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Chemical Composition of *Juniperus communis* L. Fruits Supercritical CO_2 Extracts: Dependence on Pressure and Extraction Time

BRANISLAVA BARJAKTAROVIĆ,*,† MILAN SOVILJ,† AND ŽELJKO KNEZ*,‡

Faculty of Technology, University of Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia and Montenegro, and Faculty of Chemistry and Chemical Engineering, University of Maribor, Smetanova 17, 2000 Maribor, Slovenia

Ground fruits of the common juniper (*Juniperus communis* L.), with a particle size range from 0.250−0.400 mm, forming a bed of around 20.00 ± 0.05 g, were extracted with supercritical CO_2 at pressures of 80, 90, and 100 bars and at a temperature of 40 °C. The total amount of extractable substances or global yield (mass of extract/mass of raw material) for the supercritical fluid extraction process varied from 0.65 to 4.00% (wt). At each investigated pressure, supercritical CO_2 extract fractions collected in successive time intervals over the course of the extraction were analyzed by capillary gas chromatography, using flame ionization (GC−FID) and mass spectrometric detection (GC−MS). More than 200 constituents were detected in the extracts, and the contents of 50 compounds were reported in the work. Dependence of the percentage yields of monoterpene, sesquiterpene, oxygenated monoterpene, and oxygenated sesquiterpene hydrocarbon groups on the extraction time was investigated, and conditions that favored the yielding of each terpene groups were emphasized. At all pressures, monoterpene hydrocarbons were almost completely extracted from the berries in the first 0.6 h. It was possible to extract oxygenated monoterpenes at 100 bar in 0.5 h and at 90 bar in 1.2 h. Contrary to that, during an extraction period of 4 h at 80 bar, it was possible to extract only 75% of the maximum yielded value of oxygenated monoterpene at 100 bar. Intensive extraction of sesquiterpenes could be by no means avoided at any pressure, but at the beginning of the process (the first 0.5 h) at 80 bar, they were extracted about 8 and 3 times slower than at 100 and 90 bar, respectively. Oxygenated sesquiterpenes were yielded at fast, constant extraction rates at 100 and 90 bar in 1.2 and 3 h, respectively. This initial fast extraction period was consequently followed by much slower extraction of oxygenated sesquiterpenes.

KEYWORDS: Supercritical fluid extraction; *Juniperus communis*; the common juniper; terpenes; oxygenated terpenes; juniper berry oil; carbon dioxide

INTRODUCTION

Juniperus communis L. (Cupressaceae) is a native evergreen shrub/tree of regions in the Northern hemisphere with a substantial list of traditional uses owing to its medicinal properties and highly specific flavor, primarily associated with its volatile oil components. All of the organs of this plant contain essential oils, but it is obtained mainly from the berries, mature female cones (*Juniperi Fructus*), needles and their branches (*juniper foliage*). The berries contain 0.2−3.42% of volatile oil, depending on the geographic location, altitude, degree of ripeness, and other factors, which is traditionally yielded by steam distillation of the crushed, dried, partially dried, or fermented berries (*1*). The composition of commercially available juniper berry volatile oils varies considerably, consisting mainly of monoterpenes (58−85%) (*1*) with α-pinene as its main constituent (10−76%) and a wide range of other monoterpenes, mainly sabinene (1−28%), *β*-pinene, *β*-myrcen, limonene, and terpinen-4-ol; small amounts of sesquiterpenes (2−10.2%); aldehydes; alcohols; and other oxygenated compounds. The analysis of the essential oils from *J. communis* L. fruits has been a subject of many investigations (*2*−*14*).

In flavor work, oxygenated compounds are considered as the main contributors to aroma and flavor, contrary to the terpene fraction, which acts as a flavor carrier. Additionally, the terpenes have two organoleptic disadvantages: they usually irritate the skin, eyes, and mucous membranes and become unstable under the influence of light, heat, and oxygen causing undesirable changes to the flavor and aroma profile during processing or storage. Finally, the terpene fraction is less water soluble than

* To whom correspondence should be addressed. Telephone: +381-21-450277. Fax: +381-21-450413. E-mail: barjakb@uns.ns.ac.yu (B.B.); Telephone: +386-2-2294461. Fax: +386-2-2516750. E-mail: zeljko.knez@uni-mb.si (Z.K.).
† University of Novi Sad.
‡ University of Maribor.

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the oxygenated flavor fraction. Hence, terpenes are removed from the essential oils to obtain terpeneless and sesquiterpeneless oils or "folded" oils, which have a richer concentration of flavor and oxygenated compounds compared to the parent oil (*15*).

The greatest quantity of juniper berry essential oils (a sesquiterpeneless oil) is used in the food industry as a flavor component and a spice in most major food categories (*1*). Also, juniper berry oil is used in the cosmetic and perfume industry as a fragrance component (*15*).

The plant's fruits and its essential oils are well-known (*16*−*20*) for their physiological effects, first of all diuretic (because of terpinen-4-ol) and various gastrointestinal effects. As a result, juniper berries, their essential oils, infusions, decoctions, and different alcoholic extracts of the berries have been listed in some national official pharmacopoeias (*21*−*23*). The proportion of terpinen-4-ol to α- and *β*-pinene can vary from 1:4 to 1:55 in the oil. Primarily because of this monoterpene fraction, the oil has irritant properties and an antispasmolytic effect on smooth muscles (*19*). Additionally, a number of effects of the berries and their essential oils such as hypoglycemic (*24*), abortifacient (because of isocupressic acid) (*25*), antinflammatory, and their antiblood platelet stickiness effect (*26*) continue to be tested on animals and have yet to be acknowledged by human medicine. Some antibacterial and antifungal activity of the oil was reported by certain authors (*18*, *19*, *27*, *28*). Antimicrobial activity was confirmed for a certain type of juniper berry oil, probably as a result of either the specific composition of the oil or the activity of a single unidentified compound (*28*).

