AROMATIC PROFILE OF BULGARIAN GRAPE AND FRUIT (PLUM) BRANDIES

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Abstract
The aromatic profile of traditional Bulgarian brandies - grape and fruit (plum) was determined. The identification and quantification of the volatiles was performed by gas chromatography (GC-FID). The following compounds (purity > 99.0%) in the standard solution were included: acetaldehyde, acetone, ethyl acetate, methanol, isopropyl acetate, 1-propanol, 2-butanol, propyl acetate, 2-methyl-propanol, isobutanol, 1-butanol, isobutyl acetate, ethyl butyrate, butyl acetate, 2-methyl-1-butanol, 3-methyl-1-butanol, ethyl isovalerate, 1-pentanol, pentyl acetate, 1-hexanol, ethyl hexanoate, hexyl acetate, 1-heptanol, linalool oxide, phenyl acetate, ethyl caprylate, α-terpineol, nerol, β-citronellol, geraniol. In the grape brandy were identified 13 volatile compounds, and in the fruit (plum) brandy their number were 15. The total content of the groups of identified volatile compounds in grape brandy was as follows: esters - 294.10 mg/dm^3; higher alcohols - 89.50 mg/dm^3; terpenes - 3.49 mg/dm^3. In fruit (plum) brandy was found a total ester content - 321.90 mg/dm^3; higher alcohols - 64.00 mg/dm^3; terpenes - 1.01 mg/dm^3. The established content of methyl alcohol in grape (453.00 mg / dm3) and plum (973.00 mg / dm3) brandies prove their authenticity, and its concentration levels come within the legally regulated and indicate the safety of the product for consumption. The results of the analysis of fruit brandy showed higher ester content, compared with the grape brandy. Grape brandy contains more higher alcohols and terpenes, compared with the fruit brandy.

Keywords: grape brandy, plum brandy, aromatic profile, esters, higher alcohols, terpenes, methanol, gas chromatography

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1. INTRODUCTION

Increased interest is observed in global plan to aromatic characterization of distilled alcoholic beverages, also known as spirits, obtained after the fermentation process. The traditional Bulgarian brandy belongs to this group.
The brandy is a drink with high ethanol content that is produced by distilling of fermented grape or fruit pastes, juices, or winemaking by-products (Velkov, 1996, Marinov, 2005; Kostik et al., 2014). The fermentation process is a major factor responsible for the formation of a significant amount volatile aromatic compounds in the resulting brandies. On the base of the metabolic activity of cultivated yeasts *Sacharomyces cerevisiae* is obtained complex bioconversion of carbohydrate components and their transformation into ethyl alcohol (main dominant component) and various by-products - aldehydes, higher alcohols, esters, giving a specific aroma of the different groups brandies (Pishtiisky and Ivanova, 2006). Should not be ignored and must be marked the possibility of terpene compounds accumulation. They represent a diverse group aromatic compounds. It is well known that their biosynthesis begins with acetyl-CoA. Their characteristic feature is that they are not metabolized by the yeasts and pass directly from plant ingredients and thereby enrich the aromatic profile of fermented grapes or fruits musts (Peinado et al., 2004; Tao and Li, 2009). With the highest content can be established so-called high volatile terpene alcohols - linalool, nerol, geraniol, terpinelol, citronellol (Di Stefano, 1981; Itu et al., 2011; Lengyel, 2012).
Esters are compounds with high index of aromaticity. They are important component of the brandy aromatic matrix. The esters may be formed by two major pathways: the dominant

is esterification - process of coupling of alcohols with acids; the second is formation related to *Saccharomyces cerevisiae* yeasts metabolism (Ramsay and Berry, 1984a; Velkov, 1996). The largest quantitative share of this group compounds have ethyl acetate - ester of ethanol and acetic acid (Velkov, 1996; Apostolopoulou et al., 2005). According to Velkov (1996) the main part of esters in grape brandies is represented by the group of ethyl esters, explainable in terms of the fact that the dominant alcohol is ethyl. The total esters amount in grape brandies is in the range of 500-700 mg/dm^3 (0,500 - 0,700 g/dm^3) (Velkov, 1996).
Higher alcohols are compounds obtained by the amino acid metabolism of yeasts cultures in the fermentation process of the fruits (Jung et al., 2010). They have a significant contribution to the aromatic qualities of the brandies. Their total concentration in grape brandies is in the range of 1,5 - 1,8 g/dm^3, while in the fruit brandies they can reach a higher total concentration - up to 5,0 g/dm^3 (Velkov, 1996). Their normal concentrations contribute to the formation of a typical fruity or flowery aroma of the beverage (Bonte, 1987).
High concentrations as esters and of higher alcohols can have a negative impact on brandy taste (Tešević et al., 2009).
The aim of this study was to determine and analyze the aromatic profile of traditional Bulgarian brandies - grape and fruit (plum).

2. MATERIALS AND METHODS

2.1. Samples

Grape and plum brandies produced by traditional technological scheme of production were studied. Brandies samples were analyzed at the Chemical Laboratory and Scientific Laboratory of Chromatography in the Department of Enology and Chemistry at the Institute of Viticulture and Enology, Pleven, Bulgaria.

2.2. Chemicals

Chemically pure substances (>99% purity; Sigma Aldrich, Saint Louis, MO, USA and Merck, Darmstadt, Germany) were used: acetaldehyde, acetone, ethyl acetate, methanol, isopropyl acetate, 1-propanol, 2-butanol, propyl acetate, 2-methyl-propanol, isobutanol, 1-butanol, isobutyl acetate, ethyl butyrate, butyl acetate, 2-methyl-1-butanol, 3-methyl-1-butanol, ethyl isovalerate, 1-pentanol, pentyl acetate, 1-hexanol, ethyl hexanoate, hexyl acetate, 1-heptanol, linalool oxide, phenyl acetate, ethyl caprylate, α-terpineol, nerol, β-citronellol, geraniol.

