Fractionation of the essential oil from juniper (*Juniperus communis* L.) berries by hydrodistillation and rectification

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Abstract

This paper deals with fractionation of the essential oil from juniper (*Juniperus communis* L.) berries by hydrodistillation (HD) and simultaneous hydrodistillation and rectification (SHDR) at atmospheric pressure. A rectification column was filled with the NORMAG packing A type and had 36 theoretical stages. In the present study, higher essential oil yield was obtained by HD (1.34 mass %) than by SHDR (1.17 mass %). However, mass fraction distributions of monoterpene hydrocarbons (MHs), oxygenated monoterpenes (OMs) and sesquiterpene hydrocarbons (SHs), observed for the two separation methods, differed from each other. In the SHDR process increased contents of high volatile components (predominantly MHs) were obtained in the initial fractions as well as increased contents of low volatile components (dominantly SHs) in the last fractions. Also, this method increased the separation degree of OMs.

Keywords: common juniper berries, essential oil, fractionation, hydrodistillation, simultaneous hydrodistillation and rectification.

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Common juniper (*Juniperus communis* L.) is an evergreen coniferous plant of the genus *Juniperus* (Cupressaceae). It is a perennial dioecious, rarely monoecious, tough shrub or a tree with a conical crown [1]. It is widely distributed throughout mountain areas in Southern and Eastern Europe, Central Asia, North America and North Africa [2]. In Serbia, *J. communis* is mostly distributed in the areas of Kosovo and Metohija (particularly on the slopes of mountains Šar planina, Mokra Gora, Rogozna and Kopaonik) and in the central Serbia (Užice, Bajina Bašta and Prijepolje) [1].

Common juniper berries (*Fructus Juniperi*) are generally used for production of essential oil, teas, deodorizer for alcohol distillates, additives and preservatives utilized for food taste improvements [1]. By processing of ripe fruit berries, one can obtain essential oil, extracts, aqueous mixtures, alcoholic mixtures, flavoured drinks, natural alcohol distillates, organic fertilizers and briquettes [3]. According to its composition, common juniper is classified as a plant containing organic acids [4], while in respect to the pharmacological properties it is regarded as a diuretic, stomachic, carminative and antiseptic. The most important bioactive component of common juniper berries is the essential oil that consists mainly of monoterpene and sesquiterpene hydrocarbons and their derivates [5]. The content of individual components in the essential oil strongly depends on the geographical location, climate conditions, harvesting time, treatment of collected fruits, storage conditions and the procedure used to obtain the oil [1]. In fact, qualitative differences of common juniper berry essential oils are partially due to high biological variations of the raw material [6]. In addition, significant differences in composition of monoterpenes occur between juniper plants from the same location [7]. The oils in common juniper berries at high localities (~2000 m) are of sabinene chemotype while those originating from lower localities are of α-pinene chemotype [7].

As other essential oils, the common juniper essential oil is usually obtained from the ground berries by hydrodistillation (HD). Pure components and fractions of the essential oils often exhibit considerably stronger pharmacological properties and hence have a far larger commercial value than the essential oil itself [4]. Therefore, considerable effort should be focused on searching for more efficient separation procedures, which would result in isolation of pure components or oil fractions [8]. The major problems that are often encountered in the oil fractionation process are instability, oxidation and transformation of some of the components (usually less volatile) at the distillation tempe-

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rature under atmospheric pressure. These problems can be overcome by rectification under vacuum, steam rectification or fractional rectification during hydrodistillation of essential oils from plant materials. So far, supercritical fluid extraction with carbon dioxide [9–12] and fractional vacuum distillation [13–19] are most frequently employed for the fractionation of various essential oils. Common juniper essential oil has been fractionated only by vacuum distillation in a pilot distillery plant [3,14]. Up to now, to the best of our knowledge, hydrodistillation combined with fractionation of essential oils by rectification at atmospheric pressure, called here simultaneous hydrodistillation and rectification (SHDR) was not described in literature.

This work is focused on fractionation of the essential oil from common juniper berries by both conventional HD and SHDR under atmospheric pressure. The aim of the study was to compare chemical compositions of the fractions collected during HD and SHDR and to estimate the possibility of obtaining fractions enriched with some valuable components. These fractions can possibly serve then as starting raw materials for production of the desired components in pure or enriched forms.

