Phytochemical Examination of Oils Obtained from the Fruit of Mille Thistle (*Silybum marianum L. Gaertner*) by Supercritical Fluid Extraction

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Fruit of *Silybum marianum L. Gaertner*, Supercritical Fluid Extraction, Fatty Acid Content, α-Tocopherol Content, Element Concentration

Supercritical fluid extraction (solvent: CO_2 and propane) was used for mild recovery of fatty oil of mille thistle fruit (*Silybum marianum L. Gaertner*). Fatty acid compounds (palmic acid, oleic acid, linolic acid, linolenic acid, behenic acid), coloring content (expressed in pheophytin and carotene), tocopherol content and concentrations of some metals (Al, As, B, Ba, Ca, Cd,Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, Pb, S, Ti, V, Zn), boron and sulfur were determined from oils and compared with oil obtained by Soxhlet extraction. There is significant difference between supercritical oil and traditional oil in the quantity of bioactive compounds and elements. The ratio of bioactive components and elements of oils changed during the extraction. Supercritical oils have higher concentration of C18:1 oleic acid, C18:3 linolenic acid, C20:0 and C22:0 behenic acid than the oil gained by Soxhlet extraction. The oil obtained by supercritical fluid extraction at 80 bar and 25 °C with propane has the highest coloring content. Our paper is the first report to be published on element concentrations of mille thistle oils. Accumulation of some heavy metals can be observed in almost each oil sample.

Introduction

Several different investigations have been made on mille thistle (*Silybum marianum L. Gaertner*), which belongs to the *Compositae* = *Asteraceae* family and is still in the focus of interest today. Flavanolignans (silybin, silychristin, silydianin), bioactive compounds of the fruit (*Cardui mariae fructus*), are applied as hepatoprotective agents in liver therapy (Salmi and Sarna, 1982; Sonnenbichler and Zetl, 1988; Mourelle and Franco, 1991). The fruit contains other valuable compounds as betaine, amino acids (L-cysteine, L-glutamic acid, DL-leucine) (Varma *et al.,* 1980), a large number of fatty acids (linolic acid, oleic acid, palmic acid, linolenic acid, behenic acid, stearic acid, arachidic acid) (Kaczmarek and Mrugasiewicz, 1975) and vitamins (tocopherol etc.) as well.

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Mille thistle fruit cannot be pressed mildly. For the extraction of fatty acid Soxhlet extraction by hexane has been used so far. In the present paper supercritical fluid extraction (SFE) was applied for the extraction of mille thistle fruit. The quantity and quality of oil components were investigated and the supercritical oils were compared with the oil obtained by traditional Soxhlet extraction.

Materials and Methods

In our experiment one commercially available (Herbaria Trading Co., 1996) drug sample (*Cardui mariae fructus*), air dried and ground, aged 1 year, was used.

Technical grade carbon dioxide (CO_2) was obtained from Repcelak Gas Trade Co. (Hungary). Highly pure propane (99.9%) was supplied by MÁKFI Institute and hexane by Reanal Chemical Company (Hungary).

Supercritical fluid extraction was carried out in a high pressure through-flow type instrument. The solvent CO_2 and propane were applied at different

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pressures (80–400 bar) at low temperature (25–55 °C) to obtain oils rich in unsaturated fatty acids. Seven supercritical fluid extractions were made and fractions were separated during the extractions. The fractions were separated as a function of CO_2 consumption (at every third g CO_2/g fruit). The fractions were named in alphabetical order. The oil fractions were measured by weight and the rate of extractions were investigated. Then the fractions were partly or completely mixed:
sample number 1 from A to N contain 14 fractions: A, B, C, D, E, F, G, H, I, J, K, L, M, N,
sample number 2 from A to G contain 7 fractions: A, B, C, D, E, F, G,
sample number 3 from A to E contain 5 fractions: A, B, C, D, E,
sample number 4 from A to E contain 5 fractions: A, B, C, D, E,
sample number 5/1 from A to C contain 3 fractions: A, B, C,
sample number 5/2 from D to G contain 4 fractions: D, E, F, G,
sample number 5/3 from H to K contain 4 fractions: H, I, J, K,
sample number 6 from A to E contain 5 fractions: A, B, C, D, E,
sample number 7/1 from A to D contain 4 fractions: A, B, C, D,
sample number 7/2 from E to P contain 12 fractions: E, F, G, H, I, J, K, L, M, N, O, P.

