Supercritical Carbon Dioxide Extraction of Spearmint Oil from Mint-Plant Leaves

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Spearmint oil (essential oil of *Mentha spicata*) was extracted from Turkish mint-plant leaves using supercritical CO_2 in a basket-type extractor. Effects of temperature, pressure, extraction time, amount of CO_2, and entrainer (ethanol) concentration on composition of essential-oil extracts and oil yield, relative to hydro-distillation, were investigated. Relative yields were as high as 80%. Monoterpenes fraction in extracts was inversely correlated with relative oil yields. Monoterpenes were preferentially extracted by CO_2 due to their high vapor pressure, low molar mass and low polarity. Supercritical extraction presented advantages over the conventional hydro-distillation by yielding extracts lower in monoterpenes fraction and by enabling high yields at temperatures safe for the heat-sensitive essential oil.

De l'huile de menthe verte (huile essentielle de *Mentha spicata*) a été extraite de feuilles de menthe turque au moyen de CO_2 supercritique dans un extracteur de type panier. On a étudié les effets de la température, de la pression, du temps d'extraction, de la quantité de CO_2 et de la concentration du produit d'entraînement (éthanol) sur la composition des extraits d'huile essentielle et le rendement en huile, par rapport à l'hydrodistillation. Les rendements relatifs ont atteint 80%. La fraction des monoterpènes dans les extraits est inversement corrélée aux rendements relatifs en huile. Les monoterpènes ont été extraits préférentiellement par le CO_2 en raison de leur forte tension de vapeur, de leur faible masse molaire et de leur faible polarité. L'extraction supercritique présente des avantages sur l'hydrodistillation classique en donnant des extraits faibles en fraction de monoterpènes et en permettant de bons rendements à des températures sans danger pour cette huile essentielle sensible à la chaleur.

Keywords: spearmint oil, hydro distillation, supercritical CO_2 extraction, selectivity, yield.

Essential oils and its derivatives are important ingredients utilized by food, cosmetics, pharmaceutical and other related industries. Being captive within structure of their parent plants, these valuable oils have to be taken out from plant materials via some method of extraction. Steam distillation (or hydro-distillation) is one of the widely-used methods for this purpose. In this classical method, sensitive plant material is exposed to a relatively high temperature; which causes product degradation since essential oils are mainly composed of heat-labile compounds. On the other hand, in conventional solvent-extraction methods, possible use of toxic liquid solvents and their removal from final essential-oil product introduce problems. These deficiencies of the conventional methods and ever increasing demand for high-quality essential oils have resulted in considerable interest and fostered research in application of the supercritical-fluid (SCF) technology to extraction of essential oils from plants (Vollbrecht, 1982; Sankar et al., 1988; Temelli et al., 1988; Kassim and Hameed, 1990; Ondarza and Sanchez, 1990; Favati et al., 1991; Özer et al., 1992).

The advantages of using SCFs, particularly those of the supercritical CO_2, as extractive solvents have been documented in the literature (Rizvi et al., 1986). Among the advantages of SCFs, those which are pertinent to the subject of interest may be summarized as follows:

i) gas-like viscosities and diffusion coefficients that are intermediate to those of liquids and gases provide ability to penetrate the cellular structure of plants deeply and efficiently,

ii) liquid-like densities engender high essential-oil loading capacities,

iii) sensitivities to variations in temperature, pressure, solvent density, and modifier concentration allow enough degrees of freedom to control/adjust the solvent power and selectivity to obtain high-quality essential-oil products having commercially more satisfactory compositions.

In supercritical-fluid extraction, extracts can easily be recovered from the solvent phase in their pure forms by means of temperature and/or pressure perturbations applied in simple solvent-separation step(s) ensuing the extraction. However, poor recovery of highly-volatile fractions of essential oils due to aerosol formation, and recovery of oils from separator walls are among the important problems encountered during or after de-pressurization of supercritical extractors (Lorne and Adda, 1988).

In this work, spearmint oil was extracted from Turkish mint-plant leaves (*mentha spicata*; harvested in 1991 in the southeastern region of Turkey) at various operating conditions (temperature, pressure, and extent of extraction) using supercritical CO_2 as solvent in a pseudo-continuously-operated non-equilibrium extractor. The purpose of this work is to present the effects of the operating conditions on yield and quality (composition) of the spearmint-oil extracts, and to compare compositions of the essential oils obtained via supercritical-CO_2 extraction and the classical hydro-distillation (using the Clevenger apparatus) methods.

Experimental

The extraction unit consisted of two jacketed high-pressure autoclaves (10.5 cm ID, 1250 cm^3 operating volume, manufactured by Erikman Kardeşler Makina Sanayi (İstanbul, Turkey), connected in series as shown in Figure 1 (Kaya, 1990; Özer, 1993). One of the autoclaves was charged with dry-ice (manufactured from 99.9% CO_2 by Karbokimya A.Ş., İstanbul, Turkey) and used as a thermal pump to gen-

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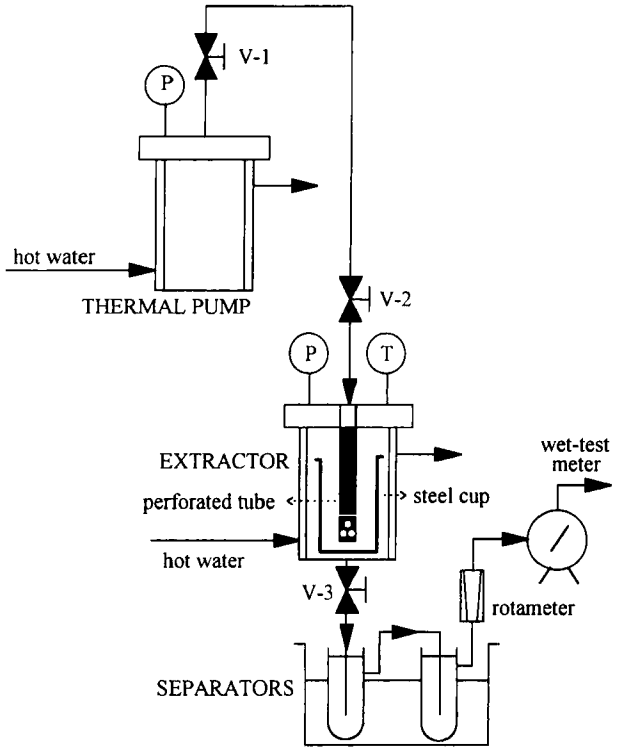