EXPERIMENTAL

Material

Ripe berries of common juniper harvested in the area of Leposavić, south side of the Kopaonik Mountain (longitude: 20°48E, latitude: 43°17N and altitude: about 800 m), in October 2005, were used. The berries were cleaned, dried in a shadow and stored in multilayered paper bags. Shortly before the study, the berries were added to a 2 L blender (Bosh MMB1001, 500 W) equipped with a stainless steel knife and ground at the high speed for 2 min. A small loss of the volatile part of the essential oil during the grinding was ignored as it was intrinsic to the very short comminution method used.

HD

A Clevenger apparatus with a 5 dm^3 round–bottom flask was employed for HD (Fig. 1a). The ground common juniper berries (1100 g) were transferred to the flask and distilled water (3300 cm^3) was added, yielding the berries-to-water ratio (hydromodule) of 1:3. The distillation rate was 8.3±0.5 cm^3/min. With the appearance of the first essential oil drop, the HD process was timed, and the volume of the extracted oil in a graduated oil separatory tube was recorded. Then, the obtained oil was drained from the graduated oil separatory tube into a receiver vessel by opening the corresponding valve. The essential oil was collected within 6 h in 20 fractions, but only the first 17 fractions collected within the initial 285 min are shown in Table 1 in order to compare the fractions collected within the same time duration in the HD and SHDR processes. After drying by using anhydrous sodium sulfate, the collected fractions were held in dark flasks in a refrigerator (4 °C) until the gas chromatography (GC) analysis. The experiments were carried out in triplicate.

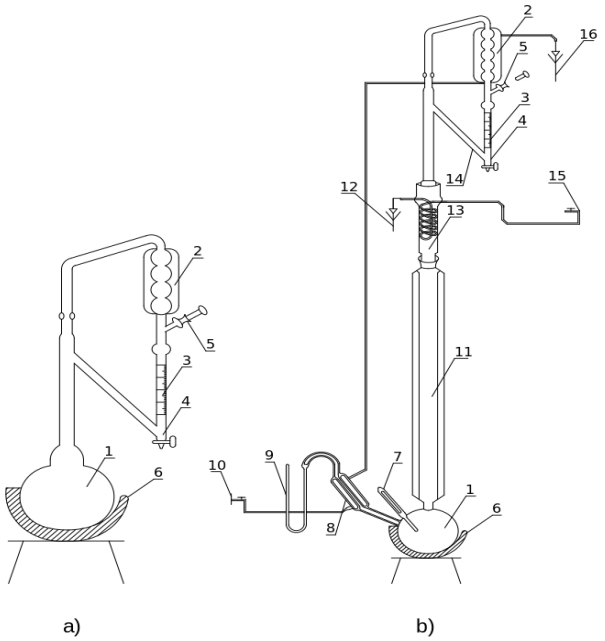

Figure 1.Experimental set-up for: a) hydrodistillation (HD) and b) simultaneous hydrodistillation and rectification (SHDR): 1) distillation flask, 2) condenser, 3) graduated oil separatory tube, 4) tap with an extension, 5) side tube with a ground stopper, 6) heating mantle, 7) thermometer, 8) condenser, 9) U–manometer, 10) taps, 11) rectifying column, 12) drain of hot water, 13) dephlegmator, 13) tap with fittings, 14) return tube, 15) water supply and 16) drain of hot water.

SHDR

For SHDR, the experimental set-up is shown in Figure 1b and consisted of a round–bottom flask (5 dm^3), a rectification column, a dephlegmator, and a Clevenger apparatus. The column (inner diameter: 29 mm and height: 2 m) filled with NORMAG packing type A (a 3 mm×18 mm rectangular mesh, made of 0.175 mm wire, bent in a cylindrical form 3 mm in diameter) was previously experimentally determined to have 36 theoretical stages by using a standard mixture of CCl_4–C_6H_6 [20]. Grounded common juniper berries along with distilled water in the same mass ratio of 1:3 as employed in HD, were added to the flask. Cooling water was fed through the condensers (2 and 8) and the dephlegmator by opening the water supplying valves (10 and 15). Then, both heaters (320 W each) were turned on while a variable transformer was set at 150 V. When the temperature in the distillation flask reached 80 °C, the voltage was reduced to 120 V in

