VOLATILE COMPOSITION OF DISTILLATES WITH ADDED EXTRACTS OF JUNIPER BERRIES (*JUNIPERUS COMMUNIS* L. – *FRUCTUS*)

Dimitar **Dimitrov**[1*], Albena **Parjanova**[2], Snezhana **Ivanova**[2], Vanyo **Haygarov**[1]
[1]Department of Selection, Enology and Chemistry, Institute of Viticulture and Enology, 1 Kala Tepe str., 5800 Pleven, Bulgaria
[2]Department of Nutrition and Tourism, University of Food Technologies, 26 Maritza bul., 4000, Plovdiv, Bulgaria
*E-mail: dimitar_robertov@abv.bg

Abstract
A gas chromatographic (GC-FID) study to determine the volatile composition of distillates with added 50% and 70% ethanol extracts of juniper berries (Juniperus communis L. - Fructus) was performed. The incorporation of the extracts not led to a quantitative growth of the total volatile compounds, higher alcohols and esters. The application of 70% ethanol extract to the distillate shown higher levels of these components compared to 50%. The most diverse species presence of basic higher alcohols (3-methyl-1-butanol, 2-methyl-1-butanol, 1-propanol, 2-propanol, 2-butanol; aromatic alcohol - 2-phenylethanol) in the experimental group with 50% ethanol extracts of the plant applied was found. Acetaldehyde was the main representative of the aldehyde fraction. In the experimental variants it was available at lower levels compared to the control. This has a positive effect on the aromatic quality. An identical trend with the basic ester - ethyl acetate was observed. The application of the extracts led to a significant quantitative increase in the terpene content of the distillates. This may indicate an increase in the biological benefits due to the biological activity of the terpenes. Five terpene alcohols have been identified - linalool oxide, α-terpineol, β-citronellol, nerol and geraniol. The application of the extracts has been shown to reduce the levels of methyl alcohol in the experimental distillates. This improves their methanol purity. Distillates with incorporated extracts of juniper berries (Juniperus communis L. - Fructus) have the capacity to be used for the production of higher alcohol beverages with a balanced aromatic profile, increased biological benefits and reduced levels of methyl alcohol.

Keywords: distillates, juniper (*Juniperus communis* L. - *Fructus*), esters, higher alcohols, terpenes, aldehydes, methanol.

Received: 25.05.2020 Reviewed: 14.09.2020 Accepted: 24.09.2020

1. INTRODUCTION

The juniper is a plant of the *Cupressaceae* family. Its essential oil has a complex chemical composition, consisting of: terpineol, cadinene, α-pinene, limonene, sabinene, myrcene, cineole, bornyl acetate, cytrole and others. The fruit of the plant also contains organic acids, resinous substances, fats and tannins. (Landjev, 2005).

The complex chemical composition of the plant, with the significant presence of valuable biocomponents, is a prerequisite for the possibility of applying its extracts to higher alcohol distillates.

The compounds that have the most significant effect on the aromatic substances formation in higher alcohol distilled beverages are esters, higher alcohols, aldehydes and terpene compounds (Kostik et al., 2014; Marku et al., 2015; Yankov et al., 2000).

Burdejova and Vitova (2019) studied the volatile composition of five medicinal plants from the Czech Republic - lavender (*Lavandula angustifolia*), mint (*Mentha piperita*), sage (*Salvia officinalis*), hyssop (*Hyssopus officinalis*) and St. John's wort (*Hypericum perforatym*). The team established 52 volatile compounds. They were dominated by monoterpenes, sesquitrenes, alcohols, esters, hydrocarbons and acids.

Veljovic et al. (2018) investigated the volatile composition of distillates (cereals, plums, grapes and wines) enriched with the medicinal mushroom *Granoderma lucidum* and plant extracts from 44 plant sources (leaves, aromatic plants, medicinal plants, fruits, dried fruits). The team found that the aromatic profile of the

beverages was determined mainly by the basic composition of the distillates, but the addition of *Granoderma lucidum* and plant extracts enriched the volatile fraction with fruit and floral ethyl esters; the content of higher alcohols 1-propanol, 2-isobutanol and isoamyl alcohol was also increased.

Rodriguez-Solana et al. (2016) conducted a study on the phenolic composition and aromatic profile of herbal liqueurs obtained after maceration of aromatic and medicinal plants in grape marc distillates. In all tested beverages, the team found the main presence of volatile components methanol, ethyl acetate, acetaldehyde, 2-butanol, 1-propanol, 2-methyl-1-propanol, 1-butanol, 2-methyl-1-butanol and 3-methyl-1-butanol.