Juniper berry volatile oil with significantly lower levels of monoterpenes and thus higher levels of active flavor components compared to traditionally obtained oil can be yielded in fluid-phase extractions with sub- (*29*, *30*) and supercritical carbon dioxide (*14*, *31*).

In the supercritical fluid extraction (SCFE) process, it is a well-known fact that, by varying the extraction pressure and consequently the density of the supercritical fluid (SCF), it is possible to change the extract composition. At lower pressures near the critical point, only volatile components, such essential oils, are extracted, while total extracts including fats, waxes, resins, and dyes are extracted at higher pressures where the fluid has a liquidlike density. The effects of pressure, temperature, particle size, and fluid flow rate on supercritical CO_2 extraction of *J. communis* L. have already been the subjects of many investigations. Some of them present the common juniper fruit supercritical CO_2 extracts' composition for a determined time (*14*, *31*). The compositions of *Juniperi Fructus* supercritical CO_2 extracts obtained after an extraction time of 1 h at 40 °C and at pressures of 90 and 125 bar (*31*) and the extract obtained after an extraction time of 2.5 h at the same temperature and a pressure of 250 bar (*14*) have been reported in the literature. This study provides information on the variation in the composition and yield of extract with time during SCFE of *J. communis* L. The aim of this study was to investigate the evolution of the composition of juniper fruit supercritical CO_2 extracts with extraction time, at different extraction pressures and to emphasize the most favorable condition for the extraction of different terpene hydrocarbon groups. A study including the effect of the length of extraction and reporting the qualitative differences among extracts collected during successive extraction time periods could give information about extraction kinetics of oxygenated and non-oxygenated terpenes.

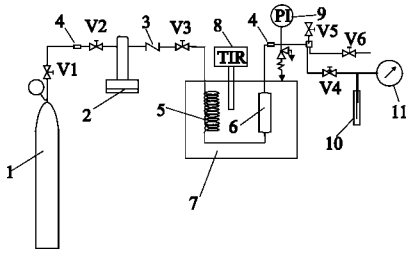

Figure 1. Experimental apparatus: (1) gas tank, (2) ISCO syringe pump, (3) check valve, (4) microfilter, (5) preheat coil, (6) high-pressure autoclave, (7) water bath, (8) temperature regulator, (9) manometer, (10) sampling trap, (11) wet flow meter, (V1–V3, V5, and V6) on–off valves, and (V4) micrometering valve.

EXPERIMENTAL PROCEDURES

Materials. The juniper berries (fruits) were supplied by Etol (Celje, Slovenia). The moisture content of the raw material, measured by a Mettler Toledo DL31 Karl-Fisher titrator, was 0.1502 kg of H_2O/kg of wet material. After the common juniper fruits were dried at 303 K in an airflow drier to reduce the water content to around 8 wt %, they were triturated closely before SCFE with a domestic blender and the material was passed through a set of sieves with mesh diameters of 0.100, 0.150, 0.200, 0.250, 0.400, 0.630, and 1.000 mm and mechanically vibrated by an agitator (Laborette, Fritschs, Germany).

n-Hexane, (p.a. grade) purchased from Merck (Darmstadt, Germany) was used as the solvent for SCF extracts during GC analyses, and acetone (technical grade) was used as a cleaning solvent for the SCFE apparatus. Authentic volatile samples were obtained through Etol (Celje, Slovenia): α-pinene (>98%) (Crystal J.S.C., Bulgaria), *β*-pinene, *γ*-terpinene, *p*-cymene, *β*-myrcene, terpinolene, terpineol (contents of terpene alcohols, >98%, including α- and *γ*-terpineol, >70%) (Crystal J.S.C., Bulgaria). (+)-Limonene (minimum 97%) was obtained from Sigma (Taufkirchen, Germany). The CO_2 (purity of 99.97%) was supplied by Messer-Griesheim (Rushe, Slovenia).

Procedure and Conditions. In all experimental runs, the fixed bed was formed with around 20 g (with deviation of ±0.05 g) of ground juniper berries, weighed with a precision of ±0.0001 g. The extraction temperature was kept constant at (40 ± 0.5) °C. **Figure 1** provides a schematic diagram of the semicontinuous, SCFE laboratory apparatus used to perform experimental runs. The apparatus was located in the Laboratory for Separation Processes of the Faculty of Chemistry and Chemical Engineering, University of Maribor, Slovenia. The equipment was built for the maximum operating pressure of 50 MPa and the maximum temperature of 100 °C. It consisted of a CO_2 supplying cylinder (1), a high-pressure pump (2) (ISCO syringe pump, model 260D, maximal pressure 35 MPa, Lincoln, NE), preheating coils (5), a 60 mL internal volume extraction vessel (6), a product collector (10), and a flow meter (11) (Elster Handel GmbH, Mainz, Germany) at the end of the assembly. The preheater and extractor were immersed in a constant temperature water bath (7) [thermostat (8) Lauda DR. R. Wobser GmbH and Co. KG, Lauda-Königshofen, Germany). A manometer (9) (Digibar PE200 Hottinger Baldwin, Darmstadt, Germany) enabled the monitoring of the extraction pressure within ±0.5 bar precision. The elements of the assembly were connected with $^1/_8''$ tubings (Dockwieler, Germany). The tubing was attached to the pump with Valco fittings (Valco, Inc., Houston, TX). All on−off valves (V1−V3, V5, and V6), a check valve (3), a micrometering valve (V4), and fittings (Autoclave Engineers) used in the system could stand pressures up to 75.8 MPa. Between the CO_2 supplying cylinder and the pump inlet, as well as on the product line emerging from the top of the extractor, two filters (10 *μ*m) (4) were placed to prevent any possible suspended particles. The line between the extractor and the product collector was equipped with a heated needle (expansion) valve (V4) and a heated on−off valve (V5). Just before the collector, the line itself was wrapped with heating tapes to prevent freezing because of gas expansion. Extracts were recovered by depressurization to atmospheric pressure in a micrometering valve (V4), and the product was precipitated

