Studii şi Cercetări Ştiinţifice
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

2024, **25** (1), pp. 091 – 099

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry

ISSN 1582-540X

ORIGINAL RESEARCH PAPER

CHARACTERIZATION OF *JUNIPERUS COMMUNIS L.* ESSENTIAL OIL OBTAINED FROM BERRIES HARVESTED FROM THE BALKAN AREA

Tiberius Opruța[1], Mihaela Tița[2], Adelina Constantinescu[2], Lăcrămioara Rusu[3], Ovidiu Tița[2*]

[1]"Lucian Blaga" University of Sibiu, Doctoral School, Bulevardul Victoriei 10, Sibiu, 550024, Romania
[2]"Lucian Blaga" University of Sibiu, Faculty of Agriculture Science, Department of Food Industry and Environmental Protection, Bulevardul Victoriei 10, Sibiu , 550024, Romania
[3]"Vasile Alecsandri" University of Bacau, Faculty of Engineering, Department of Chemical and Food Engineering, Calea Mărășești, no.157, 600115, Bacau, Romania

*Corresponding author: ovidiu.tita@ulbsibiu.ro

Received: February, 19, 2024
Accepted: March, 27, 2024

Abstract: *Juniperus communis* L. is a plant widely found as a wild crop in the South-Eastern Europe areas. Juniper berries, the fruit of *Juniperus communis* L. are a highly valued, essential oil-rich plant material used traditionally in folk medicine as antiseptic, diuretic, antirheumatic, anti-inflammatory, antibacterial and antifungicidal agent. This paper reviews information on extraction methods of the essential oil from the juniper berries, collected from different regions of Eastern Europe. The batches of essential oil obtained were characterized in terms of chemical composition using GC-FID method and organoleptic properties. Depending on the harvesting and collection areas, various results were achieved for the investigated attributes under similar production settings. Juniper berry essential oils derived from Albanian areas are rich in sesquiterpenes, which constitute key compounds with significant added value in the flavor and fragrance industries.

Keywords: *distillation, essential oil, flavors, Juniperus communis L., sesquiterpenes*

INTRODUCTION

Juniperus communis L. is a long-lived (up to 600 years or more) evergreen conifer with a high percentage of its range of woody plants in cold temperate geographic regions of the northern hemisphere, from the southern Arctic in the mountains to latitudes around 30° norths in Europe, Asia and North America. *Juniperus communis* is a globally distributed species with a wide range of ecological adaptations. Juniper plants grow singly in dry pine or mixed forests or may form a stand.

A wide geographical distribution is the main reason for the remarkable variation in morphological characteristics and chemical composition of the essential oil [1].

J. communis is the only coniferous plant that can also grow in pots. For food purposes, berries (fruits) are mainly used, while applications using the branches are less common.

Gin flavoring is probably the best-known culinary use of juniper berries. The name 'gin' has its origins in the words genièvre (French), jenever (Dutch) and ginepro (Italian), all meaning juniper. This juniper spirit base was first produced in the 16th century in Holland. At the end of the 17th century, gin spread to England causing the so-called "gin epidemic". In France and Scandinavian countries, a fermented beer-like alcoholic beverage, genevrette, flavored with juniper berries was produced from barley [2].

The aromatic properties of juniper berries are dependent on the essential oil content. Studies show that unripe fruits accumulate more essential oil (up to 4.2 %) than ripe cones (up to 1.1 %) [3, 4]. In general, it was found that the structural quality profile of essential oils of unripe and ripe juniper fruits of the same shrub is similar [5]. Two groups of terpene compounds, namely monoterpenes and sesquiterpenes, constitute the main part of the natural essential oil of *Juniper communis* L., although the balance between them is quite variable [6, 7].

Terpenes determine the strong and distinctive aroma of the essential oil obtained from juniper berries [8, 9]. The total terpene content in juniper volatile oils and their structure is influenced by several factors (geographical area, weather conditions, harvesting period, etc.) as mentioned in majority of specific literature [10 – 13].