Soxhlet extraction was carried out in Soxhlet equipment according to Hungarian Standard (MSZ 7000–83). The fruit sample (10 g) was extracted with 250 ml hexane for three hours then the solvent was evaporated.

For quantitative analysis of fatty acids gas chromatographic measurements were carried out with a Jeol 1100, equipped with a glass column (2m x 3.4 mm), packed with 3% OV-17 Chromosorb W. Temperature program: maintained at 60 °C, for 2 min, then heated with 8 °C/min. up to 230 °C.

Coloring content of the samples was determined by UV-VIS spectrometry according to the Hungarian Standard (MSZ 3649–85). This Hungarian Standard contains the determination of total carotenoids and pheophytin. For the determination of carotenoid content, the oil samples were diluted with hexane and measured against hexane at 450 nm. The extinction coefficient was given for 1% solution in 1 cm vessel 2505 (100 ml g^{-1} cm^{-1}). Coloring contents of samples for pheophytin were determined the same way at 670 nm, the extinction coefficient was 530 (100 ml g^{-1} cm^{-1}).

Tocopherol content was detected by high pressure liquid chromatography. 100 mg of each sample were saponified by refluxing with saturated methanolic KOH (5 ml) and methanol (20 ml) at the boiling point of methanol for 40 min in the presence of 0.5 g ascorbic acid. After cooling the tocopherols were extracted by gentle shaking in a separatory funnel, with 40 ml petroleum ether. The lower layer was extracted with an additional 40 ml of petroleum ether and the solvent fractions were combined, washed twice with doubly distilled water, dried on anhydrous Na_2SO_4 and evaporated under vacuum. The residues were redissolved in 5–10 ml HPLC grade hexane and injected on the normal phase HPLC column which was eluted with hexane-ethanol (99.5:0.5) as the mobile phase (Speek *et al.* 1985). The separated compounds were detected fluorometrically using a Shimazdu RF-535 fluorescence detector at 295 and 320 nm as the extinction and emission wavelength, respectively. α-, β- and δ-tocopherol were identified and determined for α-tocopherol acetate as total tocopherol content.

Concentrations of the elements of samples were determined by ICP-AES (inductively coupled plasma atomic emission spectrometer). Type of instrument: Atom Scan 25 (Thermo Jarrell Ash), a sequential plasma emission spectrometer. *Sampling*: the samples (0.5 g) were digested with a mixture of HNO_3 (5 ml) and H_2O_2 (3 ml) in teflon vessels. After digestion the samples were diluted to 25 ml, from which the following 23 elements were determined in three parallel measurements: Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, Pb, S, Ti, V, Zn.

Results and Discussion

Supercritical fluid extraction was realized with CO_2 and propane at low temperature (25–55 °C) and in a range of 80–400 bar pressure (Table I). Sample numbers 1, 2, 3 and 4 give the total amount of oil obtained from supercritical fluid extraction, while numbers 5/1, 5/2, 5/3 and 7/1, 7/2, are fractions of the same. The mixed fractions were handled as a sample. The rate of extraction was studied compared with Soxhlet extraction,

Table I. Rates and parameters of extraction.

