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Super-critical carbon dioxide extraction as an effective green technology for production of high quality rose hip oil

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The application of super-critical carbon dioxide extraction of rose hip (*Rosa canina* L.) seeds for production of high quality oil was explored. For the purpose, main oil characteristics as fatty acids composition, tocopherols and carotenoids contents, as well as the oil oxidative stability were evaluated. The results revealed that technological conditions as pressure (350–450 bar) and particles size of the milled seeds (0.4, 1 mm) did not practically affect the fatty acids composition. However, increasing the pressure from 350 to 400 bar caused slight increasing of tocopherols and carotenes contents whereas decreasing of particles size reduced their amounts. The higher quantity of these antioxidants insured better oxidative stability of the rose hip oil and thence its high quality.

Key words: rose hip oil; extraction with super-critical carbon dioxide; fatty acids; oxidative stability; tocopherols; carotenoids

INTRODUCTION

Rose hip oil is obtained from the seeds of *Rosa canina* L. fruits. Being a rich source of healthy biologically active substances as essential fatty acids, strong antioxidants as tocopherols and carotenoids, *etc.*, that oil improves lipid metabolism and possesses anticancerogenic effects along with a positive influence on dermatoses, ulcers and other skin problems [1]. Therefore the use of rose hip oil as a healthy dietary supplement and valuable cosmetic ingredient increases significantly in recent years and that requires development of effective and harmless procedures for its production.

Among the methods for extraction of oils from seeds that with super-critical carbon dioxide (CO_2) exceeds the others in the use of non-toxic, non-corrosive, non-flammable, eco-friendly and cheap solvent which can be recovered without damaging the substrate and extract. Also, the low extraction temperatures prevent thermal damage of labile compounds [2]. In recent years the application of super-critical CO_2 extraction expands significantly and put it among the leading "green" technologies.

The information published about super-critical CO_2 extraction of the oil from rose hip seeds is deficient, fragmentary and even discrepant. Therefore the aim of our work was to elucidate the effects of some technological conditions on the composition and stability of the oil and thus to reveal the potential of that "green" method for production of rose hip oil of high quality. For the purpose, basic oil features as fatty acid composition, tocopherols and carotenoids contents, as well as its oxidative stability, were investigated.

EXPERIMENTAL

Samples and reagents

Seeds of rose hip (*Rosa canina* L.) were provided by the Foundation Information and Nature Conservation [3]. Reagents and solvents used for methylation and oxidative stability determination were of analytical grade (Merck). The solvents used as mobile phase components were of HPLC grade (Merck). Reference fatty acid methyl esters, alpha- and gamma-tocopherols, and beta-carotene were from Sigma-Aldrich, Inc., delta-tocopherol was from Supelco. Carbon dioxide was 99.95% purity (Messer Ltd., Bulgaria).

Extraction of oil

Air-dried rose hip seeds (with moisture content 3.2%, determined by Electronic Moisture Analyser KERN DBS 60-3) were ground in a hammer-mill

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with 1 mm sieve and then were sorted using respective sieves to 0.4 and 1 mm particles size powders. The oil was extracted by super-critical carbon dioxide (super-critical CO_2) in SEPAREX (France) high pressure extractor equipped with extraction vessel of 5 L and working pressure up to 1000 bar. Experiments were carried out at 350, 400 and 450 bar. Effects of particles size were tested at 350 bar. After extraction at 60 ^oC for 120 min the respective samples were decanted to separate oil and eject the water. Then the oil was filtered under vacuum and stored in dark at 4 ^oC prior to analysis. Another portion of the seeds was subjected to extraction with hexane in Soxhlet apparatus for 8 hours [4] and that sample was used as a reference. The selection of all experimental conditions was based on literature data.

Analysis of fatty acids composition

Fatty acids composition of the rose hip oil was determined by gas chromatography (GC) of methyl esters (FAME). For the purpose, about 50 mg oil were transmethylated with 1% sulfuric acid in methanol [5]. The FAME were purified by preparative silica gel G thin-layer chromatography (TLC) using hexane-acetone (100:6, v/v) as a mobile phase. GC was performed on Shimadzu 17A (Shimadzu, Japan) gas chromatograph equipped with flame ionization detector and Supelcowax-10 column (100 m x 0.25 mm x 0.25 μm, SUPELCO). The column temperature was programmed from 160 ^oC to 270 °C with 4 °C/min and held at this temperature for 20 min. The injector and detector temperatures were 260 ^oC and 280 ^oC, respectively. Helium was the carrier gas at flow rate of 1.1 mL/min; sample size 15 μg, split 1:50. The peaks identification was according to retention times of the reference FAME. Analyses were performed in triplicate and the results were presented as relative percent of each fatty acid.

