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Original article

Volatile Composition of Wine Distillates with Added Thyme Extracts (*Thymus callieri*)

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Abstract

A gas chromatographic (GC-FID) study to determine the volatile composition of wine distillates with the addition of 50% and 70% ethanol extracts of thyme (*Thymus callieri*) was conducted. The incorporation of 70% ethanol extracts led to higher final levels of total volatile compounds compared to 50%. The total content of higher alcohols was also higher when 70% ethanol extracts of the plant source were added to the distillates. The main identified representatives of this fraction were 3-methyl-1-butanol, 2-methyl-1-butanol, 1-propanol, 2-butanol. Acetaldehyde has been identified at a lower concentration level compared to the control. Its quantities were in the range in which it positively affected the distillates aroma. The thyme extracts not produced the effect on increasing of the total ester content in the distillates. The basic ester was ethyl acetate. Its amount in the experimental samples was lower than the control but balanced for its positive effect on the aromatic profile. The incorporation of 50% and 70% ethanol extracts of thyme in the distillates led to a significant increase in their total terpene content. This may be an effect that increased the biological value of the distillates. Decreased methyl alcohol concentrations have been found with the used extracts in the experimental distillates. This improved their methanolic purity. The application of thyme (Thymus callieri) extracts to distillates is a prospect of obtaining new alcoholic beverages with improved and balanced volatile composition, aromatic quality and biological value..

Keywords: Distillates, Extracts, Thyme (*Thymus callieri*), Higher alcohols, Esters, Aldehydes, Methanol, Terpenes..

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INTRODUCTION

There are about 15 thyme species in the Republic of Bulgaria. They all belong to the group *Thymus* sp. div. They grow in dry, grassy and stony places. Thyme belongs to the family *Laminaceae* (Landjev, 2005). The chemical composition of the essential oil of this plant is extremely complex, consisting of a number of important bio-substances: thymol, γ-terpineol, L-borneol, L-cymol, D-pinene, caryophyllene, linalool and others. The content of organic acids, flavonoids, tannins, minerals, vitamin C, pigments and others is high (Landjev, 2005). This diverse chemical composition provides a promising opportunity to incorporate extracts of this plant into food matrices in order to enhance their qualitative and biological activities.

The distillation process results in the transformation of wine (or fermented fruits) into an alcoholic distillate.

Marku et al (2015) investigated the effect of two distillation methods (by steam and water bath) on the change of volatile Muscat Hamburg wine composition to distillates. They found that higher alcohols and acetaldehyde shown a significant increase, and methanol, citronellol, linalool and α-terpineol decreased after wine distillation. This study showed that the major components of the terpene fraction were reduced after the distillation process.

Coldea et al. (2011) conducted a study comparing three types of brandy (apple, pear and plum) from the Transylvania region, Romania. The volatile fraction consisted of: acetaldehyde, ethyl acetate, methanol, 2-butanol, 1-propanol, isobutyl alcohol, amyl-active alcohol, isoamyl alcohol and furfural. The team (Coldea et al., 2011) found that acetaldehyde, ethyl acetate and amyl alcohols were primarily responsible for the taste and quality of these distilled beverages.

Tesević et al. (2009) analyzed the aromatic components of 5 cherry distillates (*Cornus mas*. L.). They found the main presence of methanol, acetaldehyde, 1-propanol, ethyl acetate, 2-methyl-1-propanol, 1-butanol, amyl alcohols, 1-hexanol and 2-phenylethanol. Of the minor aromatic components, 84 compounds were identified.

According to Jung et al. (2010) the major volatile compounds in distilled beverages were acetaldehyde, ethyl acetate, methyl alcohol, ethyl formate, n-propyl alcohol, isobutyl alcohol and n-butanol. They found the concentration of basic volatile compounds in home-made plum brandies, respectively: acetaldehyde - 250.00 - 400.00 mg/dm^3; 1-propanol - 76.00 - 1141.00 mg/dm^3; isobutanol - 58.00 - 210.00 mg/dm^3.

Apostolopolou et al. (2005) concluded that the aromatic quality of plum brandy was due to the presence of three major volatile components in it - acetaldehyde, ethyl acetate and amyl alcohols.