order to avoid flooding of the rectification column. When the vapour appeared in the dephlegmator (after heating for about 80 min), the flow rate of cooling water was adjusted by the valve (15) to keep the condensation zone about 2 to 3 cm above the connection of the dephlegmator to the rectification column. This way, stable operation of the rectification column under total reflux was set up. The pressure drop through the rectification column was continuously monitored by an U-manometer. At the beginning of the process, the pressure drop was 5340 Pa, while during the operation under total reflux (after about 100 min from the start), it was stabilized at 4400 Pa with the temperature at the column bottom of 101 °C. From this moment on, sampling started. The valve (15) was closed to turn off the cooling water supply to the dephlegmator. As the condensate appeared in the separatory tube (3), collection of fractions was timed while the pressure drop was constant at 4005 Pa. By fully opening the valve, essential oil fraction and floral water (hydrosol) were drained into a separatory flask, after which floral water was returned into the separatory tube (3) through the side tube (5). Sampling lasted no longer than 20 s. The essential oil was collected within 285 min in 8 fractions (Table 2). After drying by using anhydrous sodium sulfate, collected fractions were held in dark flasks in a refrigerator (4 °C) until the GC analysis. The experiments were carried out in triplicate.

GC/FID and GC/MS analysis

Gas chromatography-flame-ionization detection (GC/FID) analysis of essential oils was carried out on a Hewlett-Packard, model 5890 Series II gas chromategraph (Waldbronn, Germany), equipped with split-splitless injector and automatic liquid sampler (ALS), attached to HP-5 silica capillary column (30 m×0.32 mm, 0.25 μm film thickness) and fitted to the flame ionization detector (FID). The operational qualification/performance verification (OQ/PV) test showed that the reproducibility of peak areas was within ±1%. Carrier gas flow rate (H_2) was 1 ml/min at 210 °C (constant pressure mode), injector temperature was 220 °C, detector temperature 240 °C, while the column temperature was linearly programmed from 60–240 °C (at a rate of 3 °/min), and held isothermally at 240 °C next 10 min. Solutions of essential oils in ethanol (~1%) were consecutively injected by ALS (1 μl, split mode, 1:30). Area percent reports, obtained as the result of standard processing of chromatograms, were used as the base for quantification purposes. Quantification was accomplished by a normalization method, following recommendation supplied by standard ISO 8897:2010 [21].

The same chromatographic analytical conditions as for GC/FID were employed for gas chromatography/ /mass spectrometry (GC/MS) analysis, along with the capillary column HP-5MS (30 m×0.25 mm, 0.25μm film thickness), using HP G 1800C GCD Series II Electron ionization detector (EID) system (Hewlett-Packard, Palo Alto, CA, USA). Instead of hydrogen, helium was used as the carrier gas. Transfer line was heated at 240 °C. Mass spectra were acquired in EI mode (70 eV), in *m*/*z* range 40–400. Sample solutions in ethanol (~1%) were injected by ALS (1 μl, split mode: 1:30). The constituents were identified by comparison of their mass spectra with those from Wiley275 and NIST/NBS libraries, using different search engines, Probability Merge Search (PBM) included in instruments G1701DA. ver. D.00.00.38 data station software and NIST 2.0 search program. Experimental values for retention indices were determined by the use of calibrated automated mass spectral deconvolution and identification system software (AMDIS ver.2.64), compared with those from available literature, [22] and used as an additional tool to confirm MS findings.

RESULTS AND DISCUSSION

Kinetics of extraction processes

Masses of the essential oil and oil fractions collected during HD and SHDR processes are shown in Tables 1 and 2. Figure 2 shows variations of the essential oil yield during HD and SHDR. Within the first 5 min (the extraction time interval of the first fraction), the essential oil extraction rate of SHDR was higher than that of HD. After about 110 min, extraction rates of the two methods became equal, while in the final phase the extraction rate of HD became higher than that of SHDR. The maximum essential oil yield obtained by SHDR in 285 min was 1.17±0.01mass%, which was lower by about 12.7% than that obtained by HD within the same period of time (1.34±0.01mass%). Variations in the essential oil yield and the extraction rate, and particularly the lower essential oil yield observed for SHDR, were attributed to the hold−up of the essential oil (especially its highly volatile components) in the rectification column during the initial period of operation.