Analysis of tocopherols and carotenoids

Tocopherols and beta-carotene were analyzed directly by HPLC using Agilent 1100 liquid chromatograph equipped with an autosampler injector, column oven (25 ^oC) and diode-array detector at 292 nm (for *alpha*-tocopherol), 298 nm (for *gamma*- and *delta*-tocopherols [6]) and 450 nm (for *beta*-carotene [7]). Analyses were carried out on 250 mm x 4.6 mm Nucleosil 100-5 column including an EC 4/3 Nucleosil 100-5 guard column (Macherey-Nagel). Tocopherols were eluted by mobile phase of hexane-tetrahydrofuran (96:4, v/v) at 1 mL/min flow rate (400 μg sample size) whereas beta-carotene was eluted by hexane at 0.8 mL/min flow rate (400 μg sample size). Identification was by comparison of retention times with that of reference individual isomers. Quantitation was done using respective calibration curves obtained as follows: (i) stock solution of 0.1 mg/mL *alpha*-, *gamma*- and *delta*-tocopherol, respectively, in hexane was diluted to work solutions with concentrations in the range 0.025 – 0.05 mg/mL; (ii) stock solution of 25 mg/L beta-carotene in hexane/2-propanol mixture (10:1 v/v), stored under nitrogen at -20 °C in dark, was diluted to work solutions with concentrations in the range 0.25–2.5 mg/L. Measurements were done in triplicate and the results were presented as [mg/kg oil].

Total carotenoids were determined spectrophotometrically using Cecil Series 8000 UV/VIS double beam scanning spectrophotometer (Cecil Instruments Ltd., UK) at 445 nm [8]. A calibration curve was prepared using solutions of *beta*-carotene in cyclohexane with concentrations in the range 0.5–10 mg/L. The measured samples contained about 1.2000 g oil dissolved in 25 mL cyclohexane (volumetric flask) with careful keeping of all these solutions in dark. Measurements were performed in triplicate and the results were presented as beta-carotene content [mg/kg oil].

Determination of oxidative stability

The Acid value (AV, presented as mg KOH/g) was determined by titration with ethanolic KOH [9]. Free fatty acids (FFA, given as % oleic acid) were measured titrimetrically using ethanolic NaOH [10]. The Peroxide value (PV, expressed as mEq/kg) was estimated by modified iodometric method [11]. The Induction period (IP, in hours) was found out by the following procedure: oil sample (2 g) was oxidized at 100 °C by blowing air at 50 mL/min flow rate. Aliquots were taken in fixed time intervals and the degree of oxidation was estimated by iodometric determination of the peroxide value (PV). Then kinetic curves of PV accumulation were plotted, all of them representing the mean value of three independent experiments. The Induction period (IP) was determined by the method of tangents to two parts of the kinetic curves [12]. Linear relationship between parameters investigated was obtained using the Linear fit tool of Origin software (OriginLab Corporation, MA, USA).

Statistics

The results are presented as mean values of three measurements ± standard deviation and have been compared by Student`s *t*-test (Microsoft Excel software).

RESULTS AND DISCUSSION

Fatty acids composition

Rose hip oil is one of the several seed oils which are abundant in the essential *omega*-3 (linolenic) fatty acid. With its about 23% linolenic (18:3) acid, 50% linoleic (18:2), 16% oleic (18:1) and 9% saturated (18:0 and 16:0) fatty acids the oil analyzed here (Table 1) was similar to those produced by different methods in Turkey [13–16], Romania [17], Hungary [18, 19], Poland [20]. Regarding fatty acids composition of rose hip oils obtained by super-critical CO_2 the data are scarce and inconsistent. Among four studies found in the literature [18, 19, 21, 22] only one [21] investigated effects of super-critical CO_2 extraction conditions (pressure, temperature, flow rate) on the fatty acids amounts. It was interesting that such effects were observed for 16:0, 18:0 and 18:3 only but not for 18:2. It should be noted that 18:1 was not mentioned among the results. Moreover, the unidentified components varied between 4 and 18% but these analytical flaws were not taken into account by the authors. The other three papers [18, 19, 22] compared fatty acids composition of oils obtained by super-critical CO_2 and by the standard Soxhlet extraction. According to one of them [19] there were differences in 16:0, 18:1 and 18:2 (but not in 18:3 and 18:0) amounts depending on the extraction method. The other two papers [18, 22] did not report any influence of the method of extraction on the FA composition of rose hip oil.

Table 1 presents our results about fatty acids composition of oil samples obtained by super-critical CO_2 at different conditions and by Soxhlet extraction. As can be seen, practically no differences are observed. Thus, super-critical CO_2 extraction can be successfully used for production of rose hip oil with preserved essential fatty acids.