Figure 1 — The Experimental Setup.

erate the supercritical-fluid solvent. A stainless-steel cup (8.3 cm ID, 600 cm^3 capacity, no holes on the sides and at the bottom), packed with 125 g of the mint leaves (dried and ground to 10-20 mesh-size particles), was placed inside the second autoclave (extractor) as shown in Figure 1. A closed-bottom perforated tube (length: 10.9 cm, ID: 0.5 cm, perforated length: 2.2 cm from the tip) was connected to the lid of the extractor and inserted (together with the thermocouple well) into the ground mint leaves during closure.

Extractions with pure CO_2

Initially, a block of dry-ice was placed into the first autoclave (1.5-2.0 kg; depending upon -and more than the amount required by- the planned operating pressure in the extractor). Hot water was circulated in the jacket of the first autoclave (temperature of water bath was adjusted depending on the planned operating temperature and pressure). When enough pressure (about twice of the desired extractor pressure) was developed in the first autoclave, the valves connecting the two autoclaves (V-1 and V-2 in Figure 1) were opened slowly and thus the supercritical CO_2 was transferred to the extractor. When the desired pressure was attained in the extractor, these valves were closed and the autoclaves were isolated from each other. At the end of this step, pressure in the thermal pump was in excess of that in the extractor. The system was allowed about ten minutes to come to thermal equilibrium at the desired pressure. The extractor temperature was controlled within ±0.1°C (measured by a thermocouple) of the desired value (the extractor had a separate water bath). The pressure of the extractor was controlled within ±0.25 MPa of the desired level by opening the valves (V-1 and V-2) manually and using the pressure reserved in the thermal pump.

When the extractor reached thermal equilibrium at the desired pressure, the valve (V-3) at the bottom of the extractor (which was heated by a heating tape to about 50°C) was opened slowly. Supercritical CO_2 carrying the spearmint oil was expanded through this valve and passed through two glass separators (length: 15 cm, diameter: 3 cm) placed in an ice bath. Both separators contained about 10 cm^3 ethanol and were packed with glass beads in order to enhance recovery of the solutes. About 90% of the essential oils extracted was trapped in the first separator. Temperature reduction and the use of ethanol as the trapping solvent in the separators were essential to be able to recover the highly-volatile fraction of the essential oil. Analyses showed that extracts collected in cold but dry separators had lower concentrations of the highly-volatile components. The flow rate of the gas leaving the separators was observed by a rotameter and the flow rate in the system was controlled with the valve at the bottom of the extractor (V-3). The total amount of the CO_2 passed through the extractor was measured by a wet-test meter.

Due to low solvent flow rates used in the experiments, the rate of depletion of pressure in the extractor was very slow. Hence, for the short-time extractions (two hours), only two or three manual restorations (within ±0.25 MPa accuracy) of the extractor pressure were sufficient. The excess pressure in the thermal pump was sufficient to back up the pressure in the extractor throughout the short-time extraction experiments. However, for the long-time (four hours) extractions at elevated pressures, the pressure reserved in the thermal pump was not sufficient to back up the pressure in the extractor. Thus, in these experiments, when the need arose the extractor was isolated from the thermal pump, the thermal pump was de-pressurized quickly, and charged again with 1.5-2.0 kg dry-ice. During the process of recharging and reconnection of the thermal pump to the extractor, the extractor pressure was not disturbed by more than ±0.25 MPa.

Extractions with ethanol as entrainer

Extractions using ethanol as the polar co-solvent were carried out at 40°C / 8.5 MPa / 2 h conditions. These operating conditions were determined by considering the limitations of the set-up and the phase behavior of the CO_2-ethanol system. The phase-equilibrium data (Nagahama et al., 1988) show that the critical pressure of the CO_2-ethanol system is 7.9 MPa at 40°C. Two experiments were performed by introducing 17 and 35 cm^3 ethanol together with 1.5-2.0 kg dry-ice into the first autoclave (thermal pump). These entrainer amounts translate to about 1.5 and 3.0 mol% of ethanol in the dense-gas phase. The operating conditions (40°C / 8.5 MPa), corresponding to the single-phase region of the CO_2-ethanol binary system, guarantee complete solubility of ethanol in the dense-gas phase. The experiments were performed according to the procedure explained above for pure-CO_2 extractions.

Hydro-distillation of mint leaves

Hydro-distillation experiments were performed in the Clevenger apparatus (Guenther, 1967a) using 125 g of the mint leaves before and after almost all the supercritical-fluid extraction experiments. During the early runs, relatively fresh mint leaves yielded 1.5 cm^3 of essential oil in the Clevenger apparatus. However, even though precautions were taken, mint leaves lost some of their freshness in time (about 155 days passed between the first and the last runs). Thus, essential-oil amounts as low as 0.9 cm^3 were obtained