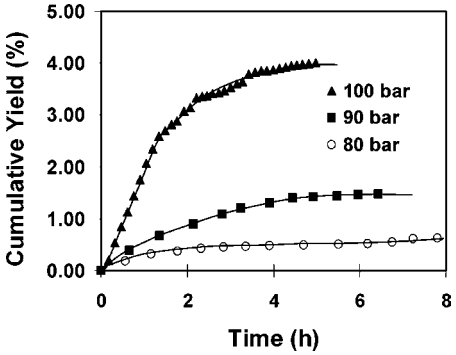

Figure 2. Effect of pressure and time on extraction yield.

in glass tubes (sampling traps) used as separators. Separator temperature was maintained at (260 ± 2) K, and pressure was kept at atmospheric level.

The apparatus was operated in a single-pass mode of CO_2 up through the bed of ground juniper berry particles. A single experiment consisted of a series of cycles, during which dense CO_2 flowed continuously up through the bed, interrupted by refill periods of the high-pressure pump cylinder (approximately 120 s). Extraction yields were measured gravimetrically (±0.0001 g) by collecting the products precipitated in glass tubes (sampling traps) used as separators.

To investigate the influence of the pressure on the total yield and juniper berry oil extraction kinetics, SCFE was carried out at pressures of 80, 90, and 100 bar with a constant CO_2 flow rate of ~0.2 kg h^{-1} and with the fraction of the ground material with particle sizes in the range of 0.250−0.400 mm. Experiments at the constant pressure were conducted until the constant mass of extract was collected (statistically insignificant mass change between two successive cycles' yields). Two replications of experiments at 90 and 100 bar were performed, and the experiment at 80 bar was repeated 3 times.

The evolution of the extracts' composition with extraction time was studied in a way that supercritical CO_2 extract fractions were collected in successive time intervals over the course of the extraction at the constant pressure. For that purpose, in the first experiment, extracts were collected every approximately 50 L of CO_2 (around a 0.6 h time interval) up to 300 L. The experiments at 80 and 90 bar were repeated keeping the same time intervals, while time intervals for extraction at 100 bar were additionally adjusted for the purpose of mathematical modeling and kept unchanged in the repeated experiment.

Extract samples were stored in the dark in the refrigerator to prevent chemical reactions before their GC analysis.

GC−FID Analysis. The 2 μL of samples, containing supercritical carbon dioxide extracts previously dissolved in *n*-hexane, were subjected to gas chromatographic analyses on a Hewlett−Packard 5890 gas chromatograph with a FID. A fused silica capillary column, with a nonpolar stationary phase (100% dimethylpolysiloxane), CP-Sil 5CB/MS, 25 m × 0.25 mm inside diameter, and 0.25 μm film thickness, was used. The purged splitless mode of sampling was implemented. The column temperature was maintained at 50 °C for 1 min and then programmed to increase as follows: at 4 °C/min to 280 °C, holding at 280 °C for 15 min, at 15 °C/min to 295 °C, and 5 min at 295 °C. The flow rate of the carrier gas (N_2) through the column was 1 mL min^{-1}, and inlet pressure was 10 psig. The injector temperature was 250 °C, and the detector termperature was 330 °C.

Quantitative presence in the extracts was determined using the method of peak-area normalization and without the application of response factor corrections. Injections were repeated in triplicates.

GC−MS Analysis. Samples prepared in the same way as for the GC analysis were subjected to GC−MS analysis on a Hewlett−Packard 5890 (series A) gas chromatograph with a mass-spectrometry detector Hewlett−Packard 5871. A fused silica capillary column, Ultra 2, 25 m × 0.20 mm inside diameter, and 0.33 μm film thickness, was used. The column temperature was maintained at 50 °C for 1 min and then programmed to increase as follows: at 4 °C/min to 280 °C and holding at 280 °C for 20 min. The linear flow velocity of the carrier gas (He) through the column was 30 cm/s. The injector temperature was 250 °C, and the detector temperature was 280 °C. Ionization voltage of 70 eV was used. The sample (1 μL) was injected in the split mode (1:60).

The identification of compounds was based on the comparison of their retention times and GC elution sequence with corresponding data of pure reference compounds or with literature data for juniper berry essential oils (*32*, *33*) and other juniper essential oils (*34*) and also by matching the mass spectra of every GC peak with those of the Wiley Library.

Table 1. Main Constituents (Area %) of the *J. communis* Supercritical CO_2 Extract Fractions Obtained in Different Extraction Time Intervals for Extraction Process at 40 °C and 80 bar