Tocopherols and carotenoids

Tocopherols and carotenoids are important biologically active substances. They are strong and effective natural antioxidants and for that reason higher their amounts are desirable feature of all oils and fats. Especially for rose hip oil which is highly unsaturated (Table 1) their contents are crucial for its stability. Unfortunately, significant part of them could be lost during oil production and that depends on both the method used and the applied technological conditions.

According to results found in the literature about tocopherols in rose hip oil (Table 2) cold-pressed oils have quite high tocopherols levels compared to other technologies, with total amounts above 1000 mg/kg oil. To the best of our knowledge, no data for tocopherols in rose hip oil extracted with super-critical CO_2 have been published yet, thence the investigations presented here reveal new and useful information.

Our results are given in Table 3. As can be seen, two isomers (*alpha*- and *gamma*-) were measured at that *gamma*-tocopherol was above five times more than the *alpha*-isomer. *Beta*-tocopherol was not expected to be present in measurable levels (Table 2), whereas *delta*-tocopherol was not detected in oil samples. Total amounts of tocopherols were above 1100 mg/kg which exceeded the best results achieved by other extraction methods. Concerning the production conditions, increasing of CO_2 pressure to 400 bar slightly increased the *gamma*-tocopherol amount. On the other hand, decreasing the particles size from 1 to 0.4 mm reduced amounts of both *alpha*- and *gamma*-isomers.

Table 1. Fatty acids composition (rel.%) of rose hip oil obtained by super-critical carbon dioxide extraction (SC-CO_2) and in Soxhlet apparatus.

Fatty acids	SC-CO_2 350bar/1mm	SC-CO_2 350bar/0.4mm	SC-CO_2 400bar/1mm	SC-CO_2 450bar/1mm	Soxhlet extraction
16:0	5.3 ± 0.7*	5.3 ± 0.7	5.2 ± 0.8	5.3 ± 0.6	5.3 ± 0.9
16:1	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01
18:0	3.6 ± 0.3	3.5 ± 0.4	3.4 ± 0.4	3.5 ± 0.3	3.4 ± 0.5
18:1	15.8 ± 0.6	15.6 ± 0.5	15.5 ± 0.7	15.8 ± 0.7	15.9 ± 0.9
18:2	50.3 ± 1.8	50.7 ± 1.7	50.6 ± 1.9	50.4 ± 2.0	50.7 ± 2.3
18:3	23.2 ± 0.9	23.1 ± 1.1	23.5 ± 1.1	23.2 ± 1.2	23.0 ± 1.5
20:0	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.2 ± 0.1
20:1	0.3 ± 0.06	0.3 ± 0.01	0.3 ± 0.0	0.3 ± 0.01	0.3 ± 0.01
22:0	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01

* Within each row, no statistically significant difference between values was found (at P=0.95).

Table 2. Data found in literature about tocopherols content in rose hip oil obtained by different methods (average values).

[Ref. No.] Extraction method	α-tocopherol (mg/kg)	β-tocopherol (mg/kg)	γ-tocopherol (mg/kg)	δ-tocopherol (mg/kg)	total tocopherols (mg/kg)
[16] cold-pressing	58	5	1060	4	1125
[20] cold-pressing	120-150	nd*	630-780	230-260	1000-1200
[23] hexane	170	nd	900	30	1100
[14] hexane	8	nd	nd	nd	-
[18] subcritical CO_2-propane	57	nd	92	27	180
[24] Soxhlet	16	1.5	nd	nd	17.5

* nd - not detected

Table 3. Tocopherols, carotenoids contents and oxidative stability parameters of rose hip oil obtained by super-critical CO_2 extraction at different conditions.

	SC-CO_2 350bar/1mm	SC-CO_2 350bar/0.4mm	SC-CO_2 400bar/1mm	SC-CO_2 450bar/1mm
tocopherols				
α-tocopherol (mg/kg)	180 ± 10^{a}*	150 ± 10^{b}	200 ± 10^{a}	190 ± 10^{a}
γ-tocopherol (mg/kg)	1040 ± 20^{a}	980 ± 20^{b}	1160 ± 20^{c}	1180 ± 20^{c}
total tocopherols (mg/kg)	1220**	1130**	1360**	1370**
carotenoids				
total carotenoids (as β-carotene, mg/kg)	16.6 ± 0.6^{a}	14.1 ± 0.6^{b}	18.4 ± 0.6^{c}	18.0 ± 0.5^{c}
β-carotene (mg/kg)	9.0 ± 0.3^{a}	3.5 ± 0.2^{b}	11.5 ± 1.0^{c}	12.5 ± 0.8^{c}
oxidative stability				
AV (mg KOH/g)	6.6 ± 0.8^{a}	6.1 ± 0.7^{a}	2.8 ± 0.5^{b}	3.2 ± 0.5^{b}
FFA (% oleic acid)	3.5 ± 0.4^{a}	3.2 ± 0.3^{a}	1.5 ± 0.3^{b}	1.7 ± 0.3^{b}
PV (mEq/kg)	9.0 ± 0.4^{a}	7.2 ± 0.4^{b}	6.1 ± 0.3^{c}	5.9 ± 0.3^{c}
IP (hours)	4.9 ± 0.3^{a}	5.6 ± 0.3^{b}	6.7 ± 0.5^{c}	6.6 ± 0.4^{c}

* Different letters within each row indicate statistically significant difference between the mean values (at P=0.95).
** Sum of the values for *alpha*- and *gamma*-isomers.