TABLE 1
Experimental Conditions and Relative Essential-Oil Yields

T (°C)	P (MPa)	Density of Pure CO_2 (g/cm^3)	Extraction Time (h)	Total Amount of CO_2 Used (g)	Average Flow Rate of CO_2 at T & P (cm^3/min)	Relative Essential-Oil Yield (%)
40	8.0	0.299	2	165.0	4.61	43.3
40	8.0	0.299	2	193.9	5.41	40.0
40	9.0	0.521	2	189.2	3.03	53.3
40	9.5	0.595	2	219.2	3.07	58.3
40	10.0	0.636	2	104.7	1.37	63.3
40	11.0	0.688	2	454.9	5.51	66.7
60	8.0	0.198	2	345.5	14.53	45.3
60	8.0	0.198	2	168.2	7.08	43.3
60	9.5	0.274	2	328.9	10.02	51.3
60	11.0	0.372	2	110.7	2.48	53.3
60	11.0	0.372	2	319.6	7.16	55.0
40	8.0	0.299	4	965.1	13.47	70.0
40	8.0	0.299	4	970.2	13.52	65.0
40	9.5	0.595	4	924.9	6.48	73.3
40	10.0	0.636	4	499.5	3.27	76.7
40	11.0	0.688	4	809.8	4.91	80.0
60	8.0	0.198	4	711.4	14.96	40.0
60	8.0	0.198	4	696.0	14.64	46.6
60	9.5	0.274	4	807.1	12.29	60.0
60	11.0	0.372	4	966.2	10.83	63.3
60	11.0	0.372	4	884.4	9.91	64.7
50	10.0	0.402	2	125.8	2.61	60.0
60	8.0	0.198	1	190.8	16.05	23.3
60	8.0	0.198	1	95.8	8.06	26.6
1.5 mol% ethanol						
40	8.5	0.383	2	177.6	3.86	50.0
3.0 mol% ethanol						
40	8.5	0.383	2	174.6	3.79	56.6

in the Clevenger apparatus during the final runs. The volumes of essential oil obtained in the Clevenger apparatus before and after the supercritical extractions were used to determine the relative volumetric essential-oil yields of the supercritical-CO_2 extraction:

$$\% \text{ Yield} = \lfloor (V_1 - V_2)/V_1 \rfloor \times 100 \quad \ldots\ldots\ldots\ldots\ldots (1)$$

where V_1 and V_2 refer to the volume of essential oil obtained in hydro distillation before and after supercritical extraction, respectively.

ANALYSIS OF OIL SAMPLES

At the end of the prescribed extraction period, the spearmint oil trapped in the separators was analyzed using gas chromatograph [HP5890 Series II (Hewlett-Packard, Palo Alto, CA, USA); FID detector at 250°C; Carbowax 20M capillary column: 30 m × 0.25 cm × 0.25 μm; carrier gas (supplied by BOS A.Ş., İstanbul, Turkey): N_2, 1 cm^3(STP)/min, 1/100 split; temperature programming: 1 min at 70°C, 5°C/min to 170°C, 1 min at 170°C, 8°C/min to 220°C, 1 min at 220°C] and mass-selective detector [HP5971 (Hewlett-Packard, Palo Alto, CA, USA); Carbowax 20M capillary column: 50 m × 0.32 cm × 0.30 μm; carrier gas (supplied by BOS A.Ş., İstanbul, Turkey): He, 1 cm^3(STP)/min, 1/50 split; temperature programming: 8 min at 70°C, 8°C/min to 130°C, 1 min at 130°C, 3°C/min to 190°C, 1 min at 190°C, 8°C/min to 250°C, 10 min at 250°C].

Spearmint oils obtained in the Clevenger apparatus were sampled and injected directly into the chromatograph immediately after the experiments. The chromatograms of these oils showed about 170 peaks, out of which 12 peaks belonging to the characterizing components which constitute the bulk of the spearmint oil (Guenther, 1967b,c) were identified by the mass-selective detector.

Results and discussion

The operating conditions and the relative essential-oil yields obtained in the supercritical-extraction experiments are given in Table 1. The volumetric flow rates of the solvent are given at the extraction conditions. The solvent densities reported are those of the pure supercritical CO_2 at the extraction conditions. Since essential oils are readily soluble in both supercritical CO_2 (Stahl et al., 1987) and subcritical CO_2 (Platin, 1993), the dense-gas phase inside the extractor is far from being dilute. Therefore, the pure-CO_2 densities reported do not represent the actual dense-gas phase densi-

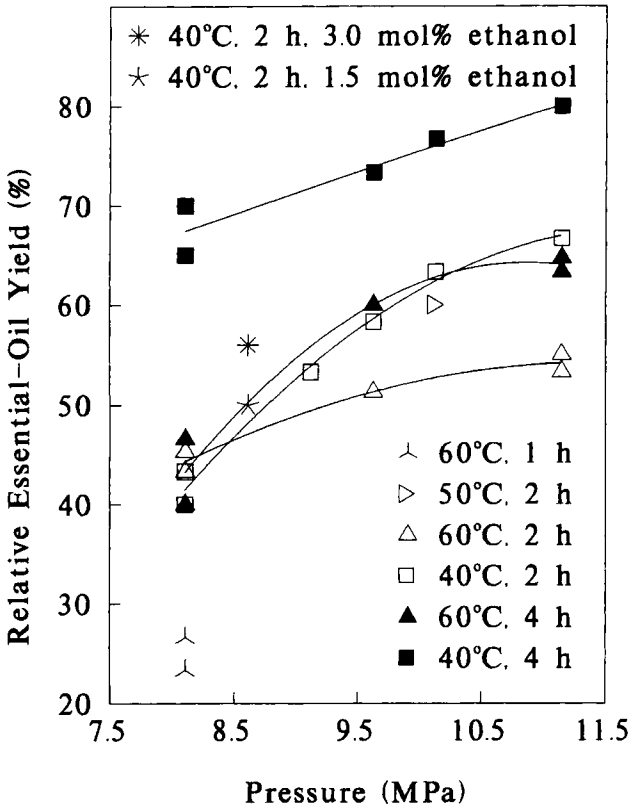


Figure 2 — Pressure Dependence of the Relative Essential-Oil Yield.

ties within the extractor. However, the reported densities should be viewed as a measure of the solvent power of the supercritical CO_2 at the corresponding operating conditions.