The aim of the present study is to define the volatile composition of distillates with added extracts of juniper berries (*Juniperus communis* L. - *Fructus*).

2. MATERIALS AND METHODS

Plant sources, fermentation, distillation and preparation of the extracts

Juniper berries were used as a plant source. There are bluish-black fleshy "fruits" with a diameter of 6-9 mm, which have a sweet taste and resinous odor. The fruits were collected in the autumn period (September-November). from the area near Dospat, Bulgaria. The geographical location of the area is following:

Bulgaria
Western Rhodopes Mts., near Dospat

Geogr. coord. (35TKG 63526 16655), October. degr.

Lat. 41.66583 **Lon.** 24.159722

UTM/MGRS KG61 **Altitude** 1214 m

Leg.: A. Parzhanova

Det.: A. Parzhanova

The plant species identification and determination was made at the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Science, Sofia, Bulgaria by Kozhuharov (1992), Delipavlov et al. (2003), Yordanova (1963-1979), Velchev (1982-1989), Peev (2012) and Turtin et al. (1964-1993). The raw material was ground into an electric robot. Six fractions with different diameter of ground particles of juniper berries (*Juniperus communis* L. - *Fructus*) were obtained: Class I $\frac{132}{0}$ µm – 13.23 %; Class II $\frac{280}{132}$ µm -23.43 %; Class III $\frac{450}{280}$ µm -17.15 %; Class IV $\frac{670}{450}$ µm -14.56 %; Class V $\frac{1000}{670}$ µm - 17.37 %; Class VI $\frac{2000}{1000}$ µm- 14.26 %.

The ethanol extracts were prepared by pouring 1 g of the ground plant raw material (juniper berries) with 20 ml of 50% ethanol and subsequently extracting using 70% ethanol in the same ratio. The purity of the ethanol used was 95%. The ratio of plant source: ethanol was calculated according to the production need of the required amounts of extract for incorporation into the distillates. The solutions prepared were stored at 18-20°C in the dark for 14 days. This is the period during which good contact was made for maximum extraction. Then the extracts were filtered and stored at 0 - 4°C.

To obtain the distillate, grapes of the Melnik-55 variety cultivated in the area of Polski Trambesh village, Sandanski region, Blagoevgrad was used. 600 kg grapes was used from which 80 liters of distillate with 63 vol. % alcohol have been obtained. The grapes were subjected to a fermentation process in accordance with the classical scheme for the production of dry red wines (Yankov, 1992).

The distillation was carried out in the licensed "Dennis-Marian Trenev Distillery 2008" Ltd., Novo Delchevo, Sandanski, Bulgaria. From the distillate obtained, experimental samples were prepared by adding (into the distillate) 50% and 70% ethanol extracts of juniper berries (*Juniperus communis* L. - *Fructus*) in quantities of 20, 50, 80, 100, 200, 400 and 600 ml. Pure distillate without extracts was used as a control sample.

Determination of ethanol content of the obtained distillates

The ethanol content of the obtained distillates was defined by specialized equipment with high precision – automatic distillation unit - DEE Destillation Unit with Densimat and Alcomat, Gibertini, Milan, Italy.

Volatile content determination by GC-FID

Gas chromatographic determination of the volatile components in distillates was done. The content of major volatile compounds was determined on the basis of stock standard solution prepared in accordance with the IS method 3752:2005. The method describes the preparation of standard solution with one congener, but the step of preparation was followed for the preparation of a solution with more compounds. The standard solution in this study included the compounds with purity > 99.0%. The 2 µl of prepared standard solution was injected in gas chromatograph Varian 3900 (Varian Analytical Instruments, Walnut Creek, California, USA) with a capillary column VF max MS (30 m, 0.25 mm ID, DF = 0.25 µm), equipped with a flame ionization detector (FID). The used carrier gas was He. Hydrogen to support combustion was supplied to the chromatograph via a hydrogen bottle. The injection was manually by microsyringe.