	extraction time (h)			
component	0–0.6	0.6–1.2	1.2–1.8	1.8–3.9
α-thujene	0.69 ± 0.03			
α-pinene	21.45 ± 1.02	0.39 ± 0.07		
α-fenchene	0.24 ± 0.02			
camphene	0.09 ± 0.01			
sabinene	11.75 ± 0.52	0.23 ± 0.04		
β-pinene	1.96 ± 0.09			
β-myrcene	4.97 ± 0.24			
δ-3-carene	0.15 ± 0.01			
α-terpinene	0.09 ± 0.01			
p-cymene	0.70 ± 0.03			
limonene	2.82 ± 0.13			
γ-terpinene	1.12 ± 0.05	1.03 ± 0.18	0.48 ± 0.08	
terpinolene	1.14 ± 0.05	1.23 ± 0.21	0.99 ± 0.17	0.26 ± 0.10
monoterpenes	47.45 ± 2.25	2.87 ± 0.50	1.47 ± 0.25	0.26 ± 0.10
α-cubebene	1.98 ± 0.08	1.72 ± 0.08	0.55 ± 0.02	
α-copaene	0.87 ± 0.04	1.13 ± 0.05	0.54 ± 0.02	
β-elemene	2.64 ± 0.11	4.66 ± 0.21	3.98 ± 0.17	2.20 ± 0.11
β-caryophillene	4.22 ± 0.17	7.19 ± 0.33	5.02 ± 0.21	1.34 ± 0.07
γ-elemene	0.64 ± 0.03	1.59 ± 0.07	2.14 ± 0.09	2.16 ± 0.10
α-humulene	3.09 ± 0.13	6.09 ± 0.28	5.20 ± 0.22	1.80 ± 0.09
cis-α-farnesene	1.14 ± 0.05	2.17 ± 0.10	0.65 ± 0.03	
γ-cadinene	12.12 ± 0.49	26.62 ± 1.22	27.17 ± 1.13	12.96 ± 0.63
β-selinene	0.72 ± 0.03	1.55 ± 0.07	1.81 ± 0.08	1.00 ± 0.05
α-farnesene		0.16 ± 0.01	0.21 ± 0.01	0.39 ± 0.02
valencene	0.81 ± 0.03	1.90 ± 0.09	2.21 ± 0.09	1.39 ± 0.07
3,7-guaidiene	0.25 ± 0.01	0.63 ± 0.03	0.78 ± 0.03	0.64 ± 0.03
viridiflorene	0.56 ± 0.02	1.33 ± 0.06	1.70 ± 0.07	1.12 ± 0.05
α-muurolene	0.61 ± 0.02	1.59 ± 0.07	2.06 ± 0.09	2.90 ± 0.14
δ-cadinene	0.76 ± 0.03	1.97 ± 0.09	2.57 ± 0.11	2.61 ± 0.13
α-calacorene		0.17 ± 0.01	0.23 ± 0.01	0.47 ± 0.02
germacrene D	3.85 ± 0.16	9.35 ± 0.46	14.22 ± 0.59	16.07 ± 0.78
germacrene B	1.08 ± 0.04	3.31 ± 0.15	4.89 ± 0.20	13.11 ± 0.64
sesquiterpenes	35.82 ± 1.46	74.42 ± 3.41	75.92 ± 3.16	60.87 ± 2.95
terpinen-1-ol	1.13 ± 0.07	0.30 ± 0.02		
β-terpineol	0.89 ± 0.06	1.06 ± 0.06	0.59 ± 0.03	
(*cis*,*trans*)-verbenol	0.92 ± 0.07	1.18 ± 0.09	0.91 ± 0.06	
pinocarveole	1.52 ± 0.09	2.38 ± 0.13	2.54 ± 0.11	1.11 ± 0.04
borneol	0.20 ± 0.01	0.38 ± 0.02	0.45 ± 0.02	0.26 ± 0.01
terpinen-4-ol	4.60 ± 0.28	6.90 ± 0.37	4.85 ± 0.21	0.90 ± 0.03
α-terpineol	1.50 ± 0.05	0.37 ± 0.02	0.32 ± 0.01	0.34 ± 0.01
γ-terpineol	0.76 ± 0.01	0.94 ± 0.02	0.46 ± 0.02	0.15 ± 0.02
bornyl acetate	0.92 ± 0.06	0.47 ± 0.03		
geranyl acetate	0.12 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.16 ± 0.01
oxygenated monoterpenes	14.03 ± 0.86	14.93 ± 0.79	10.60 ± 0.46	3.77 ± 0.13
caryophyllenol-11	0.07 ± 0.01	0.23 ± 0.02	0.34 ± 0.02	0.96 ± 0.05
nerolidol	0.65 ± 0.03	1.58 ± 0.14	2.24 ± 0.14	2.45 ± 0.14
caryophyllene oxide		0.59 ± 0.05	0.68 ± 0.04	1.98 ± 0.11
spathulenol	0.43 ± 0.02	1.20 ± 0.10	1.79 ± 0.11	3.01 ± 0.17
humulen oxide (I)	0.11 ± 0.01	0.36 ± 0.03	0.55 ± 0.03	1.16 ± 0.07
T-cadinol			0.21 ± 0.01	0.50 ± 0.03
δ-cadinol	0.12 ± 0.01	0.35 ± 0.03	0.62 ± 0.04	1.70 ± 0.10
β-eudesmol				0.15 ± 0.01
α-cadinol	0.16 ± 0.01	0.45 ± 0.04	0.74 ± 0.05	2.11 ± 0.12
oxygenated sesquiterpenes	1.93 ± 0.09	5.81 ± 0.50	8.99 ± 0.54	17.51 ± 0.99
other compounds	0.26 ± 0.04	1.97 ± 0.08	3.01 ± 0.15	17.60 ± 1.01

RESULTS AND DISCUSSION

CO_2 Extraction. The content of juniper berry essential oils [d(20 °C) = 861.2 kg m^{-3}] determined by an official procedure (*22*) was 0.70 mL/100 g of the material. **Figure 2** shows the overall SCFE curves at a temperature of 40 °C as functions of