Samples		Parameter of extraction			Rate of
Sample number	Fractions	Pressure [bar]	Temperature [°C]	Solvent	extraction (%)
1.	A-N	300	35	CO_2	46
2.	A-G	120–400	35	CO_2	14
3.	A-E	120–400	55	CO_2	17.6
4.	A-E	120–400	55	CO_2	26.5
5/1.	A-C	400	35	CO_2	40.5
5/2.	D-G	400	35	CO_2	64
5/3	H-K	400	35	CO_2	98.6
6.	A-E	120–400	40	CO_2	10.9
7/1.	A-D	80	25	C_3H_8	64
7/2.	E-P	80	25	C_3H_8	98.8
Soxhlet			69	hexane	100

Fractions A-N etc. see Materials and Methods.

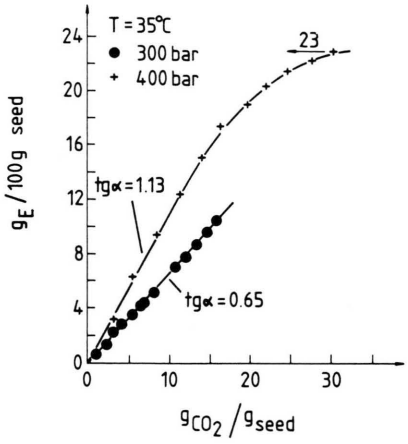

Fig. 1. Supercritical fluid-CO_2 extraction of mille thistle fruit as a function of solvent/solid ratio. Conditions: 35 °C, 300 and 400 bar pressure.

where 100% means 23 g oil from 100 g fruit. Total supercritical fluid extraction was performed at 400 bar pressure at 35 °C (Fig. 1). The points of the curves give the oil amount under the prevailing condition. From the obtained extraction curves, the tangent of the straight phase gives the equilibrum solubility. The values for this parameter in the case of 300 and 400 bar at 35 °C were 0.65 and 1.13 g extract per 100 g of CO_2, respectively. The rate of supercritical fluid extraction depends on pressure as can be seen in Table I (at high pressure the rate of extraction is higher than at low pressure).

On the basis of gas chromatography, fatty oils of mille thistle obtained by supercritical fluid extraction have two main components: C18:1 oleic acid (25.5–29.6%) and C18:2 linolic acid (34.9–44%) similarly to oil obtained by Soxhlet extraction (C18:1 oleic acid: 24%, C18:2 linolic acid: 44%). The oils contain also C16:0 palmic acid (8.2–11.7%), C18:3 linolenic acid (2.5–6.8%) and C22:0 behenic acid (4.3–8.8%) (Table II). There is no significant difference in the composition of supercritical oils or between supercritical oil and traditional oil (Fig. 2), although the ratio (quantities) of bioactive compounds of oils changed depending on the parameters of extraction. The oil obtained by Soxhlet extraction contains C 18:2 linolic acid (44%) and C 18:0 (4.8%) in higher concentration than oil obtained by supercritical fluid extraction, although C18:2 linolic acid content of supercritical oils samples 1 and 3 reach 44%. Supercritical oils have higher concentration of C18:1

Table II. Fatty acid content of oils (%).

Samples	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0
1.	10.8	-	28	44	3.9	1.4	4.3
2.	8.2	-	27	43	4.8	2.2	6.8
3.	10.4	0.06	26	44	4.1	1.3	4.3
4.	11.1	0.1	29.6	35.8	5.1	2.1	5.6
5/1.	10.7	0.3	28.8	36.3	5.1	2.2	5.8
5/2.	8.7	0.03	28	34.9	6.8	3.8	8.8
5/3	8.6	-	26.7	40	5.5	2.7	6.6
6.	11.7	0.1	27.2	37.8	2.5	2.9	8.7
7/1.	8.6	0.2	25.5	39	5.8	2.8	8.7
7/2.	9.1	0.2	26.3	40.7	4.5	2.5	7.5
Soxhlet	10.8	4.8	24	44	3.4	1.2	2.9

C16:0 palmic acid, C18:0 unidentified compound, C18:1 oleic acid, C18:2 linolic acid, C18:3 linolenic acid, C20:0 unidentified compound, C22:0 behenic acid.

