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Study of the volatile fraction of distillates with added donkey thistle (*Onopordum acanthium* L.) extracts

Изследване на летливата фракция на дестилат с добавени екстракти от магарешки бодил (*Onopordum acanthinum* L.)

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ABSTRACT

A study to determine the volatile fraction of distillates with added 50% and 70% ethanol extracts of donkey thistle (*Onopordum acanthium* L.) was conducted. The extracts were added to the pure distillate from the grapes of Melnik-55 grapevine variety in amounts of 20, 50, 80, 100, 200, 400 and 600 ml. The remaining amount to liter was a pure distillate. A distillate of Melnik-55 grapes (without extracts) was used as a control. The addition of 50% ethanol extract of *Onopordum acanthium* L. increased the total volatile composition of variants 1000:20 and 1000:50, in comparison to the control sample. Higher levels of esters and higher alcohols were found in the same variants. Incorporation of the 50% and 70% ethanol extracts of donkey thistle resulted in increased final total terpene content of the distillates. By increasing the amount of the added 50% ethanol extract, a reduction in the concentrations of acetaldehyde and ethyl acetate was found in the distillates. The basic ester in all the distillates analyzed was ethyl acetate, and the major higher alcohols were 2-methyl-1-butanol, 3-methyl-1-butanol, 1-propanol and 2-butanol. By applying of 50% and 70% ethanol extracts of the plant, a reduction in methanol content in the final distillates was observed. A prospect was the application of 50% ethanol extracts of *Onopordum acanthium* L, especially variants 1000:20 and 1000:50. This two variants showed higher final levels of aromatic compounds (beverage quality) and slightly higher final levels of terpenes (biological value) compared to the control. The other variants of this group had proven lower levels of methanol (improved toxicological quality) compared to the control.

Keywords: volatile compounds, esters, higher alcohols, aldehydes, terpenes

РЕЗЮМЕ

Проведено е изследване за определяне на летливата фракция на дестилат с добавени 50% и 70% етанолни екстракти на магарешки бодил (*Onopordum acanthinum* L.). Екстрактите са добавени в чист дестилат от грозде на сорта Мелник-55 в количества 20, 50, 80, 100, 200, 400 и 600 ml. Останалото количество до литър е дестилат. Като контрола служи чистия дестилат (без екстракти) на грозде от сорта Мелник-55. Установено е, че добавянето на 50% етанолен екстракт на *Onopordum acanthinum* L. повишава общия летлив състав на варианти 1000:20 и 1000:50, значително над установеното в контролата. Прилагането на 50% и 70% етанолни екстракти на магарешки бодил води до повишаване на финалното общо терпеново съдържание на дестилатите. С увеличаване количеството на добавен 50% етанолен екстракт е установено редуциране в концентрациите на ацеталдехид и етилацетат в дестилатите. Основен естер във всички анализирани дестилати е етилацетат, а основни висши алкохоли са 2-метил-1-бутанол, 3-метил-1-бутанол, 1-пропанол и 2-бутанол. При прилагане на 50% и 70% етанолни екстракти на растението, се констатира редуциране на метанолното съдържание в дестилатите. Перспектива представлява прилагането на 50% етанолни екстракти на *Onopordum acanthium* L, особено варианти 1000:20 и 1000:50. В тези два варианта се установяват високи крайни нива на ароматични съединения (качество на напитката) и малко по-високи крайни нива на терпени (биологична стойност), в сравнение с контролата. Останалите варианти на тази група са с доказани по-ниски нива на метанол (подобрено токсикологично качество), в сравнение с контролата.

Ключови думи: летливи съединения, естери, висши алкохоли, алдехиди, терпени

INTRODUCTION

The donkey thistle (*Onopordum acanthium* L.) is a prickly plant widespread in Bulgaria. It is of interest to nutritional science and technology because of the presence of components with functional and healthy activities in its chemical composition. Landjev (2005) states that the plant contains several flavone glycosides (quercetin, isorhamnetin, apigenin), alkaloids, saponins, vitamin C, inulin, tannins and flavones.