Veljovic et al. (2019) conducted a study to determine the volatile composition of distillates (cereals, plums, grapes and wine) enriched with medicinal mushrooms *Granoderma lucidium* and plant extracts (from 44 plant sources). The scientists from the team found that the aromatic profile of the drinks obtained was highly dependent of the main aromatic compounds of the distillate. However, the addition of *Granoderma lucidium* and the plant extracts led to an increase in the ethyl esters with a fruity and flower aroma, and the main representatives of higher alcohols were isoamyl alcohol, 2-isobutanol and 1-propanol.

The aim of this study is to define the volatile composition of distillates with added thyme extracts (*Thymus callieri*).

Materials and methods

Plant sources, fermentation, distillation and preparation of the extracts

Thyme was used as a plant source. It is from the Lamiaceae family, a perennial herbaceous plant. The whole plant has a pleasant smell. The usable part of the plant were the stems harvested during flowering (May-August).

The plant source (Thymus callieri) was collected from the area "Dalgata Barchina", Dospat, Bulgaria. 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), Yordanov (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 thyme (Thymus callieri) was 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 (thyme) 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 thyme (*Thymus callieri*) 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).

Results and Discussion

The results for the volatile composition of the control and experimental distillates with the addition of 50% and 70% ethanol extracts of thyme are presented in Tables 1 and 2.

The ethyl alcohol content of the control sample was 68.84 vol. %. It was noted that the alcoholic content of the variants using 50% ethanol extract of thyme (59.38 vol.% - 68.34 vol.%) and 70% ethanol extract (67.92 vol.% - 68.54 vol.%) was slightly lower than the control, which was explained by the partial quantitative replacement of part of the distillate with a certain amount of extract.

By the established total quantitative presence of volatile components, a lower final content (149.80±6.37 mg/dm^3 - 1301.98±14.62 mg/dm^3) was observed in the experimental variants where 50% ethanol extract of thyme was applied, compared to the control (1962.97±3.62 mg/dm^3).

However, when 70% ethanol extract of thyme was applied, two variants were distinguished - 1000:20 and 1000:80, in which a significantly higher amount of volatile compounds was found (2029.84±11.85 mg/dm^3; 4998.76±17.75 mg/dm^3, respectively) compared to the control. The other variants of this group shown a total volatile content lower than the control content. Comparing the final total concentrations of volatile compounds between the two experimental groups, it was observed that the incorporation of 70% ethanol extract of the plant resulted in higher final levels of volatile compounds than 50%. This was explained by the better extraction of volatile components from the plant using the higher concentrated extragent (70%).

Higher alcohols are a fraction of the volatile composition of the distillate that is usually present in the highest amount. According to Velkov (1996), the content of higher alcohols in grape distillates was observed at concentrations up to 1400.00 - 2000.00 mg/dm^3.

Table 1. Volatile compounds identified in the experimental samples with the addition of 50% ethanol extract of Thyme (Thymus callieri), *ND – Not Detected