Our main goal was to characterize the essential oil of juniper produced in industrial conditions from ripe berries collected from different areas in Eastern Europe.

MATERIALS AND METHODS

Collection of juniper berries

Considering the increasing demand for juniper berry essential oil, which exists in the market at the moment, we decided to finance through a joint-venture partnership, a study of the quality of essential oils resulting from the distillation of several types of juniper fruits collected from different areas of Eastern Europe. Thus, from September to November 2022 we organized the collection of about 2000 kg of juniper fruits from each of the following areas: Plovdiv - Bulgaria, Malesia and Madhe - Albania, Krushevo region - south-west Macedonia, Durres - Albania. The collection was organized by our local partner with their team of farmers and it was performed manually. The berries collected were then sorted, ripe fruits (dark blue ones) being separated by the needles and green berries. For the technological process of distillation was used only the fresh ripe berries.

Raw materials (juniper berries) preparation for steam distillation

From our technical experience in juniper berry oil production, in order to obtain a good yield of extraction and good quality of end product using steam distillation method, an important step in the process consists in the grinding of berries before feeding the distillation pot. For this purpose, we use a berries crusher with two stainless steel rolls of 140 cm length actioned with an electrical three-phase motor of 7 kWh. The medium grinding capacity of the vegetal material was at the level of 300 - 400 kg juniper berries$\cdot h^{-1}$. The ground material was collected in big bags and then discharged in the steam distillation still pot.

Industrial production of juniper berry essential oil. Equipment's & distillation procedure

The first stage in obtaining crude extract from plants is extraction. After that, additional analysis and component identification of the extracted material are required. Soaking, maceration, water percolation, Soxhlet extraction, hydrodistillation, simultaneous distillation-extraction and other methods are examples of conventional extraction processes. Many innovative extraction methods have been presented and studied in the last ten years, the majority of which have been said to be superior in terms of extraction time, solvent consumption, and efficiency Pressurized solvent extraction (PSE), supercritical fluid extraction (SFE), and microwave-assisted extractions (MAE) are the new methods that are currently accessible [14].

These oils appear to be very easy to isolate, yet depending on the extraction technique employed, the oil's composition can change significantly. Various research has provided extensive discussions on the benefits and drawbacks of various techniques mentioned above [14, 15].

The juniper berries collected from Macedonia and Albania regions were distilled in the facility of a local partner in Macedonia and the berries from Bulgaria were processed also locally, using a distillery unit from Plovdiv. The procedure of distillation was performed, in each case, using a steam distillation unit containing: distillation pot of 3000 L total volume, florentin vessel of 150 L volume, multitubular heat exchanger, steam boiler of 500 kg$\cdot h^{-1}$ steam production at 0.9 bar maximum pressure. From each region was collected a quantity of 2000 kg juniper berries. The standard quantity of raw material used for one batch of steam distillation was 1000 kg of ground juniper berries. We tried to respect the same process parameter for the steam distillation for each batch, as can be seen in Table 1.

Table 1. *Steam distillation process parameters*

Raw mat/batch [kg]	Steam flow [kg$\cdot h^{-1}$]	Steam pressure [bar]	Time/batch [h]
1000	150	0.9	8

Quality control. Equipment's and analytical methods

Gas Chromatography with Flame Ionization Detection (GC-FID)

The GC-FID analysis of the essential oil (EO) was performed with a gas chromatograph model 7890B (Agilent Technologies, USA) equipped with a flame ionization detector and a J&W DB-5 fussed silica capillary column (60 m × 0.325 mm × 0.5 µm; stationary phase macrogol 20000). The oven temperature was programmed as mentioned below. The detector and injector temperatures were set up to 260 °C. The carrier gas was hydrogen (99.99 %) at a flow rate of 2mL·min^{-1}. Essential oil samples (2 µL) were injected using the split mode 1:80 ratio. The percentage composition of EO was calculated using the peak normalization: the percentage composition is determined by measuring the area of each peak and dividing the individual areas by the total area. In order to be able to identify retention times for different aromatic compounds, substances of purities above 92 % (GC) 0.5 mL dissolved in 1.5 mL 99 % pure hexane solvent (e.g. myrcene, caryophyllene, sabinen, alpha-pinene, beta-pinene, limonene, eugenol etc.) were used as standards.