The distillation process leads to the transformation of fermented grape and fruit pulp, wine or wine by-products into a higher alcohol distillate (Marku et al., 2015). This transformation results in the transfer of volatile compounds from the fermented material to the obtained distillate. The major volatile components transferred to the distillate obtained are the products of yeast metabolism, during the fermentation (higher alcohols, esters, aldehydes) and those directly or indirectly derived from the fruit of the plant (methanol, terpene compounds) (Velkov, 1996; Jung et al., 2010). Coldea et al. (2011) examined the volatile fraction of three types of fruit brandy - plum, apple and pear. The team noted a major presence of 1-propanol, 2-butanol, isobutyl alcohol, active amyl alcohol, isoamyl alcohol, 1-butanol (from the higher alcohols fraction); ethyl acetate (from the esters fraction); acetaldehyde and furfural (from the aldehydes fraction). Study of the volatile composition of cherry brandy revealed the main presence of 1-propanol, 2-methyl-1-propanol, 1-butanol, amyl alcohols, 1-hexanol (fraction of higher alcohols); phenyl ethanol (aromatic alcohol); ethyl acetate (esters fraction) (Tesevic et al., 2009). In the study of grape distillates, the major presence of higher alcohols isoamyl, isobutyl and n-propyl and the dominance of ethyl acetate, the major ester representative, was found (Kostik et al., 2014).

The methyl alcohol concentrations in the distillates are associated with the presence of pectin in the fruits. The pectolytic enzyme complex of the fruits demethylates pectin, thereby releasing methyl alcohol, pectic acid and pectol (Marinov, 2005; Coldea et al., 2011). This alcohol is a constantly present component in the distillates. In grape distillates of unpressed and pressed grape marc, it is present in quantities of 1400.00 - 2200.00 mg/dm^3 (Velkov, 1996). Because it is a toxic compound, its concentrations are strictly regulated in the European and local legislation.

The aim of this study was to investigate the volatile fraction of a distillates with added extracts of *Onopordum acanthium* L. in order to improve the distillates aromatic quality, to increase their biological value and to reduce the final methanol levels.

MATERIALS AND METHODS

Plant sources and extracts preparation

The 50% and 70% ethanol extracts of donkey thistle (*Onopordum acanthium* L.) were used as a plant source for incorporation into the distillate from the grapes of Melnik-55 grapevine variety.

The usable part of the plant were the flower baskets picked during flowering (June-August). The herb was collected from one place –Dalgata Barchina Locality, Dospat, Bulgaria. It was dried in shaded airy rooms at 22-25°C. The dried parts were ground in an electric robot equipped with an electric grinder to obtain a finely ground substance from the dried herb. Six fractions with different particle diameters were obtained by determining the milled particle size of the dried donkey thistle (*Onopordum acanthium* L.):

Class I 132/0 µm - 13,23%;

Class II 280/132 µm - 23,43%;

Class III 450/280 µm - 17,15%;

Class IV 670/450 µm - 14,56%;

Class V 1000/670 µm - 17,37%;

Class VI 2000/1000 µm - 14,26%.

This diameters obtained ensured good contact between the particles and the solvent during the extraction process.

The ethanol extracts were prepared by pouring 1 g of the ground plant raw material with 20 ml of 50% ethanol. The purity of the ethanol used was 95%. The proportions of the amount of herb: solvent were calculated and the extraction was carried out to obtain the required amounts of extract for quantitative dosing to the experimental variants.

The extraction procedure was the same for the preparation of 70% ethanol extracts. The solvents (50% and 70% ethanol solutions) added to the milled plant material stayed with the plant material at 18-20°C in the dark for 14 days. This was the period during which they were in contact for better extraction. Then they were filtered and the obtained pure extracts were stored at 0-4°C.

