43
3-carene ^b	6.372	1.39	0.83	tr
fenchone	6.981	23.05	16.10	9.40
camphor	8.099	0.83	tr	tr
methylchavicol	9.356	5.44	5.25	4.42
<i>cis</i> -anethole	10.689	1.01	tr	tr
<i>trans</i> -anethole	11.579	56.15	73.03	84.75

^a tr (= trace), <0.1. ^b Identification is based on comparison of mass spectral data.

200 °C, and isotherm; the temperature of the injector and detector was 250 °C. Carrier gas was hydrogen at a flow rate of 7.5 mL/min.

Sensory Analysis. The qualities of fennel extracts produced by steam distillation, hexane, alcoholic, and SFE extraction were compared by ranking, which was performed as described by International Standard (1988). The samples were placed in a ranking order according to total impression parallel of odor and taste by comparing them. The rank sum of each sample was used to calculate Friedman value *F*. Comparing it with values given in the Friedman test (level of significance = 0.05), it can be concluded whether there is an overall difference among all the samples. Demonstrating the significant difference, the rank sums can be used to identify the significant difference between sample pairs by the critical value calculated from the Friedman test.

The concentration of fennel oil for sensory evaluation was determined in preliminary experiments. The sensory analysis of odor and taste was performed in a 2.5 mg/L concentration of fennel oil in tea of 60 °C that contained saccharose in concentration of 40 g/L.

Each sample was evaluated 10 times.

RESULTS AND DISCUSSION

Analytical Scale Extraction. The three subsequent extractions of the same raw material produced 14, 7, and 4 mg extracts, respectively. The results obtained by chromatographic analysis (GC-1 method) of the extracts prepared by three consecutive SFES of the same sample are shown in Table 1. All three fractions contained *trans*-anethole, fenchone, methylchavicol, and limonene as principal components. α -Pinene, camphene, β -pinene, β -myrcene, α -phellandrene, 3-carene, camphor, and *cis*-anethole were also found in the first fraction. Only α -pinene, β -pinene, and 3-carene appeared in the second fraction; the third fraction contained only the four principal components. With the extension of extraction period, *trans*-anethole content increased, whereas the content of other components decreased.

The results of sensory evaluation showed that the first fraction had a very pleasant delicious flavor, which smelled better than that of the second and third fractions. This indicated that reasonable extraction time should be selected. If a short period was taken, although the flavor of extracts became better, the yield was low; hence, the flavor and yield of the extract and consumption of CO₂ should be balanced from the viewpoint of quality and economics when the extraction period is selected. For quantitative determination, the extraction

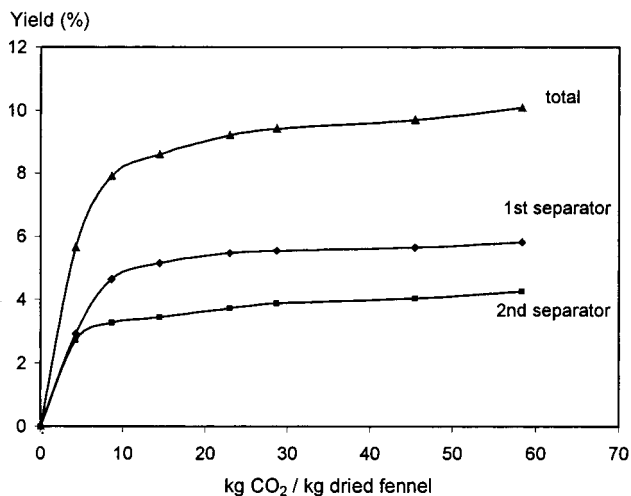


Figure 3. Extraction yields obtained for fennel seeds: run 7, flow rate = 7 kg of CO₂/kg of dried fennel per hour.

Table 2. Comparison of the Yields of Essential Oil Rich and Fatty Oil Rich Products Obtained by Stagewise Separation (Extraction at 300 bar, 40 °C)

run	1st separator			2nd separator		
	P_S (bar)	T_S (°C)	yield (%)	P_S (bar)	T_S (°C)	yield (%)
5	70	40	9.89	20	20	0.69
6	80	40	6.88	20	20	1.53
7	90	40	5.82	20	20	4.27

time that will ensure a predefined level of recovery can be calculated as detailed elsewhere (Veress, 1994).