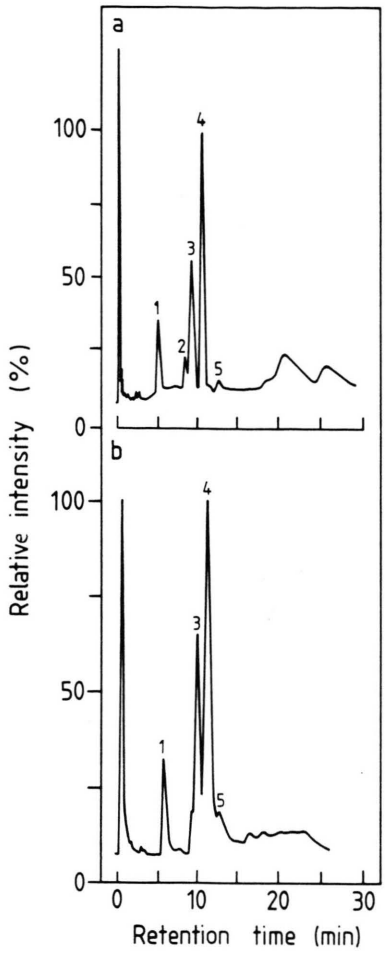

Fig. 2. Gas chromatograms of mille thistle oils obtained by
a. supercritical fluid extraction;
b. Soxhlet extraction.
1, C16:0 palmic acid; 2, C18:0 unidentified compound; 3, C18:1 oleic acid; 4, C18:2 linolic acid; 5, C18:3 linolenic acid.

oleic acid, C18:3 linolenic acid, C20:0 and C22:0 behenic acid than the oil gained by Soxhlet extraction. Linolenic acid is favourable as it significantly decreases blood triglycerrides (Langraf-Leurs *et al.,* 1990). C18:1 oleic acid and C16:0 palmic acid contents of supercritical oils decreased during the extraction (samples 5/1–5/3), while the other compounds changed at random.

Some other biologically important components of oils are: coloring materials and α-tocopherol. The coloring materials of mille thistle oils are carotenoids (mainly xanthophyll derivatives) with yellow color and porphyrin derivatives (eg. pheophytin, wich is the degradation product of chlorofill) with brown color. Carotenoids were determined as total carotenoids and all porphyrin derivatives were measured as pheophytin (Table III). The coloring contents of supercritical oils expressed in pheophytin range between 13.5–159 μg/g, while in total carotenoids: 8.2–58 μg/g. The oil obtained by SFE at 80 bar and 25 °C with propane (sample 7/1) has the highest coloring content in pheopytin. It seems that lower pressure is favourable for the extraction of porphyrins, while extraction of carotenoid coloring materials does not depend directly on pressure or temperature. The coloring contents of oils expressed in carotenoids increase during the extraction in the case of samples 5/1–5/3, while decreases in the case of samples 7/1–7/2.

Tocopherol contents of supercritical oils are significant. The total tocopherol contents are 21–59 μg/g (Table III). The tocopherol content of oils increases during the extraction in the case of samples 5/1–5/3, while decreases in the case of samples 7/1–7/2. Tocopherol has antioxidant activity (Chow, 1991) and is responsible for long durability. No change in peroxide number could be observed in the course of 2 years.

Element concentration of the samples are given in Table IV. Measurements were performed for 23 elements (Al, As, B, Ba, Ca, Cd,Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, Pb, S, Ti, V, Zn) but the concentrations of Co, Li, Pb, Ti and V were below detection limit. The amounts a Ca (81.32–879.2 ppm), Cr (12.84–248 ppm), Fe (27.85–189.28 ppm), K (6.59–149.74 ppm), Mg (22.06–173.46 ppm), Na (564.1–4634 ppm) and S (378.0–587.2 ppm) were found to be significant. The same

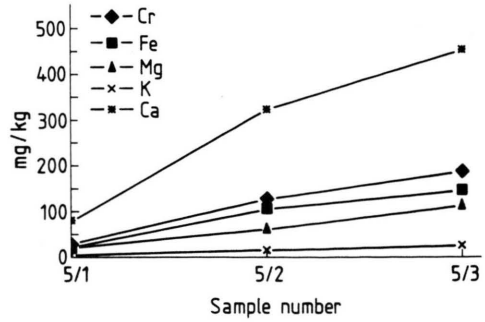

Fig. 3. Changes in element concentration in course of the extraction for sample numbers 5/1, 5/2, 5/3, see Table I and IV.