2.3. Determination of alcohol content

The alcohol content of the tested drinks was defined by specialized equipment with high precision – automatic distillation unit - Gibertiny BEE RV 10326 (Gibertiny Electronics Srl., Milano, Italy) and Gibertiny Densi Mat CE AM 148 (Gibertiny Electronics Srl., Milano, Italy).

2.4. Determination of volatile aromatic compounds

The content of major volatile aromatic compounds is determined on the basis of stock standard solution prepared in accordance with the IS method 3752: 2005 (Indian Standard, 2005). The method describes the preparation of standard solution with one congener, but the step of preparation is followed for the preparation of a solution with more compounds. The standard solution in this study include the following compounds (purity> 99.0%): acetaldehyde, acetone, ethyl acetate, methanol, isopropyl acetate, 1-propanol, 2-butanol, propyl acetate, 2-methyl-propanol, isobutanol, 1-butanol, isobutyl acetate, ethyl butyrate, butyl acetate, 2-methyl-1-butanol, 3-methyl-1-butanol, ethyl isovalerate, 1-pentanol, pentyl acetate, 1-hexanol, ethyl hexanoate, hexyl acetate, 1-heptanol, linalool oxide, phenyl acetate, ethyl caprylate, α-terpineol, nerol, β-citronellol, geraniol.
An amount of 1,0 g for each of congeners was diluted to 100 ml with 40% ethanol solution. 10 ml of this solution (with all added congeners) was diluted to 100 ml with 40% ethanol solution. From this stock solution was prepared the standard solution by adding 5 ml

of the diluted solution in the 10 ml test tube, and adding 1 ml of the previously prepared solution of octanol (internal standard). The 2 μl of resulting 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 generated and supplied to the chromatograph via a hydrogen generator Parker Chroma Gas: Gas Generator 9200 (Parker, United Kingdom). The injection is 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 resulting chromatogram of the standard solution is shown in Figure 1.

After determination of the retention times of compounds, we proceed to the identification and quantification of volatiles in the brandy samples. 5 ml of each brandy sample and 1 ml of internal standard solution (octanol) were placed in 10 ml test tubes with a stopper. Prepared samples were injected in an amount of 2 μl in a gas chromatograph and was carried out an identification and quantification of the compounds.

3. RESULTS AND DISCUSSION

The chromatographic profile of the studied brandies (grape and plum) is presented in Figure 2 a, b.

The established amounts of identified volatile aromatic compounds in the studied grape and fruit (plum) brandies are presented in Table 1.

Thirteen volatile compounds in grape brandy (alcohol - 46.72 vol. %) were identified. Identified congeners belongs to three groups of compounds with significant aromatic contribution - higher alcohols, esters and terpene alcohols.

The total content of esters found in grape brandy was 294.10 mg/dm^3. The result for the total ester content is lower than the maximum limit for grape brandies described by Velkov (1996). The lower ester content is probably due to the fact that the brandy has not undergone aging process. Aging of brandies leads to active esterification with accumulation of significant esters amounts. Ethyl acetate (170.80 mg/dm^3) showed the highest concentration of this group compounds.

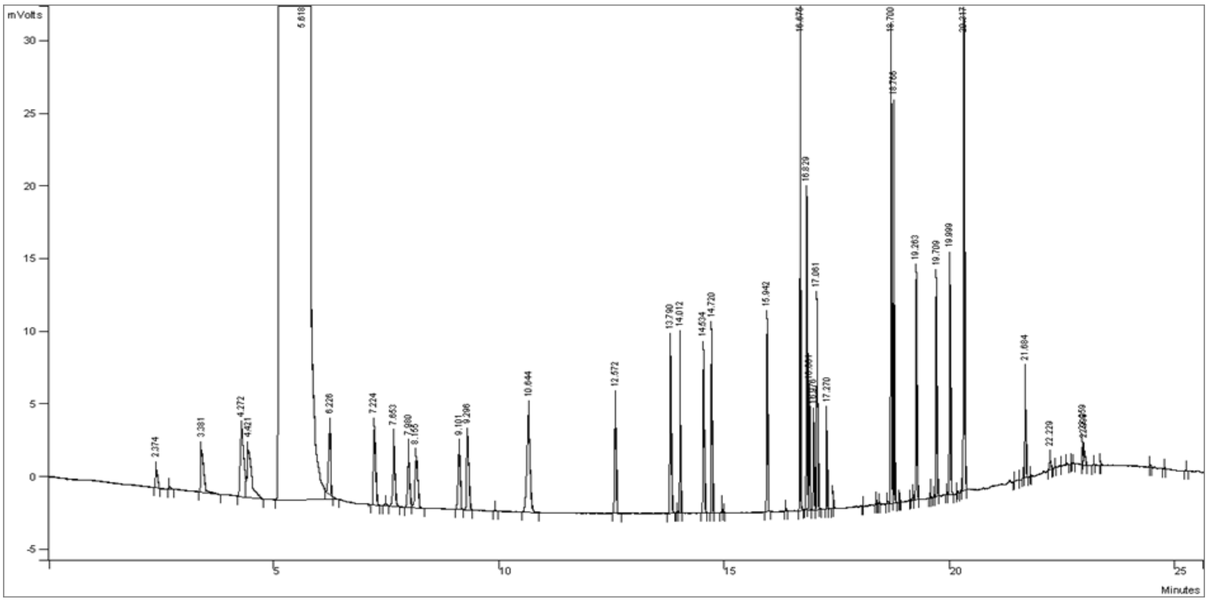
