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Recovery of Pigments from *Origanum majorana* L. by Extraction with Supercritical Carbon Dioxide

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Extraction of pigments (chlorophylls and carotenoids) from marjoram (*Origanum majorana* L.) with supercritical carbon dioxide was investigated. The aim of this study was to map the effects of extraction pressure and temperature on the yield of coloring materials by applying a 3^2 full factorial design with three repeated tests in the center of the design. For comparison, laboratory and pilot plant Soxhlet extractions were carried out using ethanol and *n*-hexane solvents. The compositions of pigments in marjoram extracts were determined by HPLC. Similar amounts of carotenoids, in addition to 40% of chlorophylls and their derivatives, were recovered from the supercritical fluid extraction, in comparison to the ethanol Soxhlet extraction.

KEYWORDS: Supercritical fluid extraction; marjoram (*Origanum majorana* L.); chlorophylls; carotenoids

INTRODUCTION

Marjoram, *Origanum majorana* L., is a tender perennial herb of the mint family (Lamiaceae or Labiatae), which was formerly classified as *Majorana hortensis* Moench. Marjoram is commonly used as a herb culinary applications. Because marjoram has been known to possess medicinal effects, it can be used in the industries of cosmetics or pharmaceuticals (*1*−*3*). The plant has been noted to exhibit antioxidant and antifungal properties (*4*, *5*).

The herb (*Majoranae herba*) contains essential oil (∼1%), bitter compounds, a large amount of tannin acids (rosmarinic acid, chlorogenic acid, and caffeic acid), flavonoids, ursolic and oleanolic acids, and waxes as well (*6*−*9*).

The color of plants is the result of the presence of chlorophyll (green) and carotenoids (yellow). Chlorophyll A is the most common type of pirrols and accounts for ∼75% of the total chlorophyll. Chlorophyll B is an accessory pigment. Pheophytin A is formed from chlorophyll A with the loss of magnesium. The term carotenoid represents a wide range of chemicals, which include two major groups of pigments: carotene and xanthophylls. β-Carotene is the precursor for vitamin A. Lutein, the main xanthophyll, represents ∼45% of the carotenoids present in plant leaves (*10*, *11*).

The extraction of the active ingredients of herbs has called for a gentle technique that does not pollute and damage these biologically active compounds. Supercritical fluid extraction (SFE) complies with these requirements. For this reason, the current applications of supercritical fluids (SCF) include cleanings, coatings, extractions, impregnations, particle formation, reactions, and separations. Carbon dioxide is probably the most widely used SCF solvent. Its critical temperature (31 °C) makes it an ideal solvent for extracting thermally labile materials, and it eliminates from the extract after extraction. CO_2 is also nontoxic, nonflammable, environmentally acceptable, and inexpensive. These properties of SFE make the products more advantageous in the fields of foods, pharmaceuticals, and cosmetics.

SFE has been proposed for the extraction of essential oil (*12*−*14*) and for the investigation of the recovery of lipids (*15*) from marjoram leaves. A process for the extraction and separation of marjoram essential oil from waxes using SFE has been reported (*16*, *17*). References of the recovery of chlorophylls and carotenoids from grass by SFE have been found with the aim of using the oil soluble color extract as a food additive. SC-CO_2 extraction was carried out at pressures in the range of 300−500 bar and at temperatures in the range of 50−60 °C. During the extraction water was subsequently added as an entrainer to the SC-CO_2. The total yield was 1.56 wt %, and the extract contained mainly pheophytins, chlorophylls, and lutein (*18*, *19*). Numerous references are presented for the isolation of carotenoids from natural plants by supercritical fluid carbon dioxide extraction applying ethanol (*20*) and chloroform solvent (*21*) as the modifier or without added entrainer (*22*−*24*).

The literature lacks precise information on the extraction of pigments from marjoram by supercritical carbon dioxide. Therefore, the aim of the present work was to examine the effect of extraction conditions (pressure and temperature) on the yield, to reveal the pigment compositions of marjoram extracts, and to evaluate the possibility of the SC-CO_2 extraction of pigments. The composition of the SFE product was compared with that of hydrodistilled oil and extracts obtained by solvent extractions.