Physico-chemical parameters determination

The main physico-chemical parameters for characterizing natural essential oils, which we also measured for the juniper berry oils produced, are: density (g·mL^{-1}, measured at 20 °C), refractive index (nD at 20 °C) and optical rotation (0). These parameters have been measured for each lot of essential oil separately, using high precision electronic and automated equipment from Anton Paar brand.
The density analysis (g·mL^{-1}, at 20 °C) was performed with the digital densimeter DMA 4500 M - Anton Paar with technical characteristics described in Table 2. The principle of the analysis method is pulse excitation method (PEM).
The determination of the refractive index (nD at 20 ºC) was carried out using the Abbemat 300/350 - Anton Paar digital refractometer with technical characteristics described in Table 3.

Table 2. *DMA 4500M Anton Paar - technical characteristics*

Measuring range	Repeatability (ISO 5725)	Accuracy	Digital resolution	*MSQ/ MAT
0 g·cm^{-3} to 3 g·cm^{-3} T(°C): 0 to 100 P (bar): up to 10	For density: 0.000005 g·cm^{-3} For temperature: 0.01 °C/0.02 °F	For density: 0.000005 g·cm^{-3} For temperature: 0.02 °C/0.04 °F	0.00001 g·cm^{-3}	1mL/30 seconds

* Minimum sample quantity required / minimum analysis time

Temperature has the biggest influence on refractive index. To ensure accurate results, the built-in Peltier temperature control adjusts the temperature at the prism/sample interface to very high accuracy within seconds. The patented T-Check abbemat calibrates and adjusts the temperature of the prism surface for accurate and traceable results. To ensure complete traceability, temperature adjustments are automatically documented in the Abbemat instrument audit library.

Table 3. *Abbemat 300/350 refractomer Anton Paar - technical characteristics*

Measuring Range [nD]	Resolution [nD]	Accuracy [nD]
1.26 to 1.72	± 0.00001	± 0.0001

The determination of optical rotation (αD 0) was performed automatically with the MCP 100 - Anton Paar digital polarimeter with technical features described in Table 4.

Table 4. *MCP 100 - Anton Paar – Technical characteristics*

Measuring Range	Resolution	Accuracy	Response time	Wavelength	*TR/TA	Temp. control
±89.9°	0.001°	0.001°	12 sec	589 nm	0.1 °C / ±0.2 °C	Peltier system

*TR/TA – Temperature resolution / Temperature accuracy; Measuring cell: Toolmaster^{TM} quartz cell 100 mm long

RESULTS AND DISCUSSION

In the technological production process, we tried as much as possible to respect the same working conditions and parameters of the plants and the same quantity of raw material per batch. From our experience, steam distillation of quantities greater than 1000 kg/batch increases the processing time by about 25 % for the same quantity of oil, but the quality of the rend product is lower from qualitative point of view. The main reason for this is the decomposition of sensitive aromatic chemical compounds such as myrcene, with the formation of polymers that negatively impact the quality of the finished product.
Thus, although we followed almost identical conditions (similar working parameters, configuration and technical specifications of the distillation and steam production plants), the results on the batches obtained are quite different in terms of composition and physico-chemical parameters. Of course, this further leads to different organoleptic characteristics (smell, taste, appearance) of the final product. It can be seen that the AB-ALBK09 batch (Figure 1. GC-FID chromatogram), has oil structure rich in sesquiterpenes, organic compounds that help a lot in obtaining "woody" notes, which is why this quality is very valuable for perfumery and flavor houses. Also, this batch has good feedback from customers who use it in the production of liquid flavors for gin.