IDENTIFIED COMPOUNDS, mg/dm^3	**DISTILLATES WITH ADDED ETHANOL EXTRACTS (50%) FROM PLANT SOURCES**						
	THYME 1000:20	THYME 1000:50	THYME 1000:80	THYME 1000:100	THYME 1000:200	THYME 1000:400	THYME 1000:600
Ethyl alcohol, vol.%	68.34	67.64	66.54	66.38	66.02	60.34	59.38
Acetaldehyde	0.05±0.01	4.12±0.64	10.48±0.54	9.11±0.72	23.96±0.36	6.93±0.23	25.06±1.21
Methanol	31.86±2.31	28.68±1.10	137.35±4.21	57.46±1.12	40.48±1.67	41.44±0.39	96.72±1.64
2-methyl-1-butanol	19.26±0.89	13.20±0.89	146.52±2.14	31.16±0.68	22.07±1.32	25.20±0.98	47.51±0.99
3-methyl-1-butanol	87.84±4.20	61.02±1.65	589.95±3.21	140.82±1.34	104.76±3.12	116.27±0.71	229.38±2.17
4-methyl-2-pentanol	ND	ND	ND	ND	ND	ND	0.05±0.01
1-propanol	5.51±0.45	2.72±0.49	22.01±0.69	5.94±0.62	4.51±0.61	4.79±0.87	9.01±0.39
2-propanol	ND	ND	0.05±0.01	0.05±0.01		8.36±0.68	0.05±0.01
1-butanol	ND	ND	ND	ND	ND	ND	0.05±0.01
2-butanol	28.12±0.65	20.58±0.67	14.44±0.87	43.42±2.14	185.32±2.10	35.09±1.25	82.89±0.72
2-methyl-1-propanol	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	ND
1-pentanol	28.66±0.98	ND	ND	ND	ND	ND	ND
1-hexanol	ND	ND	17.30±0.21	0.05±0.01	ND	0.05±0.01	12.92±0.68
2-phenylethanol	ND	ND	ND	ND	ND	7.85±0.67	41.88±0.81
Total higher alcohols	***166.44±7.18***	***97.57±3.71***	***790.32±7.14***	***221.49±4.81***	***316.71±7.16***	***197.66±5.18***	***423.74±5.79***
Ethyl acetate	25.06±0.11	19.18±0.87	47.84±0.36	38.58±0.56	8.38±0.12	19.50±0.86	68.76±0.63
Propyl acetate	ND	ND	291.02±1.02	ND	ND	ND	0.05±0.01
Isopropyl acetate	ND	ND	0.05±0.01	ND	ND	ND	ND
Isopentyl acetate	ND	ND	16.97±0.73	ND	ND	ND	ND
Ethyl butyrate	ND	ND	ND	0.05±0.01	0.05±0.01	0.05±0.01	ND
Phenyl acetate	ND	0.05±0.01	7.51±0.47	0.05±0.01	ND	0.05±0.01	0.05±0.01
Ethyl decanoate	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01
Total esters	***25.11±0.12***	***19.28±0.89***	***363.44±2.60***	***38.73±0.59***	***8.48±0.14***	***19.65±0.89***	***68.91±0.66***
α – terpineol	ND	0.05±0.01	0.05±0.01	ND	ND	0.05±0.01	ND
Nerol	ND	ND	0.05±0.01	0.05±0.01	ND	ND	0.05±0.01
β – citronellol	ND	0.05±0.01	0.24±0.10	0.05±0.01	0.05±0.01	0.05±0.01	ND
Geraniol	ND	0.05±0.01	0.05±0.01	ND	0.05±0.01	0.05±0.01	0.05±0.01
Total terpenes	-	***0.15±0.03***	***0.39±0.13***	***0.10±0.02***	***0.10±0.02***	***0.15±0.03***	***0.10±0.02***
TOTAL CONTENT	***223.46±9.62***	***149.80±6.37***	***1301.98±14.62***	***326.89±7.26***	***389.73±9.35***	***265.83±6.72***	***614.53±9.32***

Table 2. Volatile compounds identified in the control and experimental samples with the addition of 70% ethanol extract of Thyme (Thymus callieri), *ND – Not Detected