EXPERIMENTAL PROCEDURES

Materials. The dried, finely ground marjoram sample was obtained from Kalocsa, Hungary. The raw material was a grayish brown fine powder with characteristic scent. This was used for all of the extractions.

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The moisture content of the dried marjoram was 12.07 ± 0.62% (w/w). The CO_2 used was 99.5% (w/w) pure and supplied by Messer Griesheim Hungaria. Reagent grade *n*-hexane and ethyl alcohol were used for conventional Soxhlet extractions. Analytical grade reagents (Reanal) were used for chemical analysis. β-Carotene was identified and quantified by using the standard pigment (95% purity from Sigma, St. Louis, MO).

Methods. Standard methods described in the Hungarian Pharmacopoeia Ed. VII. were applied for the determination of the essential oil (by hydrodistillation), oleoresin (by hexane or ethanol Soxhlet extraction), and the moisture content of marjoram samples. A Soxhlet extraction was also carried out in a pilot plant apparatus using 96% ethanol as a solvent.

Supercritical fluid extraction was carried out in a high-pressure apparatus equipped with a 5 L volume extractor vessel and two separators connected in series. A more detailed description of the apparatus and extraction is given extensively elsewhere (*25*). Liquid CO_2 is compressed to a desired pressure by means of a pump and heated to a specified extraction temperature in order to bring it into the supercritical state before it is passed into the extraction vessel filled with the plant material. The solution leaves the extractor and through a pressure-reducing valve flows into the first separation vessel. The pasty SFE product settles at the bottom and can be collected and weighed. The solution is passed into the second separator, where the CO_2 is evaporated and the SFE product containing the mostly volatile compounds are recovered. A control system is placed just before the pump to measure the solvent flow rate. Samples of 1000 g of the plant material were weighed accurately and put into the extraction vessel. The desired temperature and pressure were adjusted, and the CO_2 feed was started. The accumulated product samples were collected and weighed at certain time intervals.

Analytical Methods. From the extract samples, ~0.1 g was weighed accurately and dissolved in the appropriate solvent with two parallel samples. An acetonitrile/methanol/isopropyl alcohol (39:43:18, v/v/v) mixture was used for the experiments. The samples were analyzed after they had been filtered through filtering paper to separate the undissolved and segregated compounds (mainly proteins).

The dissolved samples (20 μL) were injected into a Beckman liquid chromatograph equipped with a model 114 M solvent-delivery module pump, a model 340 organizer, and a model 166 UV−vis detector. The detector signals were recorded with Beckman Gold P/ACE 5000 System software.

Separations were performed on a Nucleosil 5 C_{18} stainless steel column (250 mm × 4 mm i.d.). The mobile phase was acetonitrile/methanol/isopropyl alcohol (39:43:18, v/v/v). The flow rate was 0.9 mL/min. Detection was carried out at a wavelength of 430 nm.

To identify and quantify β-carotene, a standard sample was injected and detected with the above-mentioned system. To identify chlorophyll and carotenoid-type pigments and their derivatives, authentic standards were applied taken from special previous experiment (*11*). The different pigment components were separated on cellulose thin layer plates, developed with *n*-hexane/pyridine (7:3, v/v) (*26*). Each pigment was scraped off the TLC plates and eluted in suitable organic solvent to measure their spectrums. The quantitative determination was carried out by spectophotometer using different solvents and extension coefficients (*27*). The identification of pigments was accomplished by direct scanning of the spectrum and comparison of the data with those mentioned in the literature (*11*, *26*).

RESULTS AND DISCUSSION

Influence of Extraction Conditions on Recovery. The effects of the temperature and pressure of the extractor on the yield were examined by employing a 3^2 full factorial design with three repeated tests in the center of the design. The three levels of the temperature were 40, 50, and 60 °C, whereas those for the pressure were 100, 250, and 400 bar. The dependent variable was the extraction yield, expressed in mass ratio of the extract to the starting dried material [Y = g of extract/100 g of dried material (dm), %]. During the experiments the pressures of separators 1 and 2 were kept on stable values (40 and 20 bar, respectively).