Table III. Coloring content of oils (extracts) by spectrometry and α-tocopherol content by HPLC.

Samples	Coloring content (μg/g)		Total tocopherol content
	Porphyrin derivatives expressed in pheophytin content	Total carotenoids	in α-tocopherol acetate (μg/g)
1.	71.6	21.1	21
2.	22.6	9.6	28
3.	49	33	24
4.	24	27.3	23
5/1.	19	8.2	25
5/2.	60	24	37
5/3	13.5	33.3	43
6.	44.6	43	45
7/1.	159	58	59
7/2	153	27	40
Soxhlet	146	21.6	37

Table IV. Element concentrations and ± standard deviation (n=3) of mille thistle oils (μg/g) obtained by supercritical and Soxhlet extraction.

Elements	Oil samples							
	1	2	3	4	5/1	5/2	5/3	Soxhlet
Al	2.91 ± 0.28	12.44 ± 0.41	4.82 ± 0.24	7.35 ± 0.59	1.69 ± 0.35	6.96 ± 0.55	11.20 ± 0.81	142.48 ± 0.99
As	< 0.4	< 0.6	1.46 ± 0.10	8.63 ± 0.99	< 2.1	1.49 ± 0.20	3.20 ± 0.66	< 0.6
B	2.78 ± 0.23	6.41 ± 0.022	7.03 ± 0.64	9.06 ± 0.42	2.64 ± 0.13	9.09 ± 0.08	12.26 ± 0.23	15.90 ± 0.28
Ba	0.106 ± 0.001	0.18 ± 0.001	0.264 ± 0.011	0.109 ± 0.01	0.051 ± 0.009	0.182 ± 0.009	0.317 ± 0.009	0.810 ± 0.006
Ca	100.4 ± 1.6	144.0 ± 5.9	265.8 ± 6.4	157.6 ± 4.3	81.32 ± 0.73	322.2 ± 4.1	451.8 ± 6.7	879.2 ± 2.7
Cd	< 0.02	< 0.04	< 0.06	< 0.05	0.160 ± 0.04	< 0.04	0.75 ± 0.03	1.77 ± 0.03
Cr	32.81 ± 0.27	39.86 ± 0.47	75.73 ± 0.42	12.84 ± 0.45	29.75 ± 0.80	125.0 ± 22	186.1 ± 0.7	248.0 ± 4.0
Cu	1.04 ± 0.08	0.732 ± 0.23	0.921 ± 0.195	0.769 ± 0.198	0.306 ± 0.116	0.720 ± 0.129	0.784 ± 0.102	3.60 ± 0.04
Fe	27.85 ± 0.31	37.36 ± 0.78	63.94 ± 2.39	47.16 ±1.12	22.46 ± 0.28	104.3 ± 1.9	144.6 ± 1.8	189.28±0,86
Hg	< 0.1	< 0.2	< 0.3	< 0.25	< 0.7	< 0.2	< 0.25	< 0.2
K	36.77 ± 4.09	47.76 ± 6.21	43.65 ± 8.05	34.36 ± 5.40	6.59 ± 0.51	12.85 ± 6.8	25.35 ± 1.09	149.74 ± 3.02
Mg	25.64 ± 0.37	34.29 ± 6.87	58.66 ± 0.51	39.92 ± 0.54	22.06 ± 0.28	58.90 ± 0.10	111.4 ± 0.63	173.46 ± 1.47
Mn	0.309 ± 0.009	0.45 ± 0.019	0.821 ± 0.013	0.446 ± 0.005	0.263 ± 0.006	0.654 ± 0.021	0.984 ± 0.041	2.08 ± 0.04
Mo	0.816 ± 0.07	1.03 ± 0.12	1.75 ± 0.096	0.765 ± 0.06	< 0.35	2.89 ± 0.47	4.22 ± 0.11	5.46 ± 1.10
Na	667.1 ± 6.3	869.6 ± 20.3	1557±19	1154±10	564.1 ± 3.0	2531±17	3514±55	4634±20
Ni	0.12 ± 0.07	1.47 ± 0.22	2.33 ± 0.145	0.370 ± 0.02	< 0.35	5.80 ± 0.17	8.74 ± 0.11	12.22 ± 0.18
S						378.0 ± 5.5	504.7 ± 1.6	587.2 ± 3.9
Zn	5.65 ± 0.07	11.24 ± 0.04	2.85 ± 0.06	4.89 ± 0.13	< 0.02	2.44 ± 0.19	11.22 ± 0.08	4.44 ± 0.13