The grapes from the variety Melnik-55 grown in the area of Polski Trambesh village, Sandanski municipality, Blagoevgrad region were used for the distillate preparation. The 600 kg grapes were used from which 80 liters of distillate with 63% alcohol content was obtained. The grapes were subjected to a fermentation process according to the classic scheme for the production of dry red wines: crushing and destemming, sulphitation (50 mg/kg SO_2), inoculating with pure culture dry yeasts *Saccharomyces cerevisiae* - 20 g/hl, temperature of fermentation – 28°C, separation from solids, further sulphitation, storage (Yankov, 1992).The distillation of the fermented material was carried out in a licensed distillery ("Denis-Marian Trenev 2008" Ltd, Novo Delchevo, Sandanski, Bulgaria). The experimental samples were prepared from the distillate obtained by adding of 50% and 70% ethanol extracts of donkey thistle (*Onopordum acanthium* L.) in quantities of: 20, 80, 100, 200, 400 and 600 ml. The experimental samples were poured up to one liter with distillate from Melnik-55 grapevine variety. Pure wine distillate without extracts was used as a control sample.

Determination of alcohol content of the obtained distillates

The alcohol 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 ofpreparation 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 injectedin a 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. The 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 3min. 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 the direct injection. The prepared samples were injected in an amount of 2 µl in a gas chromatograph and was carried out an identification and quantification of the volatile substances in each of them.

Statistical processing

The 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).

RESULTS AND DISCUSSION

The results obtained for the volatile fraction of the distillates with added donkey thistle extracts (*Onopordum acanthium* L.) are presented in Tables 1 and 2.

The distillates with added 50% donkey thistle extract (*Onopordum acanthium* L.), variants 1000:20 and 1000:50, shown a higher amount of total volatile compounds (3135.86±20.10 mg/dm^3 and 3259.85±18.14 mg/dm^3, respectively) in comparison with that found in the control (1962.97±3.62 mg/dm^3). The remaining experimental variants which were incorporated with this extract shown a lower total volatile content than the control. All established total concentrations of volatile compounds by used of 70% donkey thistle extract (*Onopordum acanthium* L.) were lower than the established in the control. They ranged from 322.96±6.47 mg/dm^3 - 721.28 ± 11.53 mg/dm^3.

The trend discussed was also maintained for the total content of specific groups volatile compounds. The 1000:20 and 1000:50 variants, obtained by the incorporation with 50% donkey thistle extract, shown a higher overall level of higher alcohols (2103.84±12.12 mg/dm^3 and 2282.84±12.51 mg/dm^3) and esters (427.11±5.80 mg/dm^3 and 379.62±0.98 mg/dm^3) compared to the control content of higher alcohols (1301.28±1.89 mg/dm^3) and esters (242.18±1.15 mg/dm^3). The tendency for a lower total content of higher alcohols and esters, compared to the control, in the other experimental variants of applied 50% ethanol extract of donkey thistle was maintained. When the 70% ethanol extracts of *Onopordum acanthium* L. was applied all total amounts of higher alcohols and esters were lower than the control. However, the data on the total terpenes showed a different trend. In the control sample, only one terpen - geraniol (0.05±0.01 mg/dm^3) was identified. It determined the total terpene composition of this sample. In the experimental variants with applied 50% ethanol extract of donkey thistle (*Onopordum acanthium* L.), variants 1000:20 and 1000:400 shown values of total terpenes comparable to the control. In the 1000:200 variant terpenes were not identified. All other variants shown higher total terpen content than the control. This result was an evidence of the terpenes transfer from the plant extract to the distillate. The terpenes were not identified in three variants (1000:50, 1000:80 and 1000:600) where the 70% ethanol extract of donkey thistle (*Onopordum acanthium* L.) was applied. In the 1000:200 variant, the total terpene content (0.05±0.01 mg/dm^3) was similar to the control, and in the other experimental variants it was higher (0.10±0.02 mg/dm^3).