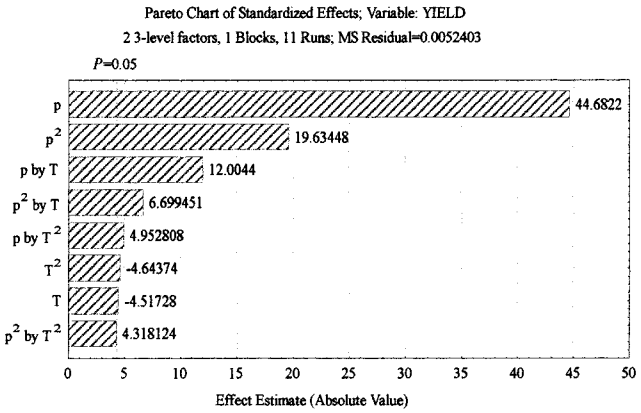

Figure 1. Pareto chart of SFE yield.

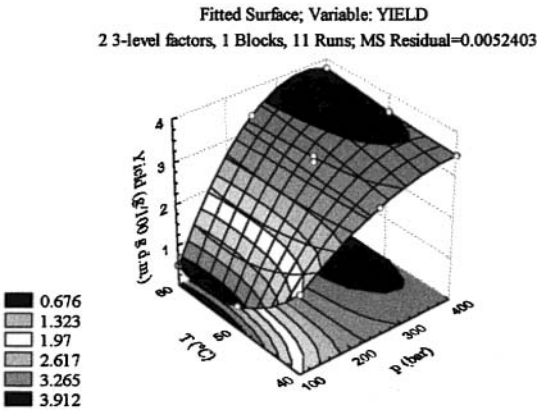

Figure 2. Three-dimensional fitted surface of SFE yields.

The effects of temperature and pressure were calculated by Statistica for Windows software (*28*). The estimated effects of the terms are demonstrated in the form of a Pareto chart (**Figure 1**) giving also the sign of the effects. The vertical dashed line, titled $P = 0.05$, gives the critical limit for significance level $P = 0.05$. Effects that have a larger absolute value than this limit are qualified as significant.

It is apparent that the pressure (p) terms (linear [p] and quadratic [p^2]) are highly significant. It is also apparent that the interaction between the linear term of temperature (T) and that of pressure [T by p] is significant as well. The linear and quadratic terms of temperature and the interactions between [T by p^2], [T^2 by p], and [T^2 by p^2] are not as significant, because the values in the rows of the above-mentioned terms are relatively lower than the values of [p], [p^2], and [T by p].

The three-dimensional response surface plot fitted to the experimental results is shown in **Figure 2**. It is seen that both the pressure and temperature of the extractor affect the yield. The curved surface in the [p] variable reflects the quadratic pressure dependence, which gives an optimal pressure within the experimental region. Due to the [T by p] interaction the surface is slightly twisted. At higher pressure ($p \geq 300$ bar) increasing the temperature produces a higher yield, whereas at lower pressures ($p \approx 100$ bar), the effect is opposite. The character of the extracts was dissimilar according to the applied conditions of SFE. At lower pressures mainly the volatile compounds were recovered, which were responsible for the scent of marjoram. At higher pressures mostly waxy compounds were achieved, containing pigments and other biological active compounds.

Table 1. Extraction Yields of Marjoram

run SFE	temp (°C)	pressure (bar)	yield (g/100 g of dm)	mg/100 g of dm chlorophyll A	pheophytin B	β-carotene	lutein
8	40	100	1.91	0.22	0.42	0.11	0.13
7	40	250	3.07	0.78	5.11	1.31	1.64
5	40	400	3.48	1.11	14.20	3.80	3.67
9	50	100	0.54	0.00	0.26	0.03	0.04
1	50	250	3.29	0.79	4.76	1.98	1.82
6	50	250	3.31	0.83	6.77	2.17	2.12
11	50	250	3.18	0.67	4.80	2.10	1.73
2	50	400	3.60	1.88	20.07	5.08	5.05
16	50	450	3.65	2.72	32.38	6.06	5.67
10	60	100	0.50	0.00	0.19	0.07	0.06
4	60	250	3.36	0.86	7.91	1.37	1.55
3	60	400	3.80	2.32	28.80	5.52	5.65
16, Sep1			2.92	2.71	31.99	6.06	5.64
16, Sep2			0.73	0.01	0.39	0.00	0.03
Soxhlet extraction with *n*-hexane			4.99	4.87	27.46	5.58	6.92
Soxhlet extraction with ethanol			13.36	6.02	196.33	9.49	9.54
pilot plant extraction with ethanol			9.07	3.65	201.07	2.88	11.15