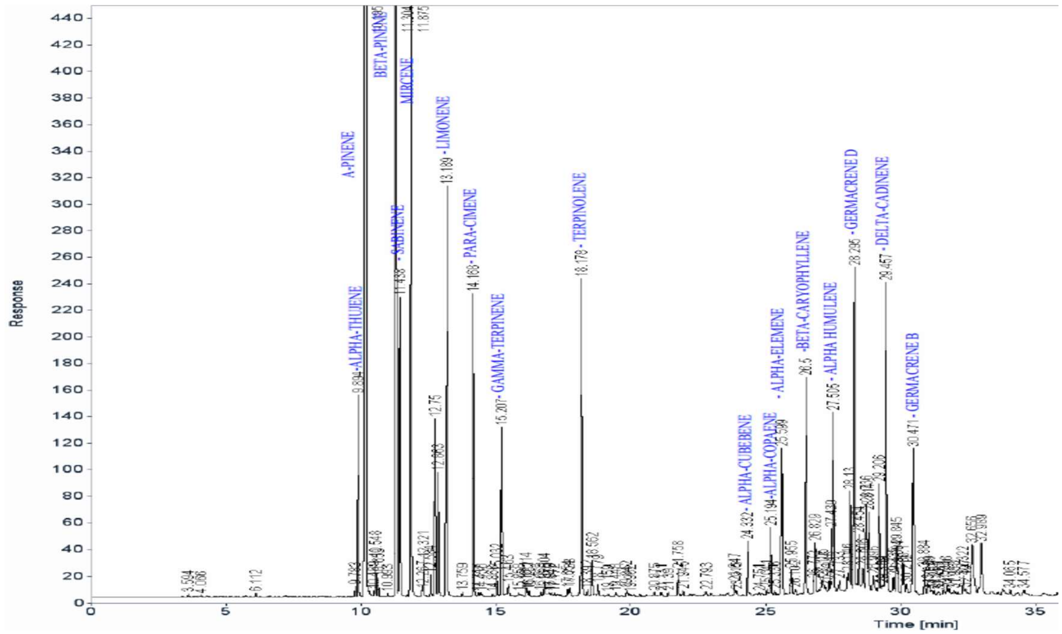

Figure 1. *GC-FID chromatogram of juniper berry essential oil AB-ALBK09 batch*

In Tables 5 and 6 it can see the results for physico-chemical parameters and also for GC-FID structure of each batch obtained.

Table 5. *Physico-chemical parameters of juniper berry EO industrial batches*

Item	**JBR BG**	**JBR MCJ AB**	**JBR MK HN**	**JBR MK HN**	**JBR AB BBS**	**JBR AB ALBK**
Lot #	**25/OJB V/22**	**2207-06**	**M-22-BE**	**OJBEO 11/22-KS**	**B9S070-810-22**	**AB-ALBK09**
EO Yield [% m/m]	1.4	1.7	1.5	1.5	1.6	1.8
Collection area	Plovdiv, Bulgaria	Malesia and Madhe, Albania	Berovo region, East Macedonia	Krushevo region, Macedonia	Durres region, Albania	Durres region, Albania
Density [$g\cdot cm^{-3}$]	0.8600	0.8723	0.8539	0.8440	0.8724	0.8724
RI	1.4739	1.4806	1.4749	1.4701	1.4765	1.4880
OR [^{0}]	(+) 6	(-) 1.8	(-) 7	(+) 13	(+) 12	(-) 2
Appearance	Mobile liq.	Mobile liq.	Mobile liq.	Mobile liq.	Mobile liq.	Mobile liq.
Color	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow
Odor	Balsamic, sweety, round	Terpenic, slightly fermented	Fresh, sweet, balsamic	Predominant terpenic, coniferous	Woody, coniferous	Terpenic, slightly fermented

From a technological point of view, we also tried in the case of the fruit batches from the Berovo area (from which the OJBEO 11/22-KS oil batch was obtained) to conduct distillation for a longer time and higher steam flow, in the hope of obtaining an oil with distillation "tails" rich in sesquiterpenes, but this did not turn out to be the case.