The aldehyde fraction was represented by acetaldehyde. With the application of 50% ethanol extract of *Onopordum acanthium* L. it was observed that its concentration gradually decreased with the increase of the extract content. Only the first two variants 1000:20 (124.52±1.25 mg/dm^3) and 1000:50 (88.94±2.64 mg/dm^3) shown higher values than the control (54.72±0.25 mg/dm^3). In all other experimental variants, its quantities were lower than the control. When it is in higher amounts (above 100.00 mg/dm^3), this aldehyde could give an oxidized tone to the taste and aroma; at lower levels it imparts an apple flavor to the drink (Chobanova, 2012). The tendency to reduce the amounts of acetaldehyde in the distillates with the addition of 50% ethanol extract of donkey thistle (*Onopordum acanthium* L.) was positive in order to improve the aromatic quality. No similar trend was observed with the incorporation of 70% ethanol extract of the plant. However, all experimental variants shown lower concentrations of acetaldehyde (11.53±0.41 mg/dm^3 - 23.38±1.93 mg/dm^3) than the control (54.72±0.25 mg/dm^3).

The higher alcohols fraction in both experimental groups (50% and 70% ethanol extracts) of donkey thistle was dominated by 2-methyl-1-butanol, 3-methyl-1-butanol, 1-propanol and 2-butanol. Of these, 2-methyl-1-butanol and 3-methyl-1-butanol were presented in the highest amounts. In the control sample, 2-methyl-1-butanol was found to be 190.38±0.12 mg/dm^3. In the experimental samples it ranged from 22.39±1.22 mg/dm^3 to 338.19±3.68 mg/dm^3 (the group with 50% ethanol extract applied); 25.84±1.13 mg/dm^3 - 108.94±2.16 mg/dm^3 (the group of 70% ethanol extract applied).

3-methyl-1-butanol was found to be 831.95±0.15 mg/dm^3 in the control sample. In the experimental samples it ranged from 119.78±2.68 mg/dm^3 to 1466.31±5.47 mg/dm^3 (with 50% donkey thistle ethanol extract applied) and 127.35±1.58 mg/dm^3 - 556.34±3.18 mg/dm^3 (with 70% donkey thistle ethanol extract applied).

The data on established main higher alcohols representatives were in correlation with those of other authors (Coldea et al., 2011).

The major representative of the ester fraction was ethyl acetate. It was found in all experimental variants analyzed. It was identified as a major compound of the ester group in higher alcohol beverages in other studies (Tesevic et al., 2009; Kostik et al., 2014). In the control sample of this study, it was found to be 242.08±1.12 mg/dm^3. With the application of 50% ethanol extract of donkey thistle (*Onopordum acanthium* L.), its concentration was increased from 1000:20 (137.94±1.54 mg/dm^3) to 1000:50 (363.27±0.74 mg/dm^3) and then gradually and regularly decreased, reaching 29.40±0.17 mg/dm^3 in the last final variant - 1000:600. No similar trend was observed with the use of the 70% ethanol extract of donkey thistle (*Onopordum acanthium* L.), but all ester levels (41.64±0.65 mg/dm^3 - 77.58±1.11 mg/dm^3) were lower than that found in the control sample (242.08±1.12 mg/dm^3). In higher quantities, this ester carried the sour aroma; at doses of 50.00 - 80.00 mg/dm^3 it provided a positive effect on the aromatic complex (Chobanova, 2012).

In the control sample, the methanol was identified in the amount of 364.74±0.32 mg/dm^3. The methanol reduction was observed when 50% ethanol extract of donkey thistle (*Onopordum acanthium* L.) was applied. A higher methanol content was found in the 1000:50 sample (508.30±1.98 mg/dm^3) and the 1000:20 sample (480.34±0.87 mg/dm^3), where both concentrations were higher than the control.

From a sample of 1000:50 a gradual and regular decrease in the methyl alcohol concentrations of each subsequent experimental sample was obtained, following the order: 508.30±1.98 mg/dm^3 (1000:50) > 204.94±1.14 mg/dm^3 (1000:80) > 155.73±2.14 mg/dm^3 (1000:100) > 134.82±2.85 mg/dm^3 (1000:200) > 105.31±4.96 mg/dm^3 (1000:400) > 50.48±0.33 mg/dm^3 (1000:600).