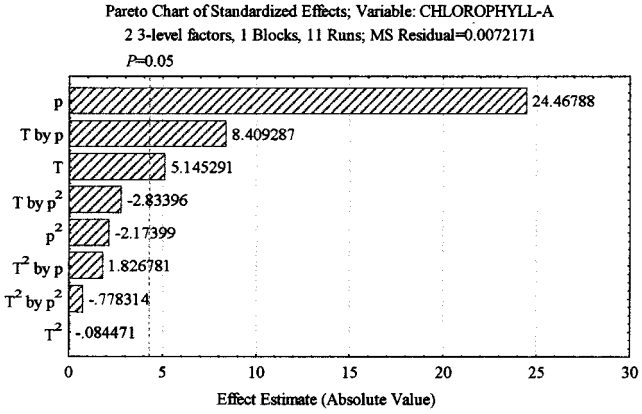

Figure 3. Effect of the extraction pressure on chlorophyll A yield.

Three SFE experiments were carried out with fractional separations at 450 bar pressure and 50 °C temperature. The pressure of the separators was kept at 78−80 bar (Sep1) and 20 bar (Sep2). Slightly higher total yield (3.66 ± 0.55 g/100 g of dried solid) was obtained compared to the SFE at 400 bar and 50 °C. Applying fractional separation, dark, brownish green wax (2.90 ± 0.65 g/100 g of dried solid) was obtained from Sep1; a yellow, marjoram-scented, oily product (0.76 ± 0.17 g/100 g of dried solid) was obtained from Sep2.

Quantitative Determination of Chlorophylls and Carotenoids. The detection of chlorophylls and their derivatives (pheophytins) and the carotenoids in marjoram extracts was achieved by HPLC. The yields of chlorophyll A, pheophytin B, and the carotenoids can be found in **Table 1**. The influence of the extraction conditions (pressure and temperature) on the yield of a particular pigment compound as dependent variable was also investigated. In the case of chlorophylls and pheophytins, the linear pressure term [*p*] had a high significant effect on the pigment yields (similar to its effect on the yield of the extract). The interaction between the pressure linear term and temperature linear term [*p* by *T*] as well as the effect of the linear term of temperature [*T*] is also significant. The chlorophyll and pheophytin compounds showed similarity; therefore, it is sufficient to show the Pareto chart of chlorophyll A (**Figure 3**). In **Figure 4**. the three-dimensional response surface of pheophytin B yield shows the largest yield at the high [*p*] high [*T*] corner. The surface of the chlorophyll A plot is analogous. It can be concluded that higher pressure and temperature of the extractor during SFE magnified the amounts of chlorophylls and pheophytins. The achieved amounts of green pigments can be increased by increasing extraction pressure, instead of by increasing the temperature. The effect of temperature on yield is significant only at a high pressure of extraction.

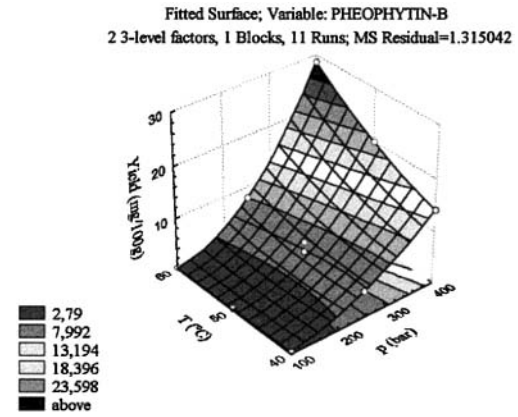

Figure 4. Three-dimensional plotted surface of pheophytin B.