Table 1 shows that the relative essential-oil yields increase asymptotically with increasing extent of extraction at constant temperature and pressure (60°C, 8 MPa conditions). Theoretically, 100% relative yields may be obtained in sufficiently long extraction periods. However, at the given operating conditions, two hours of extraction was sufficient to reach about an optimal incremental relative yield when the costs associated with extended periods of operation are considered.

Figure 2 shows the pressure dependence of the relative essential-oil yields. The effect of the extent of extraction on relative yield is more pronounced at the low temperature. Retrograde temperature effects are observed above the crossover pressure (about 8.5 MPa) at fixed extents of extraction; the relative yield increases with decreasing temperature. If there were no inverse temperature effects on solubility of the essential-oil compounds (on solvent power of the supercritical fluid), the relative essential-oil yields at 60°C / 4 h and 60°C / 2 h extraction conditions would not be lower that those at 40°C / 4 h and 40°C / 2 h conditions, respectively, and also, the relative essential-oil yields at 60°C / 4 h and 40°C / 2 h extraction conditions would not be comparable. Since similar inverse temperature effects and crossover regions are also encountered in equilibrium solubilities of pure solutes in supercritical fluids, it can be concluded that equilibrium-solubility effects were dominating in the supercritical extractions. This implies that the system was operating close to the equilibrium conditions, even though this was not particularly aimed for during design of the extractor and in selection of the operating conditions.

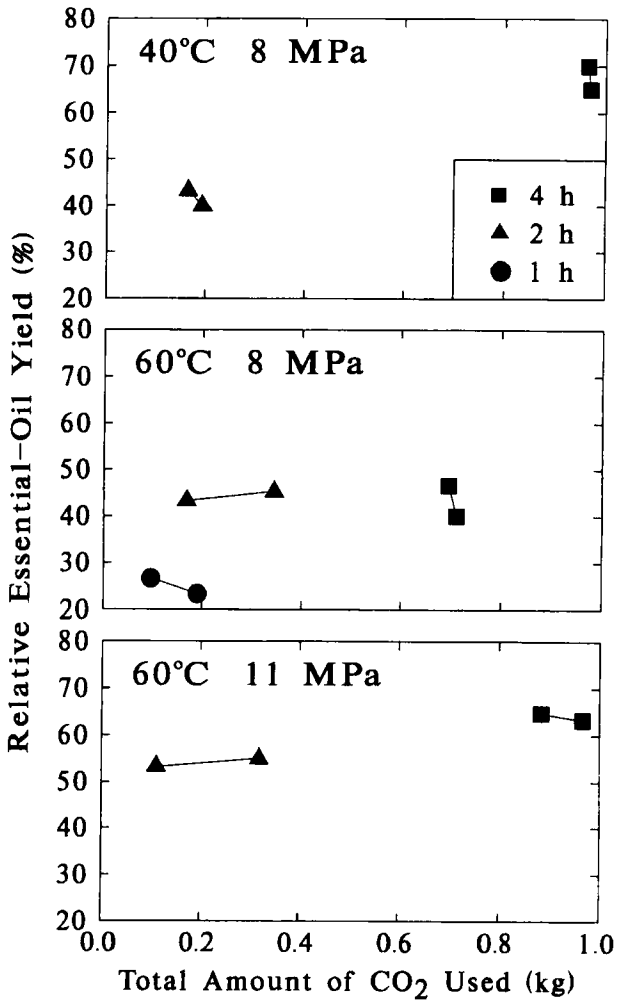


Figure 3 — Relationship between Relative Essential-Oil Yield, Extent of Extraction, and Total Mass of Solvent Used.

However, the operation of the extractor close to equilibrium conditions was plausible since the flow rates of the supercritical solvent within the extractor were quite low as can be seen in Table 1 (also, as will be discussed, external mass-transfer resistances were not controlling).

As can be seen in Figure 2 or Table 1, the increase in concentration of ethanol (polar co-solvent) increases the relative essential-oil yield as expected. Enhancement in the volumetric oil yield is significant especially with 3% ethanol; about 15% increase (from 43.3% to 50%) and 30% increase (from 43.3% to 56.6%) in the yield with 1.5% and 3% ethanol, respectively.

It is important to notice in Figure 2 that the relative essential-oil yields at 60°C / 4 h and 40°C / 2 h conditions are basically comparable within the experimental pressure range (due to inverse temperature effects within the crossover region). Also, essential-oil yield of the extraction done with 3% ethanol at 40°C is higher than that of the extraction with pure solvent at 60°C. Since essential-oils are mainly composed of heat-labile compounds it is a definite advantage to use supercritical fluids as extractive solvents (with or without a co-solvent); favorable essential-oil yields may be obtained at relatively low temperatures by capitalizing on inverse temperature effects within the crossover region and/or co-solvent effects (difficulties and cost of separation of co-solvent from the essential oil must be evaluated).