The reduction was an effective strategy for the production of a new beverages with reduced methanol content and increased toxicological safety. No similar tendency was observed with the application of the 70% ethanol extract of the plant. The methyl alcohol concentrations in these experimental variants were found in the range of 46.50±1.10 mg/dm^3 - 118.13±1.14 mg/dm^3 and were lower than that found in the control.

Table 1. Identified volatile compounds in experimental samples with the addition of 50% ethanol extract of donkey thistle (*Onopordum acanthium* L.) in different amounts

IDENTIFIED COMPOUNDS, mg/dm^3	DISTILLATES WITH ADDED ETHANOL EXTRACT (50%) FROM *Onopordum acanthium* L.						
	1000:20	1000:50	1000:80	1000:100	1000:200	1000:400	1000:600
Ethyl alcohol, vol.%	67.98	68.78	67.10	65.74	66.68	63.24	60.20
Acetaldehyde	124.52±1.25	88.94±2.64	30.50±0.69	21.23±1.10	26.15±1.54	24.27±0.74	22.86±0.10
Methyl alcohol	480.34±0.87	508.30±1.98	204.94±1.14	155.73±2.14	134.82±2.85	105.31±4.96	50.48±0.33
2-methyl-1-butanol	300.66±4.52	338.19±3.68	135.04±1.64	91.79±3.87	85.33±3.12	53.79±2.69	22.39±1.22
3- methyl -1- butanol	1403.64±2.85	1466.31±5.47	572.57±2.18	376.52±2.19	379.74±1.47	231.16±1.72	119.78±2.68
4-methyl-2-pentanol	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	ND	0.05±0.01	0.05±0.01
1-propanol	93.04±1.45	57.27±0.14	23.14±0.73	16.01±0.12	181.81±0.73	9.84±0.88	0.05±0.01
2-propanol	ND	0.05±0.01	0.05±0.01	0.05±0.01	ND	ND	ND
2-butanol	306.40±3.28	420.87±3.18	167.15±1.84	117.24±1.47	108.82±0.99	73.17±1.00	33.22±3.79
2-methyl-1-propanol	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	ND	ND	ND
1-hexanol	ND	0.05±0.01	0.05±0.01	ND	ND	ND	ND
2-phenylethanol	ND	ND	ND	ND	ND	0.05±0.01	0.05±0.01
Total higher alcohols	*2103.84±12.12*	*2282.84±12.51*	*898.10±6.43*	*601.71±7.68*	*755.70±6.31*	*368.06±6.31*	*175.54±7.72*
Ethyl acetate	137.94±1.54	363.27±0.74	120.42±2.53	106.08±1.87	84.32±0.69	71.40±1.37	29.40±0.17
Propyl acetate	ND	ND	0.05±0.01	ND	ND	105.08±2.99	ND
Isopropyl acetate	92.86±0.18	0.05±0.01	ND	0.05±0.01	ND	ND	ND
Isopentyl acetate	53.40±0.69	0.05±0.01	12.36±0.11	3.83±0.84	ND	ND	ND
Ethyl butyrate	ND	0.05±0.01	ND	ND	ND	0.05±0.01	0.05±0.01
Phenyl acetate	ND	16.20±0.21	ND	3.36±0.98	ND	ND	ND
Ethyl decanoate	142.91±3.45	ND	0.05±0.01	0.05±0.01	ND	0.05±0.01	ND
Total esters	*427.11±5.86*	*379.62±0.98*	*132.88±2.66*	*113.37±3.71*	*84.32±0.69*	*176.58±4.38*	*29.45±0.18*
α – terpineol	ND	0.05±0.01	ND	0.05±0.01	ND	ND	0.05±0.01
Nerol	ND	ND	ND	0.05±0.01	ND	0.05±0.01	ND
β – citronellol	ND	0.05±0.01	0.05±0.01	0.05±0.01	ND	ND	0.05±0.01
Geraniol	0.05±0.01	0.05±0.01	0.05±0.01	ND	ND	ND	0.05±0.01
Total terpenes	*0.05±0.01*	*0.15±0.03*	*0.10±0.02*	*0.15±0.03*	-	*0.05±0.01*	*0.15±0.03*
TOTAL VOLATILE COMPOUNDS	*3135.86±20.10*	*3259.85±18.14*	*1266.52±10.94*	*892.19±14.66*	*1000.99±8.54*	*674.27±16.40*	*278.48±8.36*