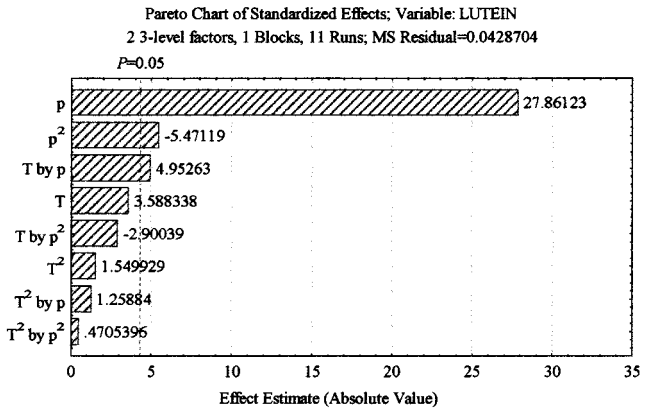

Figure 5. Effects of the extraction conditions on lutein recovery.

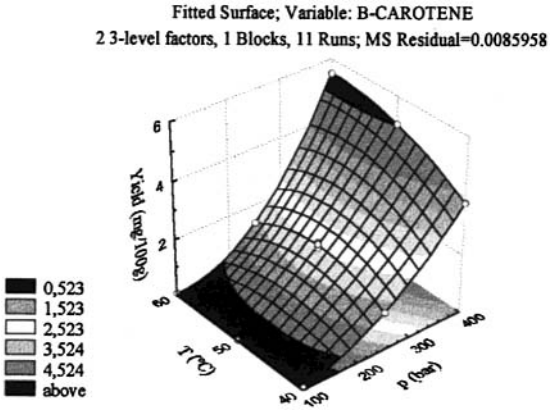

Figure 6. Effects of temperature and pressure on the plotted surface of β-carotene.

The estimated effects on lutein yield are seen in the Pareto chart (**Figure 5**). For β-carotene the Pareto chart is similar. It is apparent that the linear [*p*] and quadratic [p^2] terms of pressure have significant effects on yields of lutein and β-carotene. According to the three-dimensional response surface plot fitted to the analytical results of β-carotene (**Figure 6**), the maximum yield of carotenoids can be achieved by using high pressure and high temperature (400 bar and 60 °C).

Comparison of Different Extraction Methods. The hydrodistillation and Soxhlet extractions with ethanol, *n*-hexane

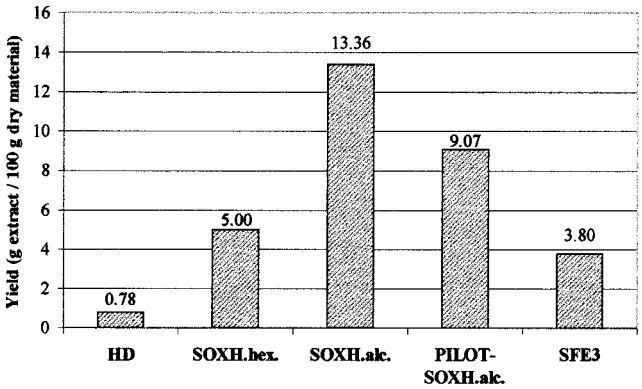

Figure 7. Comparison of extraction yield obtained by different methods.

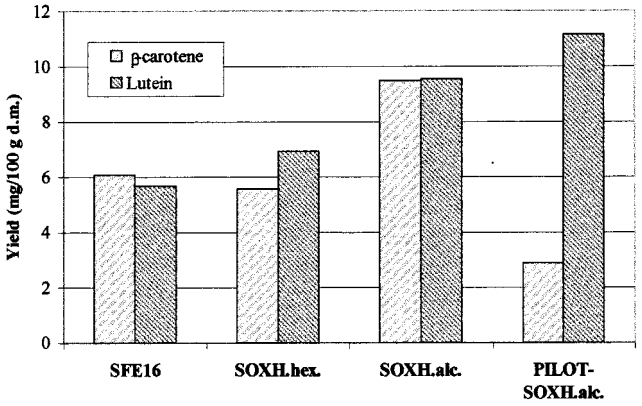

Figure 8. Comparison of carotenoids obtained by different methods.