TABLE 2
Proposed Empirical Model and Summary of Statistical Analyses

Model (partial second-order) $Y = \sum\beta_i X_i + \sum\beta_{ii} X_i^2$

Y relative essential-oil yield (%)
X_1 temperature (°C)
X_2 pressure (MPa)
X_3 extent-of-extraction (h)
X_4 total mass of solvent used (g)
X_5 flow rate of solvent (cm^3/min)

	Forward selection		Backward elimination	
Significance level	85%	95%	85%	95%
remaining significant variables	X_2, X_3, X_1^2	X_2, X_3, X_1^2	X_1, X_2, X_3, X_3^2	X_1, X_2, X_3
parameters (standard errors) of the remaining significant variables	$\beta_2 = 5.8306$ (0.436) $\beta_3 = 6.7468$ (0.988) $\beta_{11} = -0.0067$ (0.001)	$\beta_2 = 5.8306$ (0.436) $\beta_3 = 6.7468$ (0.988) $\beta_{11} = -0.0067$ (0.001)	$\beta_1 = -0.6207$ (0.090) $\beta_2 = 5.7442$ (0.859) $\beta_3 = 18.504$ (6.194) $\beta_{33} = -2.0633$ (1.104)	$\beta_1 = -0.5826$ (0.093) $\beta_2 = 6.9603$ (0.595) $\beta_3 = 7.0704$ (1.022)
Adjusted R^2	0.992	0.992	0.992	0.991
Sum of squares of residuals	544.5	544.5	492.3	582.8

When the relative essential-oil yields in Figure 2 and their corresponding operating conditions given in Table 1 are carefully examined it can be concluded that, at all temperature, pressure and extent-of-extraction levels, the relative essential-oil yields were independent of both the amount (gram) and the flow rate (cm^3/min) of the supercritical-fluid solvent. Figure 3 shows the relative essential-oil yields against the total mass of CO_2 used in the 1, 2, and 4 h extractions for the three different operating conditions (as can be seen in Table 1, only these three operating conditions have replicates at constant extents of extraction). As the figure reveals, at a fixed temperature and pressure, relative yields at constant extraction times form clusters even though in some time clusters total mass of solvent varies significantly. Therefore, rather than the total mass of solvent, the extent of extraction was the dominating variable in the experiments. The data at 60°C / 8 MPa conditions (without any replicate) especially support this conclusion (with 190.8 g of CO_2 used in the 1 h extraction only 23.3% essential-oil yield was obtained, whereas, with 168.2 g of CO_2 used in the 2 h extraction 43.3% essential-oil was obtained). However, as Figure 3 and Table 1 show, this pair of data is one of the several data points which strengthen the conclusion that rather than the total mass of solvent, the extent of extraction is the dominating variable.

Furthermore, detailed statistical analyses using all data with the exception of the data for cosolvent-aided extractions and the 50°C / 10 MPa / 2 h data reveal that the significant variables are the temperature, pressure, and the extent-of-extraction but not the total mass of the solvent or the average flow rate of the solvent. The statistical analyses were performed using "stepwise regressions" (Walpole and Myers, 1989) over the data (23 data points from Table 1) with the model shown in Table 2 which is linear in its parameters (Box and Draper, 1987). In the analyses, both the "forward selection" and "backward elimination" algorithms (Walpole and Myers, 1989) at 85% and 95% significance levels (two-tail-area probability levels) were used. In the algorithms, the variables are sequentially deleted from the model if the two-tail probabilities of the associated model coefficients are greater than the predefined significance level. Table 2 summarizes the results of the statistical analyses performed using the statistical package SYSTAT. Table 2 shows that the variables temperature, pressure, and extent-of-extraction are the statistically-significant variables. On the basis of the "adjusted R^2" and "sum-of-squares-of-residuals" values, the following model resulting from the application of the backward elimination algorithm at 85% significance level, could be selected to describe the effects of the extraction conditions on the relative oil yield:

$$Y = -0.6207\,X_1 + 5.7442\,X_2 + 18.504\,X_3 - 2.0633\,X_3^2 \quad \ldots\ldots (2)$$

This simple model, which is independent of flow rate and total mass of the solvent used, distinguishes the effects of temperature and pressure, and predicts all levels of the relative oil yield quite well.

The above observation, that the extent of extraction is the dominating variable rather than the total mass of solvent used in the experiments, points to the fact that only a fraction of the supercritical-fluid solvent could diffuse into cellular structure of the mint leaves and dissolve the essential-oil components. In other words, the amounts of supercritical-fluid solvent used in the experiments were more than the amount that was necessary to fully impregnate the cellular

TABLE 3
Composition of the Spearmint Oils Obtained via Hydro-Distillation and Supercritical Extraction

Peak ID	Component (Molar mass, kg/kmol)	Temperature (°C) at which Vapor Pressure is 0.013 MPa / 0.1 MPa	Number of Days Elapsed Starting from Run #1: 73 — Volume of Spearmint Oil Obtained in Hydro-Distillation: 1.50 cm^3 — Normalized Compositions (%) of Oils Obtained via Hydro-Distillation	Number of Days Elapsed Starting from Run #1: 99 — Volume of Spearmint Oil Obtained in Hydro-Distillation: 1.25 cm^3 — Normalized Compositions (%) of Oils Obtained via Hydro-Distillation	Number of Days Elapsed Starting from Run #1: 155 — Volume of Spearmint Oil Obtained in Hydro-Distillation: 0.90 cm^3 — Normalized Compositions (%) of Oils Obtained via Hydro-Distillation	Normalized Composition (%) of Oil Extracted via CO_2 at 40°C / 8 MPa / 4 h Conditions
A	α-pinene $C_{10}H_{16}$ (136)	90/155	0.45	0.54	0.74	0.18
B	β-pinene $C_{10}H_{16}$ (136)	94/158	0.71	0.80	1.07	0.38
C	sabinene $C_{10}H_{16}$ (136)	-/-	0.28	0.31	0.34	0.23
D	myrcene $C_{10}H_{16}$ (136)	106/171	0.34	0.33	0.39	0.26
E	α-terpinene $C_{10}H_{16}$ (136)	-/-	0.43	0.41	0.56	0.03
F	limonene $C_{10}H_{16}$ (136)	108/175	7.25	7.20	8.44	5.26
H	γ-terpinene $C_{10}H_{16}$ (136)	-/-	0.86	0.83	1.05	0.08
Total monoterpene hydrocarbons			10.32	10.42	12.59	6.42
G	1,8-cineole $C_{10}H_{18}O$ (156)	108/176	5.61	6.12	7.97	6.85
J	carvomenthone $C_{10}H_{18}O$ (156)	-/-	1.30	1.13	1.16	2.63
L	carvone $C_{10}H_{14}O$ (150)	157/228	77.90	78.94	74.55	81.15
Total oxygenated monoterpenes			84.81	86.19	83.68	90.63
I	β-bourbonene $C_{15}H_{24}$ (204)	-/-	1.53	1.11	1.15	2.32
K	caryophyllene $C_{15}H_{24}$ (204)	-/261	3.34	2.28	2.58	0.63
Total sesquiterpene hydrocarbons			4.87	3.39	3.73	2.95