Table 2. Identified volatile compounds in the control (distillate) and experimental samples with 70% ethanol extract of donkey thistle (*Onopordum acanthium* L.) added in different amounts

IDENTIFIED COMPOUNDS, mg/dm^3	DISTILLATES WITH ADDED ETHANOL EXTRACT (50%) FROM *Onopordum acanthium* L.							
	CONTROL	1000:20	1000:50	1000:80	1000:100	1000:200	1000:400	1000:600
Ethyl alcohol, vol.%	68.84	69.02	68.24	69.06	71.32	68.54	68.14	68.62
Acetaldehyde	54.72±0.25	16.85±0.19	19.08±0.32	12.12±0.19	18.91±0.14	11.53±0.41	18.77±1.25	23.38±1.93
Methyl alcohol	364.74±0.32	102.12±2.36	118.13±1.14	46.50±1.10	94.59±1.11	66.35±0.74	71.81±2.11	82.31±2.14
2-methyl-1-butanol	190.38±0.12	63.59±1.89	71.21±3.69	108.94±2.16	62.19±2.41	25.84±1.13	29.44±0.84	50.03±0.87
3-methyl-1-butanol	831.95±0.15	300.00±0.98	328.23±4.18	556.34±3.18	294.66±3.65	127.35±1.58	185.34±3.12	234.90±1.87
1-propanol	33.89±0.56	11.31±1.85	11.69±0.61	14.22±1.78	10.82±0.54	5.63±0.12	6.43±0.19	8.69±0.43
2-propanol	ND	13.34±1.21	0.05±0.01	ND	ND	0.05±0.01	ND	0.05±0.01
2-methyl-1-propanol	245.01±1.05	ND	ND	ND	ND	ND	ND	ND
2-butanol	ND	85.22±0.72	95.11±0.43	134.01±0.54	81.66±0.63	43.20±0.36	47.76±1.58	63.73±1.78
1-pentanol	0.05±0.01	ND	ND	ND	ND	ND	ND	ND
2-phenylethanol	ND	ND	ND	ND	ND	ND	0.05±0.01	ND
Total higher alcohols	*1301.28±1.89*	*473.46±6.65*	*506.29±8.92*	*813.51±7.66*	*449.33±7.23*	*202.07±3.20*	*269.02±5.74*	*357.40±4.96*
Ethyl acetate	242.08±1.12	61.16±0.12	77.58±1.11	42.59±0.68	59.67±1.74	42.91±2.10	41.64±0.65	51.43±4.20
Propyl acetate	ND	ND	0.05±0.01	ND	ND	ND	ND	ND
Isopropyl acetate	ND	ND	0.05±0.01	ND	ND	ND	ND	ND
Ethyl hexanoate	ND	0.05±0.01	0.05±0.01	ND	0.05±0.01	ND	0.05±0.01	0.05±0.01
Phenyl acetate	ND	0.05±0.01	ND	ND	ND	ND	ND	ND
Ethyl caprylate	0.05±0.02	ND	ND	ND	ND	ND	ND	ND
Ethyl decanoate	0.05±0.01	0.05±0.01	0.05±0.01	ND	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01
Total esters	*242.18±1.15*	*61.31±0.15*	*77.78±1.15*	*42.59±0.68*	*59.77±1.76*	*42.96±2.11*	*41.74±0.67*	*51.53±4.22*
Nerol	ND	0.05±0.01	ND	ND	0.05±0.01	0.05±0.01	0.05±0.01	ND
Geraniol	0.05±0.01	0.05±0.01	ND	ND	0.05±0.01	ND	0.05±0.01	ND
Total terpenes	*0.05±0.01*	*0.10±0.02*	-	-	*0.10±0.02*	*0.05±0.01*	*0.10±0.02*	-
TOTAL VOLATILE COMPOUNDS	*1962.97±3.62*	*653.84±9.37*	*721.28±11.53*	*914.72±9.63*	*622.70±10.26*	*322.96±6.47*	*401.44±9.79*	*514.62±13.25*