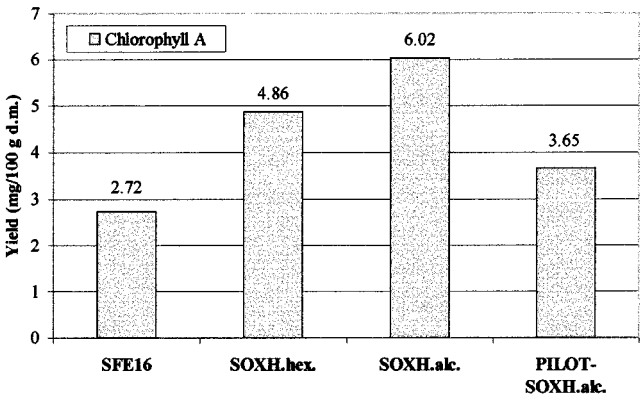

Figure 9. Recovered amounts of chlorophyll A according to the applied methods.

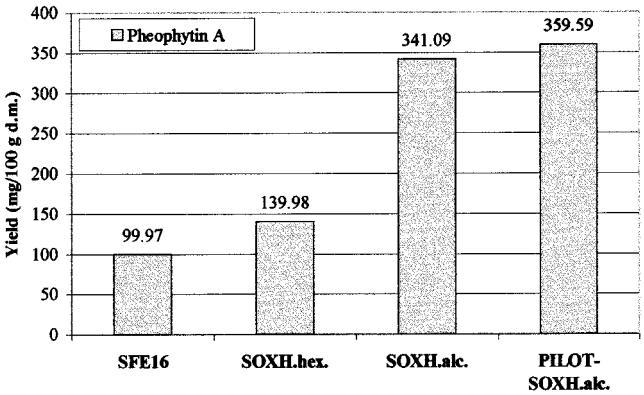

Figure 10. Achieved amounts of pheophytin A isolated by the applied methods.

solvents, and SFE were employed to extract the main volatile and nonvolatile compounds from the marjoram herb. When these methods were applied, different yields were obtained, which mainly depend on the solution power of the solvents. These yields, obtained by different methods, can be seen in **Figure 7**. It can be observed that the hydrodistillation, which is traditionally used for obtaining volatile compounds from plant materials, possesses a smaller amount of the product, although this product contains only essential oil and its main compounds. The largest amount of extract was obtained by ethanolic Soxhlet extraction, which can be explained with the good solvent property of ethanol and its polar characteristic. Ethanol dissolves a large amount of ballast material, which has to be separated from the beneficial compounds. The extract obtained through SFE is less than that obtained through ethanolic extractions, but the SFE extract is free from solvent residual and contains only the fluid CO_2 dissolved lipophilic compounds. For comparison the SFE extract contains a considerable amount of β-carotene (166.17 mg/100 g of extract), whereas in the extract obtained by ethanol the amount of β-carotene is less than half of that (71.05 mg/100 g).

Comparison of Pigments According to the Applied Extraction Methods. Different amounts of each pigment were determined, according to the applied extraction methods. Most of the pigments were found in the samples obtained by alcoholic Soxhlet extraction applying laboratory and pilot plant apparatus. In the samples obtained by Soxhlet extraction with hexane, the amounts of the examined pigments were compared to those in the extracts obtained by SFE. When SC-CO_2 was applied, the highest amounts of pigment compounds were extracted at 450 bar pressure and 50 °C temperature (SFE16).

The amounts of lutein and β-carotene (**Figure 8**) in extracts obtained by SFE (5.67 mg of lutein/100 g of dm and 6.06 mg of β-carotene/100 g of dm) were similar to those in extracts obtained by Soxhlet extraction with hexane (6.92 mg of lutein/100 g of dm and 5.58 mg of β-carotene/100 g of dm). The amount of β-carotene achieved by pilot plant Soxhlet extraction was surprisingly low despite the numerous repetitions. In addition, the beginning of the HPLC profiles in the samples obtained by ethanolic extraction contained more compounds, which assumed the presence of polar xanthophylls.

Among the examined chlorophylls, chlorophyll B was found in higher amount (53.91 mg/100 g of dm) in the extract obtained by ethanolic extraction. When SFE at 450 bar and 50 °C was applied, the maximum amount of chlorophyll B (4.25 mg/100 g of dm) was determined. The highest amount of chlorophyll A (6.02 mg/100 g of dm) was also obtained by ethanolic extraction. The determined amounts of chlorophyll A can be found in **Figure 9**.