Table 6. *GC-FID profile structure of juniper berry EO industrial batches*

Item	**JBR BG**	**JBR MCJ AB**	**JBR MK HN**	**JBR MK HN**	**JBR AB BBS**	**JBR AB ALBK**
Lot#	**25/OJBV/22**	**2207-06**	**M-22-BE**	**OJBEO 11/22-KS**	**B9S070-810-22**	**AB-ALBK09**
GC area	**%**	**%**	**%**	**%**	**%**	**%**
alpha-thujene	1.96	1.72	0.97	2.70	5.51	1.72
alpha-pinene	44.53	33.24	46.96	39.78	28.46	33.24
beta-pinene	9.50	8.22	3.18	24.80	14.29	8.22
sabinene	2.63	2.21	5.51	2.63	1.84	2.21
beta-myrcene	8.73	11.75	18.43	17.70	3.26	11.75
limonene	7.08	4.17	4.45	4.03	6.13	4.17
para-cymene	2.55	2.50	1.18	1.48	5.02	2.50
gamma-terpinene	1.57	1.39	0.96	1.37	1.50	1.39
terpinolene	2.71	3.17	1.29	0.84	5.76	3.17
alpha-cubebene	0.27	0.47	0.60	0.15	1.08	0.81
alpha-copaene	0.44	0.59	0.44	0.06	0.97	0.59
alpha-elemene	0.68	1.43	0.92	0.16	0.45	1.75
beta-caryophyllene	2.38	2.18	1.99	0.29	2.24	2.18
alpha-humulene	1.62	1.75	1.33	0.17	2.63	1.95
germacrene D	1.63	3.26	2.57	0.76	0.95	3.95
delta-cadinene	1.20	3.12	1.32	0.10	1.62	3.75
germacrene B	0.78	1.44	1.00	0.27	0.35	1.60
monoterpenes (%)	90.03	82.76	89.08	97.99	88.54	80.48
sesquiterpenes (%)	9.97	17.24	10.92	2.01	11.46	19.52

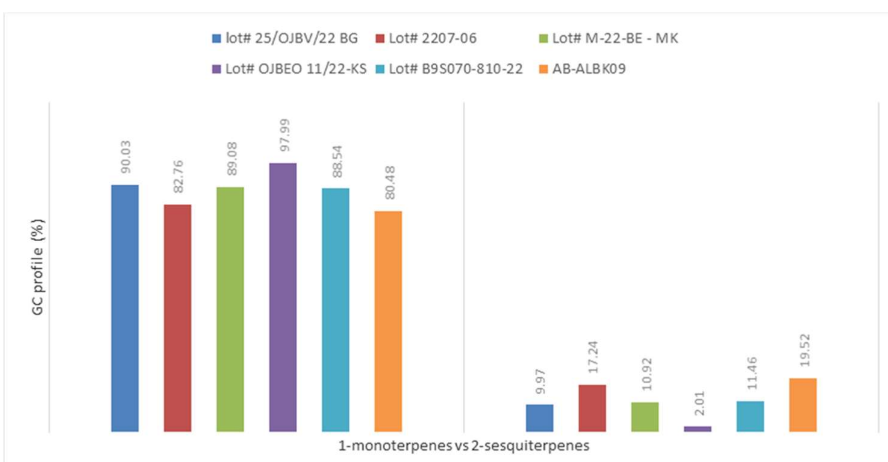

Figure 2. *GC-FID juniper berry EO distribution content per batches monoterpenes vs. sesquiterpenes*

CONCLUSIONS

Considering the above results obtained on the essential oils manufactured in the similar technological conditions, we can conclude that the area where the plant grows and the specific weather conditions are key parameters for the final quality of the finished product, and during this experiment we have drawn some valuable conclusions for our future projects that we will develop in this regard. Our team has ongoing studies to improve the distillation line and perform further industrial test with raw materials from Romania areas. The essential oils produced will be used in developing end products for the flavor and fragrances industry.