structure of the plant material (the excess amount of solvent bypassed the mint leaves). This conclusion, coupled with the equilibrium-solubility influenced temperature and pressure dependence of the relative essential-oil yields, indicates that external mass-transfer resistances were not controlling the extraction (intraparticle resistances were important). In other words, the rate of diffusion of the supercritical-fluid solvent within the solid particles were low, and thus local-equilibrium conditions prevailed within the solid structure. This conclusion also explains why the relative essential-oil yields were found independent of the average flow rate (given in Table 1) of the solvent. If the external mass-transfer resistances were controlling, increasing interparticle solvent velocity would have an effect on external mass-transfer resistance, and hence, would have increased the mass-transfer rate.

Table 3 shows the key components and normalized composition (normalized area count of chromatogram peaks) of the spearmint oils obtained by hydro-distillation of the mint leaves. As can be seen from the table, even though basic precautions were taken in the preservation of the batch of mint leaves, the plant lost some of its original freshness in time, and thus, some changes occurred in composition as well as in volumetric essential-oil yield. Components labeled as A, B, C, D, E, F, H are monoterpene hydrocarbons; G, J, L are oxygenated monoterpenes; I, K are sesquiterpene hydrocarbons. Carvone constitutes bulk of this spearmint oil; followed by limonene, 1,8-cineole, and caryophyllene. The gas chromatogram of the spearmint oil obtained via hydro-distillation (1.5 cm^3 oil yield) is given in Figure 4. Components A, B, F, H are sensitive to heat and/or air (Guenther, 1967b). Auto-oxidation and/or isomerization tendencies of these compounds are among the principal factors that cause spoilage of essential oils during conventional extraction operations. Therefore, supercritical-fluid extraction, which can be accomplished effectively at mild conditions, offers a significant advantage over the conventional methods.

It is known that the quality of essential-oil products decreases with increasing fraction of the monoterpene hydrocarbons. Decomposition of these unstable hydrocarbons generates compounds which impair the quality of the

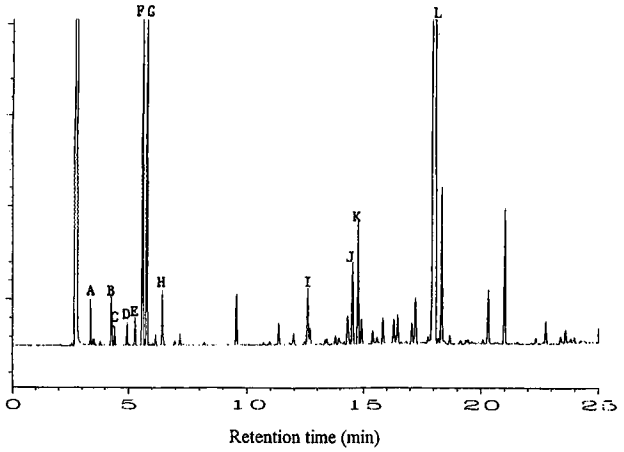


Figure 4 — Chromatogram of the Spearmint Oil Obtained via Hydro-Distillation.

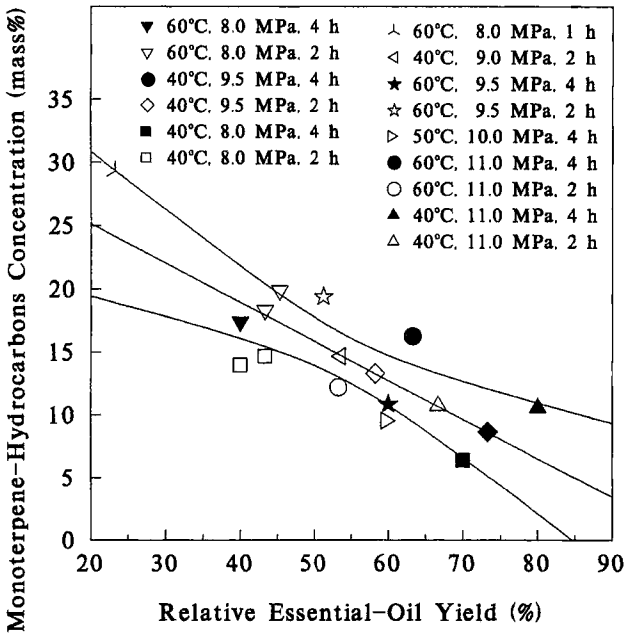


Figure 5 — Correlation between the Total Concentration of the Monoterpene-Hydrocarbons and the Relative Essential-Oil Yield (the lines show linear correlation and its 99% confidence limits).

essential-oil products. On the other hand, the real aroma of essential oils is due to oxygenated hydrocarbons. Figure 5 shows the correlation (with 99% confidence limits) between the relative essential-oil yields and the corresponding concentrations (normalized area counts) of the monoterpene hydrocarbons in the spearmint-oils obtained via supercritical extractions. The highest concentration of the monoterpene hydrocarbons corresponds to lowest relative essential-oil yield obtained in 1-h extraction period and lowest concentrations correspond to highest oil yields obtained in 4-h extractions. Table 3 shows the composition of the oil extracted by CO_2 (from the plant that yielded 1.50 cm^3 oil in hydro distillation) at 40°C / 8 MPa / 4 h conditions which resulted in an extract with lowest concentration of the monoterpene hydrocarbons. This behavior can be explained by the fact that the monoterpene hydrocarbons, being the most volatile substances among the key components (Table 3 shows the vapor pressures of the components), are extracted preferentially with most ease (Stahl et al., 1987; Platin et al.,