Chemical composition of the essential oils

Chemical compositions of oil fractions collected during HD and SHDR are compared in Figure 3. Differences among qualitative chemical compositions of the two mixtures of all collected fractions were negligible. However, their quantitative chemical compositions differed only slightly, which could be attributed to different time durations of essential oil extraction under various process conditions [23]. The HD and SHDR mixtures are mostly composed of *α*-pinene, sabinene, myrcene, *p*-cymene, 1-terpinen-4-ol, *γ*-muurolene, etc.

Table 1.Chemical composition of common juniper essential oils obtained by HD and SHDR; BP – boiling point at 760 mm Hg; na – not available, MH – monoterpene hydrocarbons; OM – oxygenated monoterpene; SH – sesquiterpene hydrocarbons

No.	Component	BP, °C	Type	Mean mass fraction, %	
				HD	SHDR
1	α-Pinene	157.0±4[a]	MH	38.0±0.35	36.4±0.35
2	Sabinene	163.7[a]	MH	17.1±0.16	8.9±0.08
3	Myrcene	167.2[a]	MH	13.3±0.13	10.8±0.10
4	β-pinene	163–166[a]	MH	1.4±0.01	1.7±0.01
5	*p*-Cymene	177±1[a]	MH	4.4±0.04	5.4±0.05
6	Limonene	178±4[a]	MH	2.3±0.02	3.1±0.03
7	γ-Terpinene	183.05[a]	MH	1.4±0.01	1.8±0.01
8	1-Terpinen-4-ol	212[a]	OM	3.6±0.03	9.1±0.09
9	α-Terpineol	217.55[a]	OM	0.3±0.00	0.7±0.00
10	α-Cubebene	245–246[b]	SH	0.5±0.00	0.7±0.00
11	β-Elemene	251–253[b]	SH	1.0±0.01	0.4±0.00
12	β-Caryophyllene	256–259[b]	SH	1.6±0.01	1.5±0.01
13	Isocaryophyllene	266–268[b]	SH	0.6±0.00	1.8±0.01
14	γ-Elemene	257–259[b]	SH	1.3±0.01	0.7±0.00
15	γ-Muurolene	171–172[b]	SH	4.4±0.04	0.2±0.00
16	Germacrene D	279–280[b]	SH	0.7±0.00	4.9±0.04
17	β-Cadinene	273–276[b]	SH	1.2±0.01	1.5±0.01
18	γ-Cadinene	271–276[b]	SH	3.1±0.03	0.6±0,00
19	δ-Cadinene	279–280[b]	SH	0.5±0.00	2.1±0.02
20	Germacrene B	287–288[b]	SH	0.3±0.00	3.6±0.03
	MH			77.7±0.40	68.0±0.35
	SH			15.7±0.08	18.0±0.09
	OM			3.9±0.02	9.8±0.05
Total hydrocarbon compounds				93.4±0.39	86.0±0.32
Total oxygenated compounds				3.9±0.02	9.8±0.05
Total (1–20)				97.3±0.39	95.8±0.33

[a]http://webbook.nist.gov/chemistry/, [b]http://www.thegoodscentscompany.com

Table 2. Separation efficiency of the three groups of compounds in the individual fractions in relation to their total mass in the essential oil

Method	Fraction	Time interval min	MHs %	Oms %	SHs %
HD	I	0–10	43.6	10.1	14.1
	II	20–40	12.5	12.4	13.4
	III	210–240	4.4	10.2	12.6
SHDR	I	0–10	89.5	47.4	10.0
	II	15–45	2.1	10.3	26.3
	III	195–285	1.3	2.6	5.1

In order to describe the influence of the applied techniques on the chemical composition of the obtained common juniper berry essential oils, only 20 components (out of 34 identified components) with mass fractions higher than 0.5% in both essential oils were analyzed. The selected components constituted about 97.3 and 95.8% of the total mass of essential oils obtained by HD and SHDR, respectively. Table 1 presents mass fractions of the selected components in HD and SHDR mixtures of all collected fractions; the components are shown in the order of appearance from the GC column. These components were further classified into three groups: high volatile monoterpene hydrocarbons (MHs): α-pinene, sabinene, myrcene, β-pinene, *p*-cymene, limonene and γ-terpinene (b.p. 157−183 °C); medium volatile oxygenated monoterpenes (OMs): 1-terpinen-4-ol and α-terpineol (b.p. 212–218 °C) and low volatile sesquiterpene hydrocarbons (SHs): α-cubebene, β-elemene, β-caryophyllene, isocaryophyllene, γ-elemene, γ-muurolene, germacrene D, β-cadinene, γ-cadinene, δ-cadineneandgermacrene B (b.p.245−288 °C). Both HD and SHDR mixtures contained mostly MHs, followed by SHs, while the contents of OMs were the lowest.