IDENTIFIED COMPOUNDS, mg/dm^3	**DISTILLATES WITH ADDED ETHANOL EXTRACTS (70%) FROM PLANT SOURCES**							
	CONTROL	THYME 1000:20	THYME 1000:50	THYME 1000:80	THYME 1000:100	THYME 1000:200	THYME 1000:400	THYME 1000:600
Ethyl alcohol, vol.%	68.84	68.54	67.92	68.44	68.52	68.04	67.96	68.46
Acetaldehyde	54.72±0.25	46.14±0.25	43.49±0.78	36.36±0.93	25.11±0.73	33.50±0.82	24.64±1.11	11.21±0.19
Methanol	364.74±0.32	308.80±2.14	219.29±1.10	603.14±4.20	141.86±1.04	180.23±3.82	126.54±2.49	44.08±0.25
2-methyl-1-butanol	190.38±0.12	219.35±3.21	123.70±1.19	653.22±4.43	79.15±1.83	125.57±0.64	84.42±0.65	27.70±0.39
3-methyl-1-butanol	831.95±0.15	967.15±1.85	557.53±3.69	2638.37±2.58	362.85±2.87	579.61±4.19	387.75±0.54	123.45±1.00
4-methyl-2-pentanol	ND	0.05±0.01	ND	0.05±0.01	ND	ND	0.05±0.01	0.05±0.01
1-propanol	33.89±056	36.09±0.95	22.73±0.89	95.48±1.16	15.10±0.99	20.75±0.14	13.94±0.19	4.48±0.09
2-propanol	ND	0.05±0.01	ND	0.05±0.01	18.88±0.67	0.05	0.05±0.01	0.05±0.01
1-butanol	ND	ND	ND	ND	ND		ND	0.05±0.01
2-butanol	ND	268.38±0.59	172.97±0.94	758.16±3.49	113.62±2.36	151.29±0.64	104.47±1.11	34.37±0.59
2-methyl-1-propanol	245.01±1.05	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	ND
1-pentanol	0.05±0.01	ND	ND	ND	ND	ND	ND	ND
1-hexanol	ND	ND	ND	0.05±0.01	ND	0.05±0.01	0.05±0.01	ND
2-phenylethanol	ND	ND	ND	0.05±0.01	ND	ND	20.72±0.69	9.22±0.83
Total higher alcohols	***1301.28±1.89***	***1491.12±6.63***	***876.98±6.72***	***4145.48±11.71***	***589.65±8.73***	***877.37±5.63***	***611.50±3.22***	***199.37±2.93***
Ethyl acetate	242.08±1.12	183.53±2.78	154.39±0.74	212.89±0.74	95.20±1.48	85.70±0.57	66.95±1.94	24.01±0.94
Propyl acetate	ND	ND	ND	ND	ND	0.05±0.01	0.05±0.01	0.05±0.01
Isopropyl acetate	ND	0.05±0.01	ND	0.05±0.01	ND	0.05±0.01	ND	ND
Isopentyl acetate	ND	ND	ND	0.05±0.01	ND	0.05±0.01	ND	ND
Ethyl butyrate	ND	0.05±0.01	0.05±0.01	ND	0.05±0.01	0.05±0.01	ND	0.05±0.01
Phenyl acetate	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	0.05±0.01
Ethyl caprylate	0.05±0.02							
Ethyl decanoate	0.05±0.01	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
Total esters	***242.18±1.15***	***183.73±2.82***	***154.54±0.77***	***213.09±0.78***	***95.35±1.51***	***85.95±0.62***	***67.10±1.97***	***24.21±0.98***
α – terpineol	ND	ND	0.05±0.01	0.64±0.12	0.05±0.01	ND	0.05±0.01	ND
Linalool oxide	ND	ND	ND	ND	ND	ND	ND	0.05±0.01
Nerol	ND	0.05±0.01	0.20±0.06	ND	0.05±0.01	0.05±0.01	0.05±0.01	0.03±0.01
Geraniol	0.05±0.01	ND	0.05±0.01	0.05±0.01	0.05±0.01	ND	0.05±0.01	0.05±0.01
Total terpenes	***0.05±0.01***	***0.05±0.01***	***0.30±0.08***	***0.69±0.13***	***0.15±0.03***	***0.05±0.01***	***0.15±0.03***	***0.13±0.03***
TOTAL CONTENT	***1962.97±3.62***	***2029.84±11.85***	***1294.60±9.45***	***4998.76±17.75***	***852.12±12.04***	***1177.71±10.90***	***829.93±8.82***	***279.00±4.38***

When 50% ethanol extract of thyme was applied, the variation of the total higher alcohols content between the different experimental variants was found in the range of 97.57±3.71 mg/dm^3 - 790.32±7.14 mg/dm^3. It correlated with the data of Velkov (1996). All total concentrations of higher alcohols found in this group of experimental variants were lower than the control (1301.28±1.89 mg/dm^3).

When 70% ethanol extract of thyme was applied, only one of the variants (1000:80) shown a very high final higher alcohols content (4145.48 ± 11.71 mg/dm^3). There was a tendency for a higher final content of higher alcohols in the experimental group with added 70% ethanol extracts of the plant compared to 50%.

Higher alcohols are compounds of volatile composition that exert aromatic influence when they are in higher amounts, which is due to their higher threshold of aromatic perception. Interpreting their total content, the experimental group of 70% ethanol extracts was more promising in terms of the aromatic expression of their aromatic influence.

The major representatives of higher alcohols in the variants analyzed were 3-methyl-1-butanol, 2-methyl-1-butanol, 1-propanol and 2-butanol. 3-methyl-1-butanol was found in the largest amounts. In the control sample, it was observed at a concentration of 831.95±0.15 mg/dm^3. In the variants with the applied 50% ethanol extract of thyme it ranged from 61.02± 1.65 mg/dm^3 - 589.95±3.21 mg/dm^3. All concentrations in these variants were lower than the control. Variations of this compound of 123.45±1.00 mg/dm^3 - 2638.37±2.58 mg/dm^3 were found when 70% ethanol extract of thyme was applied. It was evident from the results that incorporation of 70% ethanol extracts of the plant reflected in higher final levels of this higher alcohol, compared to 50% ethanol extracts.