The parameters of the gas chromatographic determination were: injector temperature – 220°C; detector temperature – 250°C, initial oven temperature – 35 °C/retention 1 min, rise to 55°C with step of 2 °C/min for 11 min, rise to 230 °C with step of 15 °C/min for 3 min. Total time of chromatography analysis – 25.67 min. The identified retention times of the compounds in the standard solution were: acetaldehyde (3.141), ethyl acetate (3.758), methanol (3.871), 2-propanol (5.170), isopropyl acetate (5.975), 1–propanol (6.568), 2–butanol (7.731), propyl acetate (9.403), 2–methyl-propanol (10.970), 1–butanol (11.509), isobutyl acetate (11.662), ethyl butyrate (12.710), butyl acetate (12.752), 2–methyl-1-butanol (13.054), 4-methyl-2-pentanol (13.629), 3–methyl-1–butanol (13.840), 1–pentanol (15.180), isopenthyl acetate (15.965), pentyl acetate (16.033), 1–hexanol (16.276), ethyl hexanoate (16.376), hexyl acetate (16.510), 1–heptanol (16.596), linalool oxide (16.684), phenyl acetate (18.055), ethyl caprylate (18.625), α-terpineol (19.066), 2-phenyl ethanol (19.369), nerol (19.694), β-citronellol (19.743), geraniol (19.831), ethyl decanoate (19.904). As an internal standard octanol was used.

After determination of the retention times of the compounds in the standard solution the identification and quantification of the volatile substances in the distillates was done. The volatile composition was determined based on direct injection of the distillates. Prepared samples were injected in an amount of 2 µl in a gas chromatograph and was carried out an identification and quantification of the substances in each of them.

Statistical processing

Statistical analysis of the data was performed by determining the standard deviation (± SD), with triplicate. It was made using Excel 2007 from the Microsoft Office Package (Microsoft Corporation, USA).

3. RESULTS AND DISCUSSION

The data of the found volatile compounds in the studied control and experimental samples using 50% and 70% ethanol extracts of juniper berries are presented in Tables 1 and 2.

Regarding the established total amount of volatile compounds, the control sample (1962.97±3.62 $mg.dm^{-3}$) shown significantly higher concentrations compared to the experimental variants with 50% ethanol extract of juniper berries (31.25±2.32 $mg.dm^{-3}$ - 209.67±6.62 $mg.dm^{-3}$). When the 70% ethanol extract of the plant was incorporated to the distillates, significantly higher levels of volatile components (238.84±3.79 $mg.dm^{-3}$ - 1242.62±12.11 $mg.dm^{-3}$) were observed, compared to the 50% ethanol extracts, but they were again lower than the control.

The results shown that the incorporation of juniper berries extracts not increase the total volatile content of the distillates as a quantitative measure. However, the application of 70% ethanol extracts was more promising.

Table 1. Identified volatile compounds in control and experimental samples with added 50% ethanol extract of juniper berries (*Juniperus communis* L. - *Fructus*) *ND – Not Detected