Table 2. Main Constituents (Area %) of the *J. communis* Supercritical CO_2 Extract Fractions Obtained in Different Extraction Time Intervals for Extraction Process at 40 °C and 90 bar

	extraction time (h)					
component	0–0.60	0.60–1.4	1.4–2.2	2.2–2.8	2.8–3.2	3.2–3.9
α-thujene	0.87 ± 0.03	0.10 ± 0.01				
α–pinene	2.76 ± 0.09	0.12 ± 0.01	0.31 ± 0.02	0.10 ± 0.01	0.08 ± 0.01	
α–fenchene	10–100 ppb					
camphene	10–100 ppb					
sabinene	2.71 ± 0.09	0.19 ± 0.01	0.60 ± 0.03	0.18 ± 0.01	0.11 ± 0.01	0.07 ± 0.01
β-pinene	0.53 ± 0.02					
β-myrcene	1.25 ± 0.04					
δ–3-carene	10–100 ppb					
α-terpinene	10–100 ppb					
p-cymene	0.05 ± 0.01					
limonene	1.12 ± 0.04					
γ-terpinene	6.16 ± 0.21	0.31 ± 0.02				
terpinolene	8.20 ± 0.28	0.74 ± 0.03	10–100 ppb	10–100 ppb	10–100 ppb	
monoterpenes	23.60 ± 0.81	1.45 ± 0.08	0.74 ± 0.04	0.28 ± 0.03	0.23 ± 0.03	0.07 ± 0.01
α-cubebene	0.77 ± 0.03	0.14 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	
α-copaene	0.43 ± 0.02	0.12 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	
β-elemene	2.56 ± 0.11	1.40 ± 0.05	0.30 ± 0.01	0.10 ± 0.01	0.29 ± 0.02	
β-caryophillene	3.29 ± 0.15	1.06 ± 0.04	0.18 ± 0.01	0.23 ± 0.01	0.23 ± 0.02	
γ-elemene	1.08 ± 0.05	1.39 ± 0.05	0.37 ± 0.01	0.14 ± 0.00	0.16 ± 0.01	
α-humulene	3.10 ± 0.14	1.24 ± 0.04	0.23 ± 0.01	0.07 ± 0.00	0.28 ± 0.02	0.14 ± 0.01
cis-β-farnesene	1.31 ± 0.06	0.28 ± 0.01	0.09 ± 0.01	0.06 ± 0.01	0.19 ± 0.02	
γ-cadinene	15.53 ± 0.68	8.45 ± 0.28	1.60 ± 0.06	0.48 ± 0.02	1.46 ± 0.06	0.27 ± 0.02
β-selinene	0.85 ± 0.04	0.63 ± 0.02	0.14 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	
α-farnesene	0.21 ± 0.01	0.32 ± 0.01	0.17 ± 0.02	0.09 ± 0.00	0.11 ± 0.01	
valencene	1.40 ± 0.06	1.01 ± 0.03	0.21 ± 0.02	0.06 ± 0.00	0.14 ± 0.01	
3,7-guaidiene	0.41 ± 0.02	0.38 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	
viridiflorene	0.84 ± 0.04	0.61 ± 0.02	0.14 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	
α-muurolene	1.11 ± 0.05	1.69 ± 0.06	1.06 ± 0.04	0.66 ± 0.02	0.61 ± 0.02	0.36 ± 0.02
δ-cadinene	1.11 ± 0.05	1.28 ± 0.04	0.38 ± 0.01	0.20 ± 0.01	0.32 ± 0.01	0.18 ± 0.01
α-calacorene	0.16 ± 0.01	0.34 ± 0.01	0.41 ± 0.01	0.45 ± 0.02	0.39 ± 0.01	0.44 ± 0.02
germacrene D	7.41 ± 0.33	9.59 ± 0.32	2.39 ± 0.09	0.79 ± 0.03	0.89 ± 0.03	0.30 ± 0.02
germacrene B	4.40 ± 0.19	12.20 ± 0.41	10.04 ± 0.36	7.40 ± 0.25	5.47 ± 0.21	4.06 ± 0.10
sesquiterpenes	47.20 ± 2.08	42.84 ± 1.43	18.54 ± 0.67	11.25 ± 0.38	11.62 ± 0.44	6.31 ± 0.27
terpinen-1-ol	0.27 ± 0.02	0.16 ± 0.01				
β-terpineol	0.63 ± 0.05	0.15 ± 0.01	0.16 ± 0.02	0.08 ± 0.01	0.06 ± 0.01	
(*cis*,*trans*)-verbenol	0.68 ± 0.05	0.21 ± 0.02	0.12 ± 0.02	0.09 ± 0.01	0.10 ± 0.01	
pinocarveole	1.27 ± 0.05	0.82 ± 0.03	0.15 ± 0.02	0.07 ± 0.01	0.14 ± 0.02	
borneol	0.30 ± 0.01	0.22 ± 0.01	0.05 ± 0.01			
terpinen-4-ol	4.97 ± 0.19	1.03 ± 0.04	0.11 ± 0.02	0.12 ± 0.02	0.15 ± 0.02	
α-terpineol	0.45 ± 0.02	0.15 ± 0.01	0.06 ± 0.01	0.05 ± 0.01		
γ-terpineol	0.42 ± 0.02	0.35 ± 0.02	0.11 ± 0.01	0.09 ± 0.01	0.05 ± 0.01	
bornyl acetate	0.35 ± 0.01	0.06 ± 0.00				
geranyl acetate	0.17 ± 0.01	0.10 ± 0.00				
oxygenated monoterpenes	12.17 ± 0.46	3.97 ± 0.14	1.02 ± 0.04	0.56 ± 0.02	1.12 ± 0.04	0.52 ± 0.02
caryophyllenol-11	0.30 ± 0.01	0.67 ± 0.03	0.76 ± 0.02	0.60 ± 0.02	0.41 ± 0.01	0.35 ± 0.02
nerolidol	1.08 ± 0.05					
caryophyllene oxide	1.00 ± 0.05	1.27 ± 0.05	0.86 ± 0.03	0.64 ± 0.03	0.57 ± 0.02	0.20 ± 0.01
spathulenol	1.45 ± 0.07	1.86 ± 0.07	0.84 ± 0.03	0.46 ± 0.02	0.56 ± 0.02	0.27 ± 0.01
humulen oxide (I)	0.44 ± 0.02	0.89 ± 0.03	1.06 ± 0.03	1.07 ± 0.04	0.88 ± 0.03	0.85 ± 0.04
T-cadinol	0.19 ± 0.01	0.39 ± 0.01	0.45 ± 0.01	0.39 ± 0.02	0.37 ± 0.01	0.25 ± 0.01
δ-cadinol	0.68 ± 0.03	1.44 ± 0.05	1.97 ± 0.06	2.18 ± 0.09	2.02 ± 0.07	1.98 ± 0.09
β-eudesmol	0.08 ± 0.00	0.20 ± 0.01	0.33 ± 0.01	0.39 ± 0.02	0.41 ± 0.01	0.41 ± 0.02
α-cadinol	0.87 ± 0.04	2.02 ± 0.08	3.15 ± 0.10	3.88 ± 0.16	3.56 ± 0.13	4.17 ± 0.19
oxygenated sesquiterpenes	8.07 ± 0.39	12.55 ± 0.47	14.04 ± 0.43	13.85 ± 0.57	11.92 ± 0.43	11.24 ± 0.52
other compounds	8.96 ± 0.34	39.18 ± 1.62	65.65 ± 2.03	74.05 ± 3.26	75.12 ± 3.12	81.86 ± 4.05