Chemical composition of the essential oil fractions

In Figure 4, fractions collected during HD and SHDR were compared regarding their chemical composition.

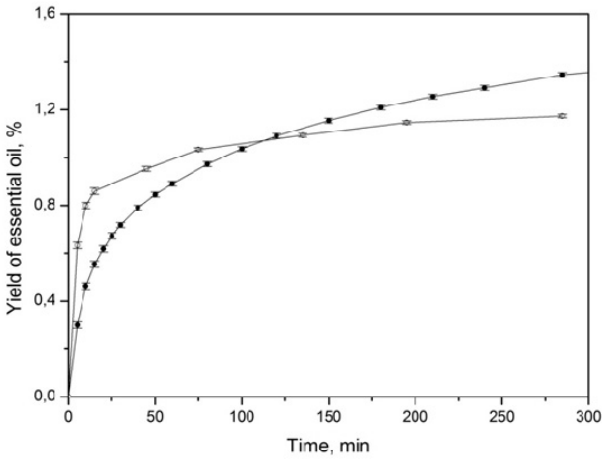

Figure 2. Variations of the essential oil yield during HD (●) and SHDR (○).

Regardless the separation technique, the initial fractions of the essential oil contained mainly high volatile MHs that reached the maximum in the first fractions (0−10 min). In HD, the MHs mass fraction decreased from 10 to 25 min, then stayed constant for the next 15 min and finally gradually decreased until the end of the process. For SHDR, the MHs mass fraction rapidly decreased in the third fraction and then gradually increased in next fractions until the end of the process. This time distribution of MHs results from their concentration profile established along the column height during the operation under total reflux. By taking out first fractions, the established concentration profile in the rectification column is disturbed. After the third fraction, MHs vapours come up from the bottom part of the rectification column and the distillation flask. The OMs mass fraction slightly increased till the fourth fraction and then stayed constant until the end of the HD process. In SHDR, the OMs mass fraction had the maximum values in the third fraction, and then rapidly decreased until reaching a plateau that continued until

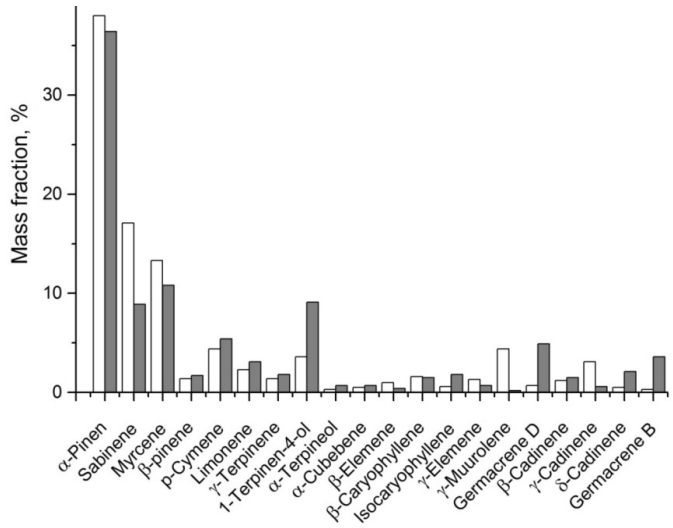

Figure 3. Comparison of average chemical compositions of the essential oils obtained during HD (white boxes) and SHDR (gray boxes).