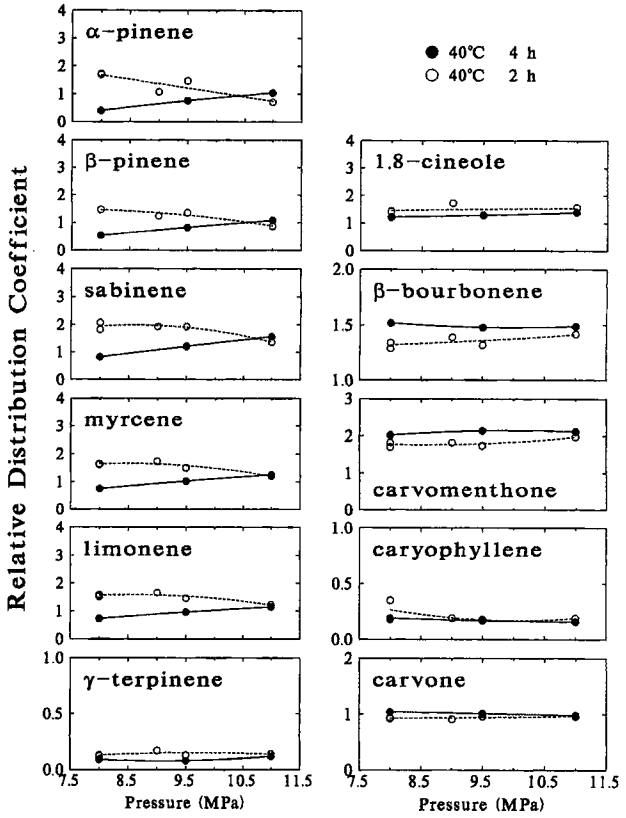


Figure 6 — Pressure and Extraction-Time Dependence of the Relative Distribution Coefficients of the Key Components at 40°C.

1994). It should be understood that the samples collected in supercritical extraction experiments are not instantaneous but integral samples. Therefore, the fraction of the monoterpene hydrocarbons in the samples collected in long-time (high-yield) extractions decreases as their amount in the mint leaves decreases with time. Concentration of harder-to-extract substances (oxygenated monoterpenes and sesquiterpene hydrocarbons; due to their higher mass and/or polarity) in the integral samples thus increases with time. This conclusion suggests possible feasibility of a two-stage supercritical extraction; a short-time extraction conducted at higher temperature and lower pressure (Platin et al., 1994) to remove the undesired monoterpene hydrocarbons from the plant structure followed by a long-time extraction conducted at higher temperature and higher pressure to obtain a spearmint-oil extract possibly free of monoterpene hydrocarbons.

Figure 6 and Figure 7 show the pressure and extraction-time dependence of the relative distribution coefficients of the key components (except α-terpinene which appeared only in trace amounts) in the spearmint oils obtained via supercritical-CO_2 extraction at 40 and 60°C, respectively. Relative distribution coefficient, R_i, of a key component is defined as: $R_i = C_i^e / C_i^d$, where C_i^e is the normalized concentration (normalized area count) of key component i in the spearmint oil obtained through supercritical-CO_2 extraction, and C_i^d is the normalized concentration (normalized area count) of key component i in the spearmint oil obtained through hydro-distillation in the Clevenger apparatus. Hence, an R_i value greater than unity indicates that concentration of component i in the spearmint oil extracted with supercritical CO_2 is higher than that in the spearmint oil obtained via hydro-distillation, or vice versa.

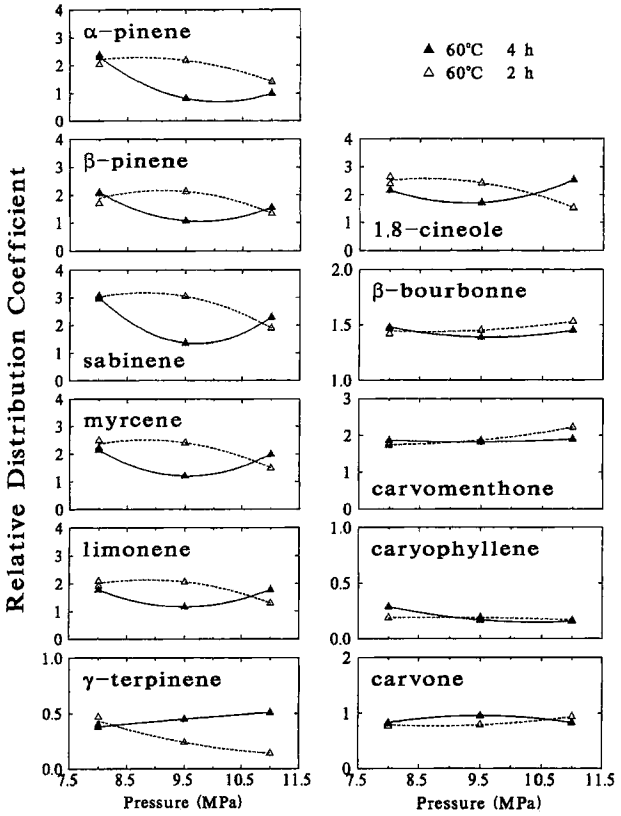


Figure 7 — Pressure and Extraction-Time Dependence of the Relative Distribution Coefficients of the Key Components at 60°C.