ACKNOWLEDGEMENTS

We are grateful to Romanian essential oil manufacturer Esentivia SRL and his partners for providing us the industrial capabilities and to food solutions company Solina Romania SRL for their full assistance in our present work.

REFERENCES

1. Adams, R.P., Pandey, R.N.: Analysis of *Juniperus communis* and its varieties based on DNA fingerprinting, *Biochemical Systematics and Ecology*, **2004**, **31**, 1271-1278;
2. Vichi, S., Riu-Aumatell, M., Mora-Pons, M., Buxaderas, S., Lopez-Tamames, E.: Characterization of volatiles in different dry gins, *Journal of agricultural and food chemistry*, **2005**, **53** (26), 10154-10160;
3. Butkienė, R., Nivinskienė, O., Mockutė, D.: Differences in the essential oils of the leaves (needles), unripe and ripe berries of *Juniperus communis* L. growing wild in Vilnius district (Lithuania), *Journal of Essential Oil Research*, **2006**, **18** (5), 489-494;
4. Ložienė, K., Labokas, J.: Effects of abiotic environmental conditions on amount and enantiomeric composition of α-pinene in *Juniperus communis* L., *Biochemical systematics and ecology*, **2012**, **44**, 36-43;
5. Lo[zbreve]ienė, K., Labokas, J., Venskutonis, P.R., Maždžierienė, R.: Chromatographic evaluation of the composition of essential oil and α-pinene enantiomers in *Juniperus communis* L. berries during ripening, *Journal of Essential Oil Research*, **2010**, **22** (5), 453-458;
6. Butkienė, R., Nivinckienė, O., Mockutė, D.: Two chemotypes of essential oils produced by the same *Juniperus communis* L. growing wild in Lithuania, *Chemija*, **2009**, **20** (3)**,** 195-201;
7. Labokas, J., Ložienė, K.: Variation of essential oil yield and relative amounts of enantiomers of α-pinene in leaves and unripe cones of *Juniperus communis* L. growing wild in Lithuania, *Journal of essential oil research*, **2013**, **25** (4), 244-250;
8. Vaičiulytė, V., Ložienė, K.: Variation of chemical and morphological characters of leaves and unripe cones in *Juniperus communis*, *Botanica Lithuanica*, **2013**, **19** (1), 37-47;
9. Marongiu, B., Porcedda, S., Piras, A., Sanna, G., Murreddu, M., Loddo, R.: Extraction of *Juniperus communis* L. ssp. nana Willd. essential oil by supercritical carbon dioxide, *Flavour and Fragrance Journal*, **2006**, **21** (1),148-154;
10. Orav, A., Koel, M., Kailas, T., Müürisepp, M.: Comparative analysis of the composition of essential oils an supercritical carbon dioxide extracts from the berries an needles of Estonian juniper (*Juniperus communis* L.), *Procedia Chemistry*, **2010**, **2** (1), 161-167;
11. Lohani, H., Haider, S.Z., Chauhan, N.K., Sah, S., Andola, H.C.: Aroma profile of two Juniperus species from Alpine region in Uttarakhand, *Journal of Natural Products*, **2013**, **6**, 38-43;
12. Burt, S.: Essential oils: their antibacterial properties and potential applications in foods-a review, *International journal of food microbiology*, **2004**, **94** (3), 223-253;

13. Merwe, M.V.D., Winfield, M.O., Arnold, G.M., Parker, J.S.: Spatial and temporal aspects of the genetic structure of Juniperus communis populations, *Molecular Ecology*, **2000**, **9** (4), 379-386;
14. Chan, C.H., Yusoff, R., Ngoh, G.C., Kung, F.W.L. : Microwave-assisted extractions of active ingredients from plants, *Journal of Chromatography A*, **2011**, **1218** (37), 6213-6225;
15. Cassel, E., Vargas, R.M.F., Martinez, N., Lorenzo, D., Dellacassa, E.: Steam distillation modeling for essential oil extraction process, *Industrial crops and products*, **2009**, **29** (1), 171-176.