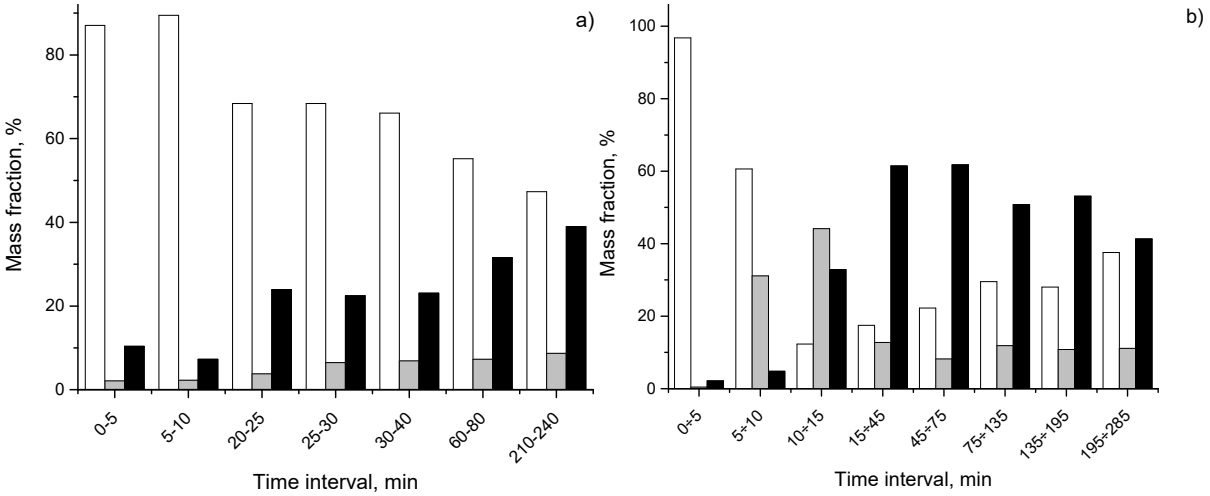

Figure 4. Time distributions of mean mass fractions of MHs (white boxes), SHs (black boxes) and OMs (gray boxes): a) HD and b) SHDR.

the end of the process. The SHs mass fraction gradually increased and reached its maximum value at the end of the HD process, and in the middle of SHDR (fractions collected from 15 to 75 min), staying at relatively high values until the end of the process, which is attributed to boiling temperatures of these components.

In order to compare separation efficiencies of the two techniques regarding the three groups of compounds (MHs, OMs and SHs) relative to their total amounts in the essential oil, three representative fractions were analyzed. As it can be seen in Table 2, independently of the applied technique, high volatile MHs were collected mainly at the beginning of the process (the first fraction), but SHDR (89.5%) was more efficient than the HD (43.6%). Also, the separation degree of medium volatile OMs was the highest in the first fraction of the SHDR. The separation degree of the least volatile SHs by SHDR was low due to their detainment in the distillation flask and column and was much lower than that obtained by HD. The separation degrees of MHs and OMs achieved by the SHDR in the second and third fractions were very low and lower than those obtained by the HD.

CONCLUSION

The present study shows a higher essential oil yield using the HD process (1.34 mass%) than the SHDR (1.17 mass%), but the essential oil obtained by the latter method is of higher quality indicated by a larger amount of more valuable, high volatile components. The SHDR increases separation of high volatile components (predominantly MHs) in fractions obtained at the beginning of the process, as well as of low volatile components (dominantly SHs) in the last fractions obtained at the end of the process. Also, this method increases the separation degree of OMs. Probably, by increasing the number of plates and by adjusting the reflux ratio (for instance, a reflux to takeoff ratio), more efficient separation of the essential oil could be achieved. In addition, the obtained fractions could be further fractionated by rectification in order to isolate individual components of high purity.

Acknowledgements

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IZVOD

FRAKCIONISANJE ETERIČNOG ULJA KLEKE (*Juniperus communis* L.) HIDRODESTILACIJOM I REKTIFIKACIJOM

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(Naučni rad)

U radu je opisano frakconisanje etarskog ulja kleke (*Juniperus communis* L.) hidrodestilacijom (HD) i istovremenom hidrodestilacijom i rektifikacijom (SHDR) na atmosferskom pritisku. Rektifikaciona kolona je napunjena pakovanjem NORMAG tip A i ima 36 teoretskih podova. Istraživanje je pokazalo da se veći prinos etarskog ulja dobija primenom HD (1,34%) nego primenom SHDR (1,17%). Međutim, vremenska raspodela masenih udela u frakcijama monoterpena (MH), oksigenovanih monoterpena (OM) i seskviterpena (SH), uočena kod dve separacione metode, međusobno se razlikuje. Kod SHDR je povećan sadržaj lako isparljivih komponenata (i to pretezno MH) u početnim frakcijama i teškoi sparljivih komponenata (dominantno SH) u poslednjim frakcijama. Takodje, ovom metodom je povećan stepens eparacije OM.

Ključne reči: • Kleka • Eterično ulje • Frakcionisanje • Simultana hidrodestilacija i rektifikacija