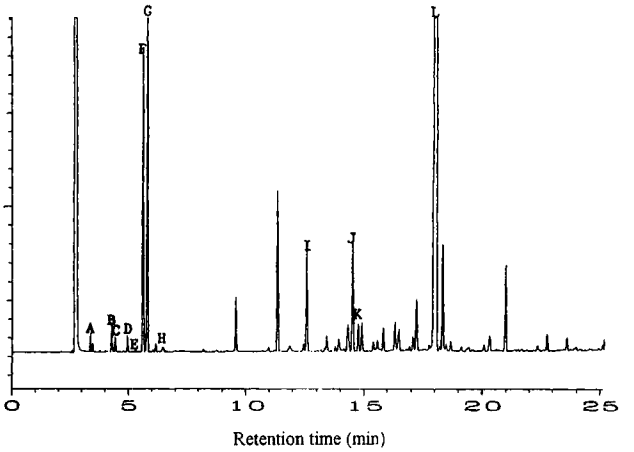


Figure 8 — Chromatogram of the Spearmint Oil Extracted at 40°C / 8 MPa / 4 h Conditions.

At 40°C (Figure 6), the monoterpene hydrocarbons, with the exception of γ-terpinene, exhibit similar pressure dependence. For short-time extractions at lower pressures, relative distribution coefficients of the monoterpene hydrocarbons (except γ-terpinene) are the highest among those of the other key components. This is because these compounds can be extracted earlier with ease by supercritical CO_2 (Stahl et al., 1987) due to their high vapor pressure, low molar mass and low polarity (CO_2 is a non-polar solvent). It is known that, in phase equilibria between spearmint-oil (oil of *Mentha cardiaca*) and CO_2, solubility of the monoterpene hydrocarbons go through maxima due to dominating effect of their high vapor pressures as solvating power of CO_2 goes through minima around either 45°C / 2.7 MPa or 55°C / 3.5 MPa conditions (Platin et al., 1994). Therefore, the monoterpene hydrocarbons can be extracted preferentially even at low pressures (by subcritical CO_2). At 60°C (Figure 7), the monoterpene hydrocarbons, with the exception of γ-terpinene, exhibit similar pressure dependence as well. Compared to the behavior observed in Figure 6, since the vapor pressure effect of the monoterpene hydrocarbons is higher at 60°C, their preferential loading into supercritical phase continues even in long-time extractions at low pressures. The argument based on vapor-pressure effect of the compounds also explains the behavior of 1,8-cineole (an oxygenated monoterpene) which is similar to those of the monoterpene hydrocarbons at both 40 and 60°C; as can be seen in Table 3, its vapor pressure is close to those of the monoterpene hydrocarbons (its boiling point is lower than that of the γ-terpinene).

The spearmint oil obtained in supercritical extraction at both 40 and 60°C, has lower concentration of γ-terpinene (α-terpinene is in trace amounts) and caryophyllene compared to the spearmint oil obtained via hydro-distillation of the mint leaves. Concentrations of the other compounds (carvone, β-bourbonene, carvomenthone) in the spearmint oil obtained in supercritical extraction are either equal to or greater than their concentrations in the hydro-distillation product. Since, γ-terpinene and α-terpinene are also monoterpene hydrocarbons, and caryophyllene should have vapor pressure, molar mass and polarity values close to those of β-bourbonene (as both being sesquiterpene hydrocarbons), the lower relative distribution coefficients of γ-terpinene, α-terpinene and caryophyllene suggest that these compounds may be contained in the hard-to-reach (for supercritical CO_2) regions of the cellular structure of the mint plant, and/or, that they may be associated with hard-to-extract substances contained within the cellular structure.

It is seen also from Figure 6 that the spearmint oil with the lowest monoterpene-hydrocarbons fraction was obtained at 40°C / 8 MPa / 4 h extraction conditions (Figure 8 shows the chromatogram of this essential-oil extract). As can be seen in Figure 5, the relative essential-oil yield corresponding to these operating conditions is among the highest yields of supercritical extraction. Therefore, in designing supercritical-fluid-aided essential-oil extractors and in optimization of its operating policies, the trade-off between essential-oil yield and monoterpene-hydrocarbons fraction should be prudently taken into consideration.

Based on the results of this work, the optimum of the operating conditions for a single-cycle supercritical extraction of the spearmint oil (low in monoterpene-hydrocarbons fraction) from mint leaves are at 40°C / 8 MPa / 4 h. However, a two-cycle supercritical extraction of the mint leaves may be more feasible: a short pre-treatment of the plant by supercritical extraction at 60°C / 8 MPa / 2 h conditions to remove the undesired monoterpene hydrocarbons from the plant structure followed by extraction at 40°C / 8 MPa / 4 h conditions to obtain a spearmint-oil extract possibly free of monoterpene hydrocarbons. The use of multi-stage separators with temperature and/or pressure programming coupled with optimum extraction conditions may significantly enhance product quality. For example, Reverchon (1992) demonstrated that via fractional separation (two separators in series, operating at different temperature and pressure) of co-extracted essential-oil components and cuticular waxes, it was possible to obtain high-quality (wax-free) essential oils from marjoram leaves.

Unfortunately, due to some problems encountered in the sample-collector part of the set-up, spearmint oils obtained in extractions with pure CO_2 and with CO_2-ethanol mixture cannot be compared in terms of their compositions. However, since the same problem was present in both of the entrainer-aided extractions, the effect of entrainer concentration on composition of the extracts can be discussed (Özer, 1993). When the concentration of ethanol in the solvent phase increased from 1.5 to 3.0 mol%, concentrations of α-pinene, β-pinene, sabinene, myrcene, limonene, 1,8-cineole, and β-bourbonene showed an average increase of about 50%, no significant change was observed for carvomenthone, but concentrations of caryophyllene and carvone showed about 10% decrease. Similar co-solvent effect has been reported in the literature in extraction of pepper using supercritical CO_2 as solvent and ethanol as entrainer (Duboc et al., 1988).

Acknowledgements

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