Pilot-Scale Preliminary SFE and Fractionation.

All of the preliminary extractions were carried out at 300 bar and 40 °C. Typical extraction curves are shown in Figure 3, in which the total yield and the yields of fatty oil rich product and essential oil rich product (mass of extract/mass of dried material) were plotted versus the specific solvent mass of CO₂ passed through the extractor. Under different separation conditions, the yields of various products are listed in Table 2. It can be shown that the ratio of the essential oil rich product (second separator) and fatty oil rich product (first separator) depended on the separation parameters. With increase in the pressure from 70 to 90 bar in the first separator, the yield of fatty oil rich product decreased by 41.2% in the first separator, whereas the yield of essential oil rich product increased by as many as 6 times in the second separator.

Fractionation of Fennel Oil. Small-scale and preliminary pilot-scale extractions of fennel oleoresin using supercritical CO₂ indicated that there is a feasibility to extract and fractionate the oleoresin from ground fennel seeds. The yield and the quality of the separated products depended on the extraction pressure, temperature, and time (or the amount of CO₂) and on the pressure and temperature in the first separator, respectively.

The purpose of the additional experiments was to investigate the effects of four variables on the quality of products and CO₂ amount consumed at the same level of yield during the extraction and fractionation of fennel oil from ground seeds with supercritical CO₂. The variables studied were the extraction pressure and temperature and the separation pressure and temperature in the first separator. A central composite design with $\alpha = \pm 1.547$ star (axial) points was realized once;

Table 3. Extraction of Fennel Seeds: Factor Levels

factor	star (axial)	low	center	high	star (axial)
(coded)	-1.547	-1	0	1	1.547
pressure P_E (bar)	220	237.7	270	302.3	320
temp T_E (°C)	31	37.7	50	62.3	69
pressure P_S (bar)	70.7	74	80	86	89.3
temp T_S (°C)	20	23.5	30	36.5	40

Table 4. ANOVA Table^a

	SS	df	MS	F	p
(1) P_E (L)	0.29702	1	0.297020	0.284430	0.603550
P_E (Q)	4.19605	1	4.196047	4.018189	0.068110
(2) T_E (L)	0.54446	1	0.544462	0.521384	0.484086
T_E (Q)	0.36677	1	0.366775	0.351229	0.564420
(3) P_S (L)	0.24430	1	0.244300	0.233945	0.637320
P_S (Q)	4.64676	1	4.646760	4.449798	0.056586
(4) T_S (L)	9.10639	1	9.106386	8.720394	0.012076
T_S (Q)	8.69967	1	8.699667	8.330915	0.013669
1L by 2L	0.17787	1	0.177868	0.170329	0.687098
1L by 3L	0.34280	1	0.342798	0.328268	0.577259
1L by 4L	0.03247	1	0.032470	0.031094	0.862972
2L by 3L	0.46373	1	0.463728	0.444072	0.517771
2L by 4L	0.30631	1	0.306314	0.293330	0.598006
3L by 4L	1.86668	1	1.866684	1.787561	0.206017
error	12.53116	12	1.044263		
total SS	43.82252	26			

^a SS, sum of square; df, degrees of freedom; MS, mean square; F , F test value; p , p level.

Table 5. Comparison of Extract Yields (Percent) Obtained from Fennel Seeds by Different Methods

	steam distillation	Soxhlet extraction		SFE (run 40) ^a		
		hexane	alcohol	1st separator	2nd separator	total
yield	3.04	10.59	15.44	7.93	2.05	9.98

^a Run 40: extraction 270 bar, 50 °C; 1st separator 80 bar, 40 °C.

three repeated experiments were performed in the center of the design. The factor levels are given in Table 3.

The CO₂ consumption was compared at various conditions. When the highest extraction temperature and pressure were chosen, the CO₂ consumption was at the lowest level (10% yield was obtained with 30–32 kg of CO₂/kg of dried fennel solvent consumption).