extraction pressure and time. The total yield of the supercritical CO_2 extraction, calculated by the weight of the material charged in the extractor at pressures of 80, 90, and 100 bar was 0.65 (±0.03), 1.48 (±0.04), and 4.00 (±0.10)%, respectively. At the constant temperature, an increase in supercritical carbon dioxide (from 280, through 490, to 630 kg m^{-3}) by increasing pressure resulted in a solubility increase and hence a solvent power increase. Therefore, the total yield increased with pressure. However, at higher densities, an intensive co-extraction of high-boiling nonvolatile components with volatile components was taking place.

The constituents of the extracts are grouped into five main groups: the first four are monoterpenes (M), sesquiterpenes (S), oxygenated monoterpenes (OM), and oxygenated sesquiterpenes (OS). The fifth group, labeled as the O group, comprises higher molecular mass compounds (such as fats, waxes, and some other unidentified peaks). More than 200 constituents were detected in various juniper berry extracts, and the contents of 50 identified compounds are reported in this work: 13 monoterpenes, 18 sesquiterpenes, 10 oxygenated monoterpenes, and 9 oxygenated sesquiterpenes.

The chemical composition of the extract fractions obtained during successive extraction time intervals at 80, 90, and 100 bar pressures are presented in **Tables 1**, **2**, and **3**, respectively. The dependence of the percentage yields of the investigated

Table 3. Main Constituents (Area %) of the *J. communis* Supercritical CO_2 Extract Fractions Obtained in Different Extraction Time Intervals for Extraction Process at 40 °C and 100 bar