IDENTIFIED COMPOUNDS, mg/dm^3	DISTILLATES WITH ADDED ETHANOL EXTRACTS (50%) FROM PLANT SOURCES							
	CONTROL	JUNIPER BERRIES 1000:20	JUNIPER BERRIES 1000:50	JUNIPER BERRIES 1000:80	JUNIPER BERRIES 1000:100	JUNIPER BERRIES 1000:200	JUNIPER BERRIES 1000:400	JUNIPER BERRIES 1000:600
Ethyl alcohol, vol.%	68.84	68.54	67.74	67.22	66.94	64.48	61.46	59.68
Acetaldehyde	54.72±0.25	4.41±0.28	4.12±0.59	4.49±0.24	0.05±0.01	2.88±0.27	7.19±0.87	5.45±0.16
Methanol	364.74±0.32	5.34±0.95	22.90±0.98	24.68±1.84	15.77±1.36	24.07±1.32	44.19±1.93	37.19±0.83
2-methyl-1-butanol	190.38±0.12	2.97±0.46	13.19±0.67	13.52±0.58	6.79±0.67	13.12±0.39	24.22±1.62	17.59±0.57
3-methyl-1-butanol	831.95±0.15	12.35±0.18	58.28±2.10	14.07±1.17	31.68±1.89	57.94±2.32	25.21±0.56	75.17±1.89
4-methyl-2-pentanol	ND	ND	0.05±0.01	0.05±0.01	ND	0.05±0.01	0.05±0.01	0.05±0.01
1-propanol	33.89±0.56	0.38±0.09	2.33±0.11	2.34±0.13	1.44±0.64	2.31±0.19	4.13±0.19	3.08±0.09
2-propanol	ND	ND	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	7.85±0.67	11.39±0.27
2-butanol	ND	3.43±0.17	17.66±0.93	18.08±0.63	10.84±0.82	17.63±0.90	31.26±0.83	23.33±1.76
2-methyl-1-propanol	245.01±1.05	ND	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01
1-hexanol	ND	ND	ND	0.05±0.01	ND	ND	0.05±0.01	ND
2-phenylethanol	ND	0.05±0.01	ND	0.05±0.01	0.05±0.01	0.05±0.01	2.99±0.19	6.39±0.27
Total higher alcohols	***1301.28±1.89***	***19.18±0.91***	***91.61±3.84***	***48.26±2.56***	***50.90±4.05***	***91.20±3.84***	***95.81±4.09***	***137.05±4.87***
Ethyl acetate	242.08±1.12	2.27±0.17	15.58±1.10	18.01±0.74	10.08±0.64	14.22±0.39	28.15±1.01	20.58±0.20
Propyl acetate	ND	ND	ND	0.05±0.01	ND	0.05±0.01	0.05±0.01	0.05±0.01
Isopropyl acetate	ND	ND	ND	ND	ND	ND	0.05±0.01	ND
Isopentyl acetate	ND	ND	0.05±0.01	ND	ND	0.05±0.01	ND	2.81±0.08
Ethyl butyrate	ND	ND	0.05±0.01	ND	0.05±0.01	ND	0.05±0.01	ND
Ethyl caprylate	0.05±0.02	ND	0.05±0.01	ND	0.05±0.01	ND	2.22±0.32	0.05±0.01
Ethyl hexanoate	ND	0.05±0.01	ND	ND	ND	ND	ND	2.77±0.12
Hexyl acetate	ND	ND	ND	ND	ND	ND	0.50±0.05	ND
Phenyl acetate	ND	ND	ND	ND	ND	ND	1.60±0.18	3.57±0.31
Total esters	***242.18±1.15***	***2.32±0.18***	***15.73±1.13***	***18.06±0.75***	***10.18±0.66***	***14.32±0.41***	***32.62±1.59***	***29.83±0.73***
α – terpineol	ND	ND	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01
Nerol	ND	ND	0.05±0.01	0.0016±0.0002	ND	0.05±0.01	0.02±0.01	0.05±0.01
β – citronellol	ND	ND	ND	ND	0.05±0.01	ND	ND	ND
Geraniol	0.05±0.01	ND	0.05±0.01	ND	0.05±0.01	ND	0.05±0.01	0.05±0.01
Total terpenes	***0.05±0.01***	ND	***0.15±0.03***	***0.051±0.01***	***0.15±0.03***	***0.10±0.02***	***0.12±0.03***	***0.15±0.03***
TOTAL CONTENT	***1962.97±3.62***	***31.25±2.32***	***134.51±6.57***	***95.54±5.40***	***77.05±6.11***	***132.57±5.86***	***179.93±8.51***	***209.67±6.62***

The total higher alcohols content of 1301.28±1.89 mg.dm^{-3} was found in the control sample. In the experimental variants with incorporated 50% juniper berries ethanol extract, these concentrations were significantly lower (19.18±0.91 mg.dm^{-3} - 137.05±4.87 mg.dm^{-3}). In the other experimental group (with 70% ethanol extract of the plant applied) higher quantitative levels of total higher alcohols (157.58±2.48 mg.dm^{-3} - 906.29±8.73 mg.dm^{-3}) were observed, but they were again lower than the established in the control sample.

From the obtained results it was clear that the application of 50% and 70% ethanol extracts of juniper berries to the distillate was not led to a quantitative complication in the final total concentration of higher alcohols.

Table 2. Identified volatile compounds in the experimental samples with added 70% ethanol extract of juniper berries (*Juniperus communis* L. - *Fructus*) *ND – Not Detected