trend can be observed for the elements as it was experienced for total carotenoid and tocopherol content of oils (Table IV). In oil samples, the concentration of almost each element increases during the extraction (Fig. 3). The oil obtained by Soxhlet extraction contains most of the elements (Al, B, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, S) in higher concentration than measured for supercritical oils. The presence of antioxidant type metals or of the metal components of the antioxidant system are particularly relevant (Cr, Mn, Zn) (Zidenberg-Cherr and Keen, 1991). Accumulation of some heavy metals can be observed in almost each oil sample. The occurrence of chromium, molybdenum, nickel at different concentrations is particularly significant in oils.

Table V contains the element concentration of mille thistle fruit and the residues of extractions. Element concentrations in samples are markedly different. The data obtained for mille thistle fruits are in good agreement with the average concentrations of plants (Kabata-Pendias and Pendias, 1984), although some element concentrations (Ca, K) are relatively low while chromium concentration is relatively high. Some enreachment in element concentration (boron, barium, coppper, po-

Table V. Element concentration (μg/g) of mille thistle fruit and its residues after extractions.

Elements	Mille thistle fruit	Residue after supercritical fluid extraction	Residue after Soxhlet extraction
Al	62.55 ± 1.74	35.40 ± 1.54	60.17 ± 1.80
As	1.65 ± 0.25	< 1.5	2.79 ± 1.44
B	16.8 ± 0.03	22.01 ± 0.71	17.87 ± 0.73
Ba	1.82 ± 0.08	1.74 ± 0.017	2.20 ± 0.019
Ca	8664 ± 103	8596 ± 93	8750 ± 58
Cd	0.47 ± 0.045	< 0.1	< 0.1
Cr	5.32 ± 0.17	< 0.2	< 0.2
Cu	18.51 ± 0.24	23.01 ± 0.45	19.80 ± 0.035
Fe	112 ± 8.2	43.50 ± 0.36	248.9 ± 3.1
Hg	< 0.5	< 0.5	< 0.5
K	6886 ±58.2	7819 ± 108	7744 ± 179
Mg	4351 ± 81	4976 ± 62	5062 ± 74
Mn	29.95 ± 1.12	35.39 ± 0.31	36.73 ± 0.25
Mo	0.738 ± 0.056	< 0.25	0.639 ± 0.55
Na	220 ± 9.4	376.0 ± 3.1	275.6 ± 5.3
Ni	3.29 ± 0.25	9.97 ± 0.52	11.98 ± 0.59
S	1818 ± 86	1965 ± 25	1992 ± 35
Zn	51.97 ± 0.35	52.16 ± 0.14	55.13 ± 0.28

tassium, magnesium, manganese, sodium, nickel, sulfur, zinc) can be observed in the residues.

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