	extraction time (h)					
component	0–0.30	0.30–0.45	0.45–0.60	0.60–0.75	0.75–0.90	0.90–1.20
α-thujene	0.12 ± 0.02	0.07 ± 0.01				
α-pinene	7.17 ± 0.26	0.13 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.08 ± 0.00	0.07 ± 0.00
α-fenchene	0.10 ± 0.02					
camphene	10–100 ppb					
sabinene	3.33 ± 0.11	0.08 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.10 ± 0.01	0.13 ± 0.01
β-pinene	0.67 ± 0.03	10–100 ppb	10–100 ppb	10–100 ppb	10–100 ppb	0.07 ± 0.00
β-myrcene	1.90 ± 0.06					
D3-carene	10–100 ppb					
α-terpinene	10–100 ppb					
limonene	0.23 ± 0.03					
p-cymene	0.69 ± 0.06	10–100 ppb	10–100 ppb	10–100 ppb	10–100 ppb	0.06 ± 0.00
γ-terpinene	0.78 ± 0.06	0.07 ± 0.01				
terpinolene	0.80 ± 0.06	0.15 ± 0.01				
monoterpenes	16.08 ± 0.73	0.52 ± 0.05	0.25 ± 0.02	0.30 ± 0.02	0.19 ± 0.01	0.33 ± 0.02
α-cubebene	0.81 ± 0.04	0.13 ± 0.00	10–100 ppb	0.06 ± 0.00		
α-copaene	0.54 ± 0.03	0.14 ± 0.00	10–100 ppb	0.05 ± 0.00		
β-elemene	2.93 ± 0.11	1.86 ± 0.06	0.42 ± 0.02	0.12 ± 0.01	0.05 ± 0.00	
β-caryophillene	2.68 ± 0.15	1.17 ± 0.04	0.20 ± 0.01	0.05 ± 0.00		
γ-elemene	2.64 ± 0.09	1.70 ± 0.05	0.54 ± 0.02	0.19 ± 0.01	0.06 ± 0.00	
α-humulene	3.22 ± 0.17	1.43 ± 0.04	0.28 ± 0.01	0.06 ± 0.00		
cis-β-farnesene	0.62 ± 0.03	0.34 ± 0.01	10–100 ppb	0.09 ± 0.00	10–100 ppb	0.08 ± 0.00
γ-cadinene	12.43 ± 0.72	10.08 ± 0.31	2.20 ± 0.08	0.47 ± 0.02	0.23 ± 0.01	0.09 ± 0.00
β-selinene	1.04 ± 0.04	0.70 ± 0.02	0.22 ± 0.01	0.05 ± 0.00		
α-farnesene	0.48 ± 0.03	0.30 ± 0.01	0.21 ± 0.01	0.11 ± 0.00		
valencene	2.17 ± 0.07	1.25 ± 0.04	0.34 ± 0.01	0.07 ± 0.00	0.06 ± 0.00	
3,7-guaidiene	0.61 ± 0.03	0.41 ± 0.01	0.14 ± 0.01	0.04 ± 0.00		
viridiflorene	0.73 ± 0.04	0.63 ± 0.02	0.22 ± 0.01	0.05 ± 0.00		
α-muurolene	2.82 ± 0.09	1.73 ± 0.05	1.36 ± 0.05	0.76 ± 0.04	0.48 ± 0.02	0.14 ± 0.01
δ-cadinene	2.35 ± 0.08	1.44 ± 0.04	0.61 ± 0.02	0.24 ± 0.01	0.13 ± 0.01	0.15 ± 0.01
α-calacorene	0.26 ± 0.01	0.34 ± 0.01	0.43 ± 0.02	0.54 ± 0.03	0.38 ± 0.01	0.29 ± 0.01
germacrene D	8.85 ± 0.45	8.97 ± 0.28	3.94 ± 0.15	1.05 ± 0.05	0.40 ± 0.02	0.09 ± 0.00
germacrene B	10.60 ± 0.57	13.07 ± 0.40	13.16 ± 0.50	8.10 ± 0.38	5.80 ± 0.23	2.13 ± 0.08
sesquiterpenes	56.80 ± 2.77	46.37 ± 1.43	24.96 ± 0.95	12.75 ± 0.60	8.06 ± 0.32	3.13 ± 0.11
terpinen-1-ol	0.19 ± 0.03	0.16 ± 0.02				
β-terpineol	0.46 ± 0.04	0.11 ± 0.01	0.15 ± 0.02	0.06 ± 0.01		
(*cis*,*trans*)-verbenol	0.53 ± 0.05	0.11 ± 0.01	0.05 ± 0.01			
pinocarveole	1.47 ± 0.07	0.42 ± 0.02	0.15 ± 0.02	0.06 ± 0.01		
borneol	0.27 ± 0.01	0.13 ± 0.01	0.05 ± 0.01			
terpinen-4-ol	4.63 ± 0.22	0.87 ± 0.04	0.10 ± 0.02	0.05 ± 0.01		
α-terpineol	0.47 ± 0.02	0.15 ± 0.02	0.11 ± 0.01			
γ-terpineol	0.44 ± 0.02	0.21 ± 0.01	0.18 ± 0.02	0.05 ± 0.01		
bornyl acetate	0.25 ± 0.01	0.05 ± 0.00	0.05 ± 0.01	0.05 ± 0.01		
geranyl acetate	0.16 ± 0.01	0.13 ± 0.01				
oxygenated monoterpenes	9.97 ± 0.48	2.96 ± 0.14	0.76 ± 0.03	0.80 ± 0.06	0.14 ± 0.04	0.21 ± 0.03
caryophyllenol-11	0.53 ± 0.03	0.51 ± 0.02	0.75 ± 0.03	0.62 ± 0.02	0.43 ± 0.02	0.15 ± 0.01
spathulenol	0.92 ± 0.03					
thujyl alcohol	1.36 ± 0.05	1.04 ± 0.04	0.94 ± 0.03	0.64 ± 0.02	0.50 ± 0.02	0.12 ± 0.01
caryophillene oxide	1.37 ± 0.07	1.68 ± 0.07	0.95 ± 0.03	0.52 ± 0.02	0.25 ± 0.01	0.13 ± 0.01
gleenol	0.72 ± 0.03	0.74 ± 0.03	0.92 ± 0.03	0.90 ± 0.03	0.72 ± 0.03	0.46 ± 0.02
cedrol	0.41 ± 0.02	0.41 ± 0.02	0.44 ± 0.02	0.62 ± 0.02	0.36 ± 0.01	0.16 ± 0.01
T-cadinol	1.16 ± 0.05	1.27 ± 0.05	1.68 ± 0.06	1.91 ± 0.06	1.70 ± 0.06	1.25 ± 0.05
torreyol	0.23 ± 0.02	0.17 ± 0.01	0.23 ± 0.01	0.20 ± 0.01	0.22 ± 0.01	0.25 ± 0.01
α-cadinol	1.54 ± 0.08	1.75 ± 0.07	2.65 ± 0.09	3.15 ± 0.11	3.16 ± 0.12	2.65 ± 0.11
oxygenated sesquiterpenes	8.94 ± 0.39	11.00 ± 0.45	12.72 ± 0.44	12.08 ± 0.41	9.82 ± 0.36	6.48 ± 0.27
other compounds	8.21 ± 0.26	39.24 ± 1.43	61.32 ± 2.30	74.06 ± 3.38	81.80 ± 2.72	89.85 ± 4.15

compound groups on extraction time are shown in **Figures 3**, **4**, and **5** for extractions at 80, 90, and 100 bar, respectively.

Extract yielded during the first 0.6 h (**Table 1**) consisted predominately of monoterpenes (47.45%) with the highest levels of α-pinene (21.45% of the total yield), sabinene (11.75%), β-myrcene (4.97%), limonene (2.82%), β-pinene (1.96%), terpinolene (1.14%), and γ-terpinene (1.12%). The content of sesquiterpenes was at the level of 35.82%, and among sesquiterpenes, the following dominated: γ-cadinene (12.12%), β-caryophillene (4.22%), germacrene D (3.85%), and α-humulene (3.09%). Oxygenated monoterpenes were found in this extract in the content of 14.03% whose main components were terpinen-4-ol (4.60%), pinocarveole (1.52%), and α-terpineol (1.50%). In this extract, the quantities of oxygenated sesquiterpenes were very low and their total amount was 1.93%. Other compounds such as fats, waxes, and some other unidentified peaks of compounds with higher molecular mass were present at the level of 0.26%.