Outstanding amounts of the pheophytins were found, especially in the extracts obtained by ethanolic Soxhlet extraction. A comparison of the amounts of pheophytin A can be seen in **Figure 10**. Alcoholic laboratory and pilot plant Soxhlet extractions resulted in significant amounts of pheophytin A (341.09 and 359.59 mg/100 g of dm). Only the third part of these amounts was obtained by applying SFE. In the case of pheophytin B the yields of the different extraction methods were in relation to the amounts of pheophytin A. The highest amount of pheophytin B was 201.07 mg/100 g of dm obtained by alcoholic pilot plant Soxhlet extraction. In the samples of SFE only 32.38 mg of pheophytin B/100 g of dm was found.

Finally, in the case of samples obtained by fractionated SFE, pigments mainly were found in the samples of Sep1. Chlorophyll

pigments were not found in significant amounts in the samples from Sep2.

The experimental results indicated that the aroma from marjoram leaves could be efficiently extracted by SFE. The extracts possess all of the characteristics of the natural herb including its main volatile compounds, which are responsible for the scent and flavor. The pigment analysis revealed the main pigments and their amounts in the marjoram extracts. The amounts of chlorophylls and carotenoids can be enhanced with the optimization of the extraction conditions (pressure and temperature). Further experiments are required to estimate the usage of these supercritical fluid extracts in the areas of foods, cosmetics, and pharmaceuticals.

ABBREVIATIONS USED

SFE, supercritical fluid extraction; SCF, supercritical fluids; SC-CO_2, supercritical carbon dioxide; ANOVA, analysis of variance; *P*, value belongs to the *F*-test statistic during ANOVA; *T*, extraction temperature (°C); *p*, extraction pressure (bar); Sep1, first separator; Sep2, second separator; HD, hydrodistillation; SOXH.hex, Soxhlet extraction with *n*-hexane; SOXH.alc, Soxhlet extraction with ethanol; PILOT-SOXH.alc, pilot plant Soxhlet extraction with ethanol; SFE3, supercritical fluid extraction with carbon dioxide at 400 bar pressure and 60 °C temperature; SFE16, supercritical fluid extraction with carbon dioxide at 450 bar pressure and 50 °C temperature; dm, dry material.

LITERATURE CITED

(1) Bauer, K.; Garb, D.; Surburg, H. Natural Raw Materials in the Flavor and Fragrance Industry. *Common Fragrance and Flavor Materials*; VCH Press: Weinheim, Germany, 1990; p 163.

(2) Price, S. *Aromatherapy Workbook*; Thorsons Press: London, U.K., 1995; pp 107−109.

(3) Vera, R. R.; Chane M. J. Chemical composition of the essential oil of marjoram (*Origanum majorana* L.) from Reunion Island. *Food Chem.* **1999**, *66*, 143−145.

(4) Daferea, D. J.; Ziogas, B. N.; Polissiou, M. G. GC-MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on *Penicillium digitatum*. *J. Agric. Food Chem.* **2000**, *48*, 2576−2581.

(5) Deans, S. G.; Svoboda, K. P. The antimicrobial properties of marjoram (*Origanum majorana* L.) volatile oil. *Flavour Fragrance J.* **1990**, *5*, 187−190.

(6) Rápóti, J.; Romváry, V. *Pharmaceutical Plants*; Medicine Press: Budapest, Hungary, 1977; pp 213−214.

(7) Pino, J. A.; Rosado, A.; Estarron, M.; Fuentes, V. Essential oil of marjoram (*Origanum majorana* L.) grown in Cuba. *J. Essent. Oil Res.* **1997**, *9*, 481−482.

(8) Lawrence, M. B. Progress in essential oil: Marjoram oil. *Perfum. Flavor*. **1994**, *19*, 39−40.

(9) Baser, K. H. C.; Kirimer, N. Composition of the essential oil of *Origanum majorana* L. from Turkey. *J. Essent. Oil Res.* **1993**, *5*, 577−579.