The yield of essential oil rich product and its essential oil content and the yield of fatty oil rich product and its essential oil content were determined at each run. The essential oil percentage of the products was obtained by steam distillation. The total content of unwanted components (the sum of the mass of the essential oil in the first separator and of nonessential oil in the second separator related to the mass of dried raw material) was used as a dependent variable. For example, the first and the second separator products of run 40 (yields are given in Table 5) contained 19.5 and 76.2% steam volatile components, respectively. Hence, the amount of total unwanted components was $7.93 \times 0.195 + 2.05 \times (1 - 0.762) = 2.03\%$.

From the ANOVA table (Table 4) it is seen that the linear and quadratic components of the separator temperature effect [$T_S(L)$ and $T_S(Q)$] are significant at $p = 0.012$ and 0.013 level, respectively. The quadratic components of the extractor pressure [$P_E(Q)$] and the separator pressure [$P_S(Q)$] effects are close to the usual significance level ($p = 0.068$ and 0.057, respectively).

Figure 4 is a contour plot showing the effect of the separation pressure (P_S) and temperature (T_S) on the

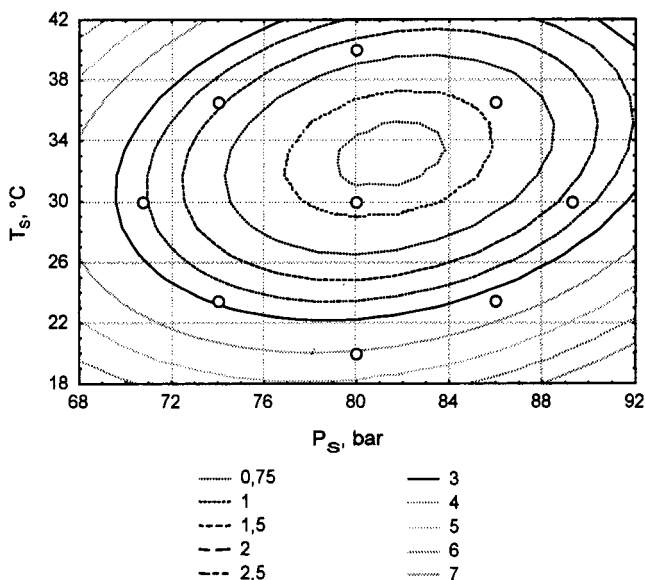


Figure 4. Effect of the separation temperature (T_S) and pressure (P_S) on the amount of total content of unwanted components in both separators ($P_E = 302$ bar, $T_E = 62$ °C): (O) experimental design.

Table 6. Composition of Major Volatile Components of Different Fennel Seed Extracts (GC-2 Method, Percent of Peak Area)

compound	distilled oil	hexane extract	alcoholic extract	SFE		
				run 20 ^a	run 13 ^b	run 44 ^c
α -pinene	2.53	0.90	tr ^d	0.98	0.67	0.52
β -pinene	0.46	0.44	tr	0.52	0.40	0.35
limonene	3.23	1.58	0.55	1.21	0.93	0.85
fenchone	17.38	17.31	14.03	15.71	16.57	13.88
methylchavicol	5.30	3.54	3.37	3.14	3.05	3.14
<i>trans</i> -anethole	64.21	71.38	70.93	77.55	77.98	77.85

^a Run 20: extraction 238 bar, 38 °C; 1st separator 74 bar, 36 °C. ^b Run 13: extraction 270 bar, 50 °C; 1st separator 80 bar, 30 °C. ^c Run 44: extraction 320 bar, 50 °C; 1st separator 80 bar, 30 °C. ^d tr (= trace), <0.1%.

amount of total unwanted components in both separators. From Figure 4 it can be found that the minimum level of total unwanted components occurred at the pressure between 80 and 84 bar and temperature between 31 and 35 °C.

Analysis of the Volatile Compounds and Fatty Acid Composition of Fennel Extracts Prepared under Different Conditions. The yield obtained by SFE compared with those by steam distillation and Soxhlet extraction is presented in Table 5. Table 5 indicates that the yield of the essential oil rich product was 67.4% of the steam distilled oil, whereas the total yield of SFE was almost the same as that of hexane extraction; however, it was 64.6% of the alcoholic extract.