2-methyl-1-butanol was the second most abundant higher alcohol identified in the distillates. Its concentration in the control sample was 190.38±0.12 mg/dm^3. In the variants with 50% ethanol extract of thyme, the concentrations of this compound (13.20±0.89 mg/dm^3 - 146.52±2.14 mg/dm^3) were found to be lower than the control and to their content in the other experimental group - distillates with incorporated 70 % ethanol extract of thyme (27.70±0.39 mg/dm^3 - 653.22±4.43 mg/dm^3).

1-propanol was established at the lowest concentration of the major identified representatives of higher alcohols. In the control sample, it was found at concentration of 33.89±0.56 mg/dm^3. In the variants where 50% ethanol extract of thyme was used, it varied from 2.72±0.49 mg/dm^3 - 22.01±0.69 mg/dm^3. Its concentrations in this experimental group were lower than the control and also lower from its presence in the other experimental group, where 70% plant ethanol extract was used (4.48±0.09 mg/dm^3 - 95.48±1.16 mg/dm^3).

From the established results regarding the fraction of higher alcohols, it was clear that the incorporation of 70% ethanol extract of the plant was more promising and complicates the quantitative presence of the basic higher alcohols. The data were in correlation with the results found by Veljović et al. (2019), who applied herbal extracts and fungus extract from *G. lucidum* and identified isoamyl alcohol, 2-isobutanol and 1-propanol as the main representatives of this fraction.

Acetaldehyde is a compound that is the major and quantitatively dominant representative of the aldehyde fraction. In higher alcohol beverages at levels of 50.00 - 300.00 mg/dm^3, it exerts a positive influence, above these concentrations its effect is negative, related to the manifestation of oxidized tone in taste and aroma (Velkov, 1996). In the control sample, this compound was found at concentrations of 54.72±0.25 mg/dm^3. In the variants where 50% ethanol extract of thyme was used, its concentrations ranged from 0.05±0.01 mg/dm^3 - 25.06±1.21 mg/dm^3, while in the variants with application of 70% ethanol extract it was found in higher quantities (11.21±0.19 mg/dm^3 - 46.14±0.25 mg/dm^3). In both experimental groups, its concentrations were lower than that in the control and were within the range in which it exerted a positive influence on the aromatic quality of the drink.

The esters are the compounds that have the most significant impact on the aromatic profile of alcoholic beverages. This is due to their diverse aromatic nuances and their low threshold of aromatic perception.

In the control sample, the total content of these components was 242.18±1.15 mg/dm^3. In the experimental variants with the addition of 50% ethanol extract of thyme only in variant 1000:80, their total content (363.44±2.60 mg/dm^3) was higher than the control. In all other variants, the total amounts of esters were lower. When 70% ethanol extract of the plant was applied, the total ester content in the experimental samples varied from 24.21±0.98 mg/dm^3 to 213.09±0.78 mg/dm^3 and was also lower than the control.

From the results obtained, it was clear that the incorporation of thyme extracts did not lead to significant quantitative growth of the ester fraction. According to Velkov (1996), the content of esters in grape distillates can be observed in concentrations up to 500.00 - 700.00 mg/dm^3. The results obtained in this study correlated with these data.

The main representative of the ester fraction, practically identified in all the variants analyzed, was ethyl acetate. The ester has a dual effect - according to Apostolopolou et al. (2005) to a concentration of 150.00 mg/dm^3 it exerts a positive influence on the aroma (fruity). According to Velkov (1996) the concentration of its normal presence is 500.00 - 700.00 mg/dm^3. Above these values it has negative effect (acetic acid taste). In the control sample, ethyl acetate was found at a concentration of 242.08±1.12 mg/dm^3. In both experimental groups, using 50% ethanol extract of thyme (8.38±0.12 mg/dm^3 - 68.76±0.63 mg/dm^3) and 70% ethanol extracts (24.01±0.94 mg/dm^3 - 212.89±0.74 mg/dm^3) its concentrations were lower than the control concentration. In the 50% ethanol extracts variants, ethyl acetate was found to be in lower content than 70%.