* ND: not detected

CONCLUSIONS

The following conclusions can be established from the study:

- The incorporation of 50% ethanol extract of donkey thistle (*Onopordum acanthium* L.) resulted in an effective increase of the total volatile composition in two experimental variants - 1000:20 1000:50. They exceeded the total volatile content of the control. The incorporation of the 70% ethanol extract of the plant was not led to a quantitative increase in the volatile composition.
- The application of 50% and 70% ethanol extracts of donkey thistle was effective in order to increase the total terpene content of the distillates from Melnik-55 grapevine variety.
- When the 50% *Onopordum acanthium* L. extract was applied, a decrease in the concentrations of acetaldehyde and ethyl acetate was observed with an increase in the amount of added extract. This was a trend that could improve the aromatic quality of the distillates.
- The main higher alcohols typical for the higher alcohol beverages were identified. 2-methyl-1-butanol, 3-methyl-1-butanol, 1-propanol and 2-butanol dominated quantitatively.
- The reduction of methanol concentrations with the application of 50% and 70% ethanol extracts of *Onopordum acanthium* L. was observed. This resulted in the formation of a beverage with reduced methanol levels and improved toxicological safety.

REFERENCES

Chobanova, D. (2012) Enology. Part I: Composition of wine. Bulgaria: Academic Press of University of Food Technologies.

Coldea, T., Socaciu, C., Parv, M., Vodnar, D. (2011) Gas-chromatographic analysis of major volatile compounds found in traditional fruit brandies from Transylvania, Romania. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 39(2), 109-116. DOI: https://doi.org/10.15835/nbha3926053

Indian Standard 3752:2005. Alcohol Drinks – Methods of Test (Second Revision).

Jung, A., Jung, H., Auwärter, V., Pollak, St. Fárr, A., Hecser, L., Schiopu, A. (2010) Volatile congeners in alcoholic beverages: analysis and forensic significance. Romanian Journal of Legal Medicine, 18, 265 – 270. DOI: https://doi.org/10.4323/rjlm.2010.265

Kostik, V., Gjorgeska, B., Angelovska, B., Kovachevska, I. (2014) 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), 41-46. DOI: https://doi.org/10.11648/j.sjac.20140204.12

Landjev, I. (2005) Encyclopedia of medicinal plants in Bulgaria, herbs, diseases. Bulgaria: Editor "Trud".

Marinov, M. (2005) Technology of alcoholic beverages and spirit. Bulgaria: Academic Press of University of Food Technology.

Marku, K., Kongoli, R., Mara, V. (2015) 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),617-621.

Tešević, V., Nikićević, N., Milosavljević, S., Bajić, D., Vajs, V., Vučković, I., Vujisić, L., Đorđević, I., Stanković, M., Veličković, M. (2009) Characterization of volatile compounds of "Drenja", an alcoholic beverage obtained from the fruits of cornelian cherry. Journal of Serbian Chemical Society, 74(2),117-128. DOI: https://doi.org/10.2298/jsc0902117t

Velkov, E. (1996) Encyclopedia of alcoholic beverages. Bulgaria: Poligrafia Ltd.

Yankov, A. (1992).Winemaking technology. Bulgaria: Zemizdat.