(10) Törley, D. *Food Chemistry and Classification*; Müegyetem Press: Budapest, Hungary, 1999; pp 72−98.

(11) Daood, G. H.; Czinkotai, B.; Hoschke, Á.; Biacs, P. High-performance liquid chromatography of chlorophylls and carotenoids from vegetables. *J. Chromatogr*. **1989**, *472*, 296−302.

(12) Koller, W. D. Einfluss der Extraktionsbedingungen bei der CO_2-Hochdruckextraktion auf die Zusammensetzung des Kopfraumgases und den Geruch der Extrakte aus gerebeltem Majoran. *Lebensm.-Wiss. -Technol.* **1986**, *19*, 56−58.

(13) Quirin, K.-W.; Gerard, D. Hochdruckextraktion von Gewürzen. *ZFL* **1990**, *11*, 731−734.

(14) Nitz, S.; Kollmannsberger, H.; Punkert, M. CO_2-Hochdruckextraktion von Gewürzen. *Chem. Mikrobiol. Technol. Lebensm.* **1992**, *14*, 108−116.

(15) Tietz, U.; Meusel, D.; Tschirnich, R. Untersuchungen zur Extraktion von Lipiden des Marjorans (*Majorana Hortensis* M.) mit fluidem Kohlendioxid. *Chem. Mikrobiol. Technol. Lebensm.* **1991**, *13*, 153−159.

(16) Reverchon, E. Fractional separation of SCF extracts from marjoram leaves: mass transfer and optimization. *J. Supercrit. Fluids* **1992**, *5*, 256−261.

(17) Reverchon, E. Supercritical fluid extraction and fractionation of essential oils and related products. *J. Supercrit. Fluids* **1997**, *10*, 1−37.

(18) Mukhopadhyay, M. Natural Food Colors. *Natural Extracts Using Supercritical CO_2*; CRC Press: Bombay, India, 2000; pp 254−255.

(19) Jay, A. J.; Steytler, D. C.; Knights, M. Spectrophotometric studies of food colors in near critical CO_2. *J. Supercrit. Fluids* **1991**, *4*, 131−141.

(20) Careri, M.; Furtattini, L.; Mangia, A.; Musci, M.; Anklam, E.; Theobald, A.; von Holst, C. Supercritical fluid extraction for liquid chromatographic determination of carotenoids in Spirulina Pacifica algae: a chemometric approach. *J. Chromatogr. A* **2001**, *912*, 61−71.

(21) Chandra, A.; Nair, M. G. Supercritical fluid carbon dioxide extraction of α- and β-carotene from carrot (*Daucus carota* L.). *Phytochem. Anal.* **1997**, *8* (5), 244−246.

(22) Franca, F. L.; Reber, G.; Meireles, A. A. M.; Machado, T. N.; Brunner, G. Supercritical extraction of carotenoids and lipids from buriti (*Mauritia flexuosa*), a fruit from the Amazon region. *J. Supercrit. Fluids* **1999**, *14*, 247−256.

(23) Jarvis, A. P.; Morgan, D. Isolation of plant products by supercritical fluid extraction. *Phytochem. Anal.* **1997**, *8* (5), 217−222.

(24) Modey, W. K.; Mullholland, D. A.; Raynor, M. W. Analytical supercritical fluid extraction of natural products. *Phytochem. Anal*. **1996**, *7* (1), 1−15.

(25) Simándi, B.; Deák, A.; Rónyai, E.; Yanxiang, G.; Veress, T.; Lemberkovics, É.; Then, M.; Sass-Kiss, Á.; Vámosi-Falusi, Zs. Supercritical carbon dioxide extraction and fractionation of fennel oil. *J. Agric. Food Chem.* **1999**, *47*, 1635−1640.

(26) Sievers, G.; Hynninen, P. Thin-layer chromatography of chlorophylls and their derivatives on cellulose layers. *J. Chromatogr*. **1977**, *134*, 359−364.

(27) Lichtenthaler H. K. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods Enzymol.* **1987**, *148*, 350−382.

(28) *STATISTICA for Windows* (computer program manual); StatSoft, Inc.: Tulsa, OK, 1995; e-mail info@statsoft.com, URL http://www.statsoft.com.

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