After an extraction period of 0.6 h at a pressure of 80 bar, concentrations of the monoterpenes in the extract fractions decreased significantly, while the quantities of the other groups increased (**Table 1** and **Figure 3**). Considering the masses of the yielded oil fractions (data not shown), it could be calculated that before the end of the first 0.6 h, 99% of monoterpenes had already been extracted from the berries at all pressures. It was possible to extract oxygenated monoterpenes almost completely

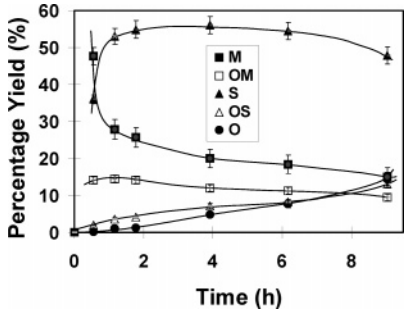

Figure 3. Time change of the compound groups' percentage yields for extractions at 80 bar and 40 °C. Compound groups: monoterpenes (M), (■); oxygenated monoterpenes (OM), (□); sesquiterpenes (S), (▲); oxygenated sesquiterpenes (OS), (△); and other (O), (●).

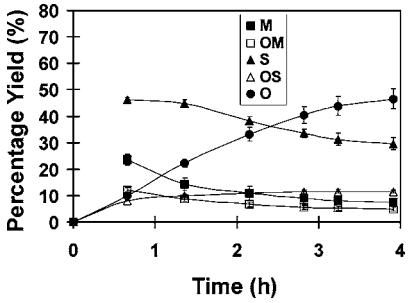

Figure 4. Time change of the compound groups' percentage yields for extractions at 90 bar and 40 °C. Explanation for used abbreviations and symbols is the same as the one given in **Figure 2**.

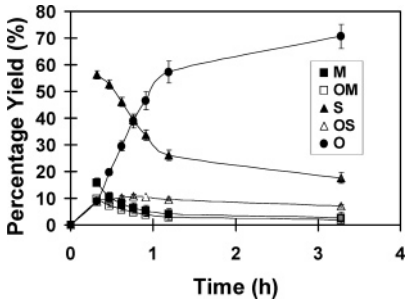

Figure 5. Time change of the compound groups' percentage yields for extractions at 100 bar and 40 °C. Explanation for used abbreviations and symbols is the same as the one given in **Figure 2**.

at 100 bar in 0.5 h and at 90 bar in 1.2 h. Contrary to that, during an extraction period of 4 h at 80 bar, it was possible to extract only 75% of the maximum yielded value of OM at 100 bar. The content of OMs in the fractions collected after the first extraction period of 0.6 h at 90 and 100 bar was 12.17 (±0.49) and 5.54 (±0.38)%. Intensive extraction of sesquiterpenes could be by no means avoided at any pressure, but at the beginning of the process (the first 0.5 h) at 80 bar, they were extracted about 8 and 3 times slower than at 100 and 90 bar, respectively. Oxygenated sesquiterpenes were yielded at fast, constant extraction rates at 100 and 90 bar in 1.2 and 3 h, respectively. This initial fast extraction period was consequently followed by a much slower extraction of OS.

Fractions enriched with high-boiling components were obtained by increasing the supercritical CO_2 density. After extraction at 80 bar in the period of up to 3 h, cuticular waxes, fats, resins, and other compounds of high molecular masses could be avoided to a high degree. The contents of these compounds (O) in the extracts obtained during the first 0.6 h of extractions at 90 and 100 bar were 9.96 (±0.69) and 30.49

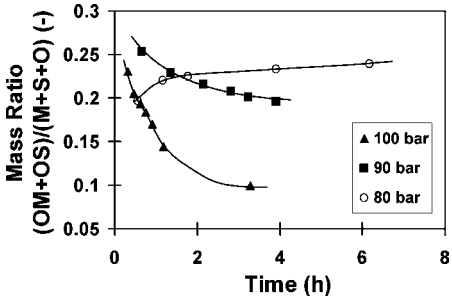

Figure 6. Mass ratio of the oxygenated terpenes to the other compound groups in the extracts obtained at 40 °C and pressures of 80, 90, and 100 bar.

(±1.55)%. Therefore, SCFEs of juniper berries at higher pressures should be conducted in an apparatus with two separators. On the other hand, in the process of essential oil deterpenation at 80 bar pressure and 40 °C, monoterpenes might easily be removed after a short extraction period. This monoterpene fraction might be used for other purposes, antimicrobial or in the future for ecologically acceptable pest control.

During the first 0.6 h of extractions at 90 and 100 bar, the fractions yielded consisted respectively of 47.20 (±1.74) and 45.45 (±2.36)% sesquiterpenes and 8.07 (±0.35) and 10.65 (±0.52)% oxygenated sesquiterpenes. Because the oxygenated terpenes give a more relevant contribution to fragrance than the non-oxygenated ones, the percentage of oxygenated terpenes in the extracts was used as an indicator of the quality of the oil. The mass ratio of oxygenated terpenes to the sum of monoterpenes, sesquiterpenes, and other compounds in the extracts obtained at 40 °C and pressures of 80, 90, and 100 bar is shown in **Figure 6**.

Because of an increased extraction of higher molecular mass compounds (O) with extraction time, the ratio (OM + OS) to (M + S + O) decreased in time for extractions at 90 and 100 bar. Results indicate that extraction, at 90 bar and 40 °C, lasting up to 2 h (∼40 g of CO_2/g of material), could give the highest values of the desired mass ratio. Because of decreased sesquiterpene extraction rates after 2 h of extraction and a rate of OS extraction which is still high, a process lasting up to 3 h (∼60 g of CO_2/g of material) would increase the OS/S mass ratio.

Fractions with different contents of various terpenoid groups were obtained by varying the extraction pressure and time in the process of supercritical CO_2 extraction of *J. communis* L. The concentration of terpene compounds, which are extracted equally fast and, as a rule, show synergy and have similar activities, would result in obtaining genuine natural products, which could serve desired purposes.

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