IDENTIFIED COMPOUNDS, mg/dm^3	DISTILLATES WITH ADDED ETHANOL EXTRACTS (70%) FROM PLANT SOURCES						
	JUNIPER BERRIES 1000:20	JUNIPER BERRIES 1000:50	JUNIPER BERRIES 1000:80	JUNIPER BERRIES 1000:100	JUNIPER BERRIES 1000:200	JUNIPER BERRIES 1000:400	JUNIPER BERRIES 1000:600
Ethyl alcohol, vol.%	68.50	68.74	68.70	69.42	68.06	68.54	67.86
Acetaldehyde	0.05±0.01	35.53±0.57	22.00±0.27	19.76±0.29	17.42±0.27	9.84±0.14	29.13±0.24
Methanol	161.73±1.28	190.65±0.69	189.20±1.45	117.66±2.47	105.79±0.72	43.08±0.61	10.16±0.75
2-methyl-1-butanol	66.59±1.96	112.42±2.28	122.74±3.53	74.97±1.69	77.61±0.64	19.44±0.63	16.86±0.85
3-methyl-1-butanol	329.36±2.54	541.89±3.65	573.73±2.73	350.88±3.87	357.62±2.71	94.50±0.79	81.26±0.34
1-propanol	16.13±1.12	22.07±0.59	22.09±0.39	13.14±0.32	12.24±0.33	ND	ND
2-propanol	0.05±0.01	0.05±0.01	23.30±0.97	0.05±0.01	9.99±0.68	23.36±0.18	30.05±0.67
2-butanol	119.38±1.53	166.61±1.82	164.43±1.11	96.04±0.94	94.78±0.53	9.15±0.51	25.43±0.26
1-hexanol	ND	ND	ND	ND	ND	ND	119.10±1.01
2-phenylethanol	ND	ND	ND	0.05±0.01	24.36±0.14	11.13±0.37	36.06±0.73
Total higher alcohols	***531.51±7.16***	***843.04±8.35***	***906.29±8.73***	***535.13±6.84***	***576.60±5.03***	***157.58±2.48***	***308.76±3.86***
Ethyl acetate	103.81±1.74	119.80±3.21	97.98±1.27	75.77±1.77	46.52±0.44	24.74±0.29	10.45±0.17
Propyl acetate	ND	ND	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01
Ethyl caprylate	0.05±0.01	ND	ND	ND	0.05±0.01	ND	0.05±0.01
Ethyl hexanoate	0.05±0.01	10.72±0.74	ND	ND	ND	ND	ND
Phenyl acetate	ND	ND	0.05±0.01	ND	5.05±0.13	2.99±0.17	9.78±0.29
Ethyl decanoate	0.05±0.01	0.05±0.01	26.95±0.35	0.05±0.01	15.07±0.27	0.05±0.01	0.05±0.01
Total esters	***103.96±1.77***	***130.57±3.96***	***125.03±1.64***	***75.87±1.79***	***66.74±0.86***	***27.83±0.48***	***20.38±0.49***
Linalool oxide	ND	ND	ND	ND	ND	0.41±0.06	ND
α – terpineol	ND	0.05±0.01	ND	0.05±0.01	0.05±0.01	ND	0.05±0.01
Nerol	ND	0.05±0.01	0.05±0.01	0.05±0.01	ND	0.05±0.01	0.05±0.01
β – citronellol	0.05±0.01	0.05±0.01	0.05±0.01	ND	0.05±0.01	ND	ND
Geraniol	0.05±0.01	0.05±0.01	ND	0.05±0.01	ND	0.05±0.01	0.05±0.01
Total terpenes	***0.10±0.02***	***0.20±0.04***	***0.10±0.02***	***0.15±0.03***	***0.10±0.02***	***0.51±0.08***	***0.15±0.03***
TOTAL CONTENT	***797.35±10.24***	***1199.99±13.61***	***1242.62±12.11***	***748.57±11.42***	***766.65±6.90***	***238.84±3.79***	***368.58±5.37***

4. CONCLUSION

The following conclusions can be made from the study:

• The incorporation of juniper berries extracts into the distillate not increase the total volatile content. However, 70% ethanol extracts were more promising in this direction, compared to 50%.

• The application of juniper berries extracts not complicate the total quantitative content of higher alcohols in the distillates. The application of 70% ethanol extracts of the plant shown higher final levels of higher alcohols in the experimental variants of this group, compared to 50%.

• The most diverse species presence of higher alcohols was found in the variants of the experimental group with 50% ethanol extracts of juniper berries applied. They were 3-methyl-1-butanol, 2-methyl-1-butanol, 1-propanol, 2-propanol, 2-butanol and the aromatic alcohol 2-phenylethanol.

• The main representative of the aldehyde fraction was acetaldehyde. The application of the extracts led to a reduction of its levels in

the experimental distillates, balancing the aldehyde content, with positive effect on the aromatic quality.

• The application of juniper berries extracts not led to quantitative growth in the final ester content. However, the incorporation of 70% ethanol extracts resulted in higher final amounts of esters compared to 50%.