Major components of oil samples obtained by different extraction methods were analyzed by using the GC-2 method, and the results are shown in Table 6. The SFE extracts recovered from the second separator are listed. It can be found that the products contain the same components; however, the compositions of those are different. The distilled essential oil contains more α -pinene, limonene, and methylchavicol and less anethole than the extracted oils.

From Table 6 it can be seen that among the supercritical CO₂ extracts, when the extraction temperature, separation pressure, and temperature were kept con-

Table 7. Composition of Fatty Acids of Extracts (Percent)

fatty acid	hexane extract	alcoholic extract	SFE		
			run 41 ^a	run 31 ^b	run 44 ^c
C _{12:0}	0.07	0.09	0.17	0.06	0.08
C _{14:0}	0.15	0.19	0.12	0.17	0.15
C _{16:0}	5.23	5.72	5.08	5.80	5.31
C _{16:1}	0.52	0.54	0.55	0.62	0.58
C _{18:0}	0.91	0.90	0.80	0.88	0.81
C _{18:1}	78.92	78.21	79.32	78.15	79.16
C _{18:2}	13.53	14.01	13.56	13.62	13.42
C _{18:3}	0.39	0.42	0.42	0.40	0.41
C _{20:0}	0.41	0.38	0.23	0.28	0.29

^a Run 41: extraction 220 bar, 50 °C; 1st separator 80 bar, 30 °C. ^b Run 31: extraction 270 bar, 50 °C; 1st separator 80 bar, 30 °C. ^c Run 44: extraction 320 bar, 50 °C; 1st separator 80 bar, 30 °C.

Table 8. Sensory Evaluation of Fennel Seed Extracts^a

product	SFE run 20 ^b	SFE run 40 ^c	distilled oil	hexane extract	alcoholic extract
valid answers	10	10	10	10	10
rank sum	30	17	20	40	43

^a $F_{\text{calcd}} (21.4) > F_{\text{tabd}} (9.21)$ (at level of significance of 0.05). Critical value of difference of rank sums between sample pairs: 13.9. ^b Run 20: extraction 238 bar, 38 °C, 1st separator 74 bar, 36 °C. ^c Run 40: extraction 270 bar, 50 °C, 1st separator 80 bar, 40 °C.

stant, the change of extraction pressure had no significant effect on the composition of the oils in the second separator.

The compositions of fatty acids of fennel oleoresins prepared under different conditions are shown in Table 7. They are only slightly different for the extracts obtained by SFE and conventional solvent extraction.

Sensory Evaluation of Fennel Extracts Produced by Different Separation Methods. The SFE extracts obtained in the second separator were examined.

The quality of fennel oils prepared under various extraction and separation conditions showed a significant overall difference when subjected to sensory evaluation. The rank sums of the samples studied are shown in Table 8. The value $F (21.5)$ calculated from rank sums as described by the Friedman test was greater than the tabulated one (9.49 at a level of significance of 0.05). From a comparison of sample pairs, the difference of rank sum was 10 or 3 between steam distilled oil and CO₂ extracts produced at runs 20 and 40, respectively. This means that there were no significant differences between them because these values were less than the critical value of a different rank sum, 13.9 (level 0.05), calculated from the Friedman test (International Standard, 1988). On the basis of difference of rank sum, significant differences could be found among hexane, alcoholic extracts, and steam distilled oil or CO₂ extract produced at 270 bar and 50 °C. The hexane and alcoholic extract have the last places among samples studied. The results indicated that the first place was shared by steam distilled oil with CO₂ extract of fennel produced at run 40.

CONCLUSION

Supercritical CO₂ has been successfully employed as a solvent for the extraction of fennel oil. Stagewise precipitation using two separators in series was found to be an effective separation method to fractionate the CO₂ extracts into essential oil rich and fatty oil rich

products. The results from designed experiments indicated that the CO₂ amount consumed for a given overall yield was at the lowest level when the extraction pressure and temperature were above 302 bar and 62 °C, respectively. The minimum level of total unwanted components in both separators appeared at pressures of 80–84 bar and temperatures of 31–35 °C in the first separator.

Supercritical CO₂ extraction resulted in the same total yield as hexane extraction.

The results from sensory evaluation of fennel oil and oleoresins showed that the supercritical CO₂ extraction product and distilled essential oil had better quality than the alcohol and hexane extracts.

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