However, even the lowest tocopherols contents are comparable to the best results obtained by other extraction methods (cold-pressing, hexane extraction, *etc*.).

Carotenoids and specially *beta*-carotene in rose hip oil are presented in several publications. Depending on the producing technology, their amounts vary from 40 to 150 mg/kg for total carotenoids [15, 19, 20, 23] and from 0.2 to 2 mg/kg for *beta*-carotene [14, 18, 23]. According to our results (Table 3), super-critical CO_2 extraction at the investigated conditions ensured rose hip oil with 14–18 mg/kg total carotenoids and 4–13 mg/kg beta-carotene. Comparing to the results mentioned above, total carotenoids were far less but the beta-carotene content was significantly higher than the available data. As with the tocopherols, increasing the pressure from 350 to 400 bar caused slight increasing of carotenes contents whereas decreasing of particles size reduced their amounts. Comparing to other methods, the extraction with super-critical CO_2 ensures rose hip oil rich in beta-carotene along with tocopherols.

Oxidative stability

The oxidative stability of oils depends mainly on their fatty acids composition and the presence of antioxidants. Because of its high content of linolenic acid the rose hip oil is expected to have rather low oxidative stability. In literature have been found data only about acid value (AV) and peroxide value (PV) of two cold-pressed rose hip oils, in the range respectively 0.1–0.6 mg KOH/g and 1.2–2.1 mEq/kg [20]. Our results about oxidative stability are given in Table 3. Concerning AV and PV, the values of oil produced by super-critical CO_2 (2.8–6.6 mg KOH/g and 5.9–9.0 mEq/kg) are higher than cold-pressed rose hip oils cited above. Nevertheless, AV and free fatty acids (FFA) are below the maximum values permitted for virgin olive oil [25]. The induction periods (IP) of tested rose hip oils (4.9–6.7 hours) are typical for oils with similar unsaturation, *e.g.* linseed oil [26]. Regarding conditions for super-critical CO_2 extraction and samples content discussed in the previous sections, it should be expected decreasing

of AV, FFA and PV and respective increasing of IP with increasing of the CO_2 pressure because of the same trends in the main antioxidants along with no alteration in fatty acids (Tables 1 and 3). Indeed, the results for oxidative stability of rose hip oil samples (Table 3) confirm that assumption. So, pressures above 400 bar could be recommended for production by super-critical CO_2 extraction of rose hip oil with higher stability, *i.e.* of higher quality.

CONCLUSION

The extraction of rose hip seeds with super-critical CO_2 enables production of high quality glyceride oil containing significant amounts of unchanged essential fatty acids and natural antioxidants (mainly tocopherols and carotenoids). Extraction conditions such as pressure (350–450 bar) and particles size of the milled seeds (0.4, 1 mm) do not practically affect the fatty acids composition. On the other hand, increasing the pressure from 350 to 400 bar causes slight increasing of tocopherols and carotenes contents whereas decreasing of particles size reduced their amounts. The higher quantity of these antioxidants insures better oxidative stability of the rose hip oil and thence its high quality.

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ЕКСТРАКЦИЯТА СЪС СУПЕР-КРИТИЧЕН ВЪГЛЕРОДЕН ДИОКСИД КАТО ЕФЕКТИВНА „ЗЕЛЕНА“ ТЕХНОЛОГИЯ ЗА ПОЛУЧАВАНЕ НА ВИСОКОКАЧЕСТВЕНО ШИПКОВО МАСЛО

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(Резюме)

Изследвано е приложението на екстракцията със супер-критичен въглероден диоксид за получаване на висококачествено масло от семки на шипка (*Rosa canina* L.). За целта са определени основните характеристики на маслото като мастно-киселинен състав, съдържание на токофероли и каротеноиди, както и окислителната му стабилност. Резултатите показват, че технологични условия като налягането (350–450 бара) и размер на частиците на смлените семки (0.4, 1 мм) не влияят върху мастно-киселинния състав. Обаче, повишаване на налягането от 350 до 400 бара предизвиква леко увеличение в токоферолното и каротеноидно съдържание, докато намаляване размера на частиците води до намаляване на количеството токофероли и каротеноиди. По-високото съдържание на тези антиоксиданти осигурява по-висока окислителна стабилност на шипковото масло и така повишава неговото качество.