Ethyl acetate has been found to be a major ester representative of the higher alcoholic beverage composition in other studies (Tesević et al., 2009; Coldea et al., 2011; Jung et al., 2010). It was noted that the concentration of ethyl acetate in the experimental variants was reduced compared to the control. This was a positive trend due to the partial substitution of the distillate portion with thyme extract and this reflected in the balancing of the content of the ester, allowing its positive aromatic effect to be expressed.

The esters propyl acetate, isopropyl acetate, isopentyl acetate, ethyl butyrate, phenyl acetate, ethyl decanoate were also identified, but in lower amounts.

Terpenes are not fermentation products, but pass from the fruit (fermentable) to the distillate. These compounds have extensive biological and pharmacological activities (Gonzalez-Burgoz and Gomes-Seramilos, 2012).

Only one terpene - geraniol was identified in the control sample. Its concentration (0.05±0.01 mg/dm^3) determined the total concentration of identified terpenes in the control sample.

In the experimental variants with 50% ethanol extract of thyme, a total terpene content varying between samples in the range of 0.10±0.02 mg/dm^3 - 0.39±0.13 mg/dm^3, significantly exceeding the control was found. The same trend was observed in the other experimental group using 70% ethanol plant extracts (0.05±0.01 mg/dm^3 - 0.69±0.13 mg/dm^3).

A probable reason for the higher terpene content in the experimental variants was the terpene composition of the plant, the extract of which was incorporated into the distillates. The presence of higher terpene levels in the experimental distillates may be an effect that increases their biological value. The terpene fraction was represented by the identified 5 terpene alcohols - α-terpineol, linalool oxide, nerol, β-citronellol and geraniol.

The question for the presence of methyl alcohol in the distillates have great importance. This compound is formed upon degradation of the fruit pectin caused by the pectolytic enzyme complex (Marinov, 2005). According to Velkov (1996), the normal presence of this volatile component in grape distillates is 400.00 - 2000.00 mg/dm^3.

In the control sample, methanol was found at a concentration of 364.74±0.32 mg/dm^3. In the variants where 50% ethanol extract of thyme was used, the concentrations of this compound ranged from 28.68±1.10 mg/dm^3 - 137.35±4.21 mg/dm^3. It can be seen that they are significantly lower than the control. This was due to the substitution of a part of the distillate with a plant extract.

With the application of 70% ethanol extract of the plant only in variant 1000:80 a concentration of methanol (603.14±4.20 mg/dm^3) higher than the control one was found. All other variants of this experimental group shown methanol levels below the control. The methanol concentrations found in this experimental group were higher than those reported in the first group (with 50% ethanol plant extracts).

All concentrations of methyl alcohol found in this study met the safety criteria. It is apparent that incorporation of extracts that replacing portions of the distillate resulted in reduced final methanol levels. This improved the methanol purity of the distillate.

Conclusions

The following conclusions can be made from the study:

- The incorporation of 70% ethanol extracts of thyme resulted in higher total final levels of volatile components in distillates than 50% ethanol extracts. This is due to the better extraction of volatile components using a higher concentrated extragent (70%).
- The incorporation of 70% ethanol extract of thyme resulted in increased higher alcohols content in the experimental distillates compared to 50%. The main representatives of this fraction in both experimental groups were: 3-methyl-1-butanol, 2-methyl-1-butanol, 1-propanol and 2-butanol.
- Acetaldehyde (as the major aldehyde) was found to be lower in the two experimental groups compared to the control. Its concentrations were in the range in which it exerts a positive influence on the beverage sensory chracteristics.
- The incorporation of thyme extracts not led to significant quantitative growth of total ester content. The basic ester was ethyl acetate. Its concentrations in the experimental variants were lower than the control. This was a positive trend that reflected in the balancing of the content of this ester, which allows a positive expression of its aromatic influence.
- Higher total terpene content of distillates with thyme extracts was observed compared to the control. This can have an effect leading to an increase in the biological value of the distillate.
- The incorporation of thyme extracts to replace parts of the distillate resulted in reduced final methanol levels. This improves the methanolic purity of the experimental distillates.

The application of thyme extracts (*Thymus callieri*) to distillates is a prospect of obtaining new alcoholic beverages with improved and balanced volatile composition, aromatic quality and biological value.

Additional Declaration

Research and publication ethics principles were comply with in this study. Authors contributed equally to the study.

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