• The basic ester was ethyl acetate. It was present in reduced amounts in the experimental variants compared to the control. This trend balanced its presence and allowed a positive effect of the ester on the aromatic quality of the distillates.

• The application of 50% and 70% ethanol extracts of juniper berries led to higher final terpene content in the distillates, compared to the control. This complicated the terpene composition and can be an indicator of increased biological benefits, as terpene compounds have biological activity. In the experimental group with incorporated 70% ethanol extracts of the plant very high final terpene content was observed. Five terpene alcohols have been identified: linalool oxide, α-terpienol, β-citronellol, nerol and geraniol.

• It has been proven that the application of juniper berries extracts led to the production of higher alcohol distillates with lower levels of methyl alcohol. This improved the distillates methanolic purity.

The distillates with incorporated extracts of juniper berries (*Juniperus communis* L. - *Fructus*) can be a prospect for the production of higher alcohol beverages with a balanced aromatic profile, increased biological benefits and reduced levels of methyl alcohol.

5. REFERENCES

[1]. Apostolopoulou, A.A., Flouros, A.I., Demeritzis, P.G., Akrida-Demeritzi, K. Differences in concentration of principal volatile constituents in traditional Greek distillates, *Food Control*, 16, 2005, p: 157-164.

[2]. Burdejova, L., Vitova, E. Assessment of volatile compounds with emphasis on volatile allergens in selected dried medicinal plants using solid phase microextraction coupled with gas chromatography–mass spectrometry, *Chemical Papers*, 74, 2019, p: 1679-1690.

[3]. Kostik, V., Gjorgeska, B., Angelovska, B., Kovachevska, I. Determination of some volatile compounds in fruit spirits produced from grapes (*Vitis vinifera* L.) and plums (*Prunus domestica* L.) cultivars, *Science Journal of Analytical Chemistry*, 2(4), 2014, p: 41-46.

[4]. Kozhukharov, S. Determinant of Higher Plants, Science and Art, 1992, Sofia. (BG)

[5]. Landzhev, I. Encyclopedia of Medicinal Plants in Bulgaria, Herbs, Diseases. Editor "Trud", 2005, p: 122 – 123. (BG)

[6]. Marinov, M. Technology of alcoholic beverages and spirits. Plovdiv, Bulgaria, 2005, Academic Publishing of University of Food Technologies, ISSN 0477-0250. (BG)

[7]. Marku, K., Kongoli, R., Mara, V. Influence of the Distillation Process on the Aromatic Compounds of the Distillate Produced by ″Muschat Hamburg″ Cultivated in Durres, *International Journal of Advanced Research in Science, Engineering and Technology*, 2(5), 2015, p: 617-621.

[8]. Peev, D., Yordanov, D., Kozhuharov, S., Anchev, M. Flora of Bulgaria, 2012, Volume 11, BAS, Sofia. (BG).

[9]. Rodríguez-Solana, R., Manuel Salgado, J., Manuel Domínguez, J., Cortés-Diéguez, S. Phenolic compounds and aroma-impact odorants in herb liqueurs elaborated by maceration of aromatic and medicinal plants in grape marc distillates, *Journal of the Institute of Brewing*, 122, 2016, p: 653–660.

[10]. Standard 3752:2005. Alcohol Drinks – Methods of Test (Second Revision).

[11]. Tutin, V., Heywood, N., Burgers, D., Moore, D., Valentine, S., Walters, D., Webb, H. Flora Europea, 1964-1993, 1-5, Cambridge.

[12]. Velchev, V. Flora of Bulgaria, 1982-1989, Volume 8-9, BAS, Sofia. (BG)

[13]. Veljović, S., Tomić, N., Belović, M., Nikićević, N., Vukosavljević, P., Nikšić, M., Tešević, V. Volatile Composition, Colour, and Sensory Quality of Spirit-Based Beverages Enriched with Medicinal Fungus *Ganoderma lucidum* and Herbal Extract, *Food Technology and Biotechnology*, 57(3), 2019, p: 408–417.

[14]. Velkov, E. Encyclopedia of Alcoholic Beverages. Plovdiv, Bulgaria, Poligrafia Ltd, 1996, ISBN 954-698-002-1. (BG)

[15]. Yordanova, D. Flora of Bulgaria, 1963-1979, Volume 1-7, BAS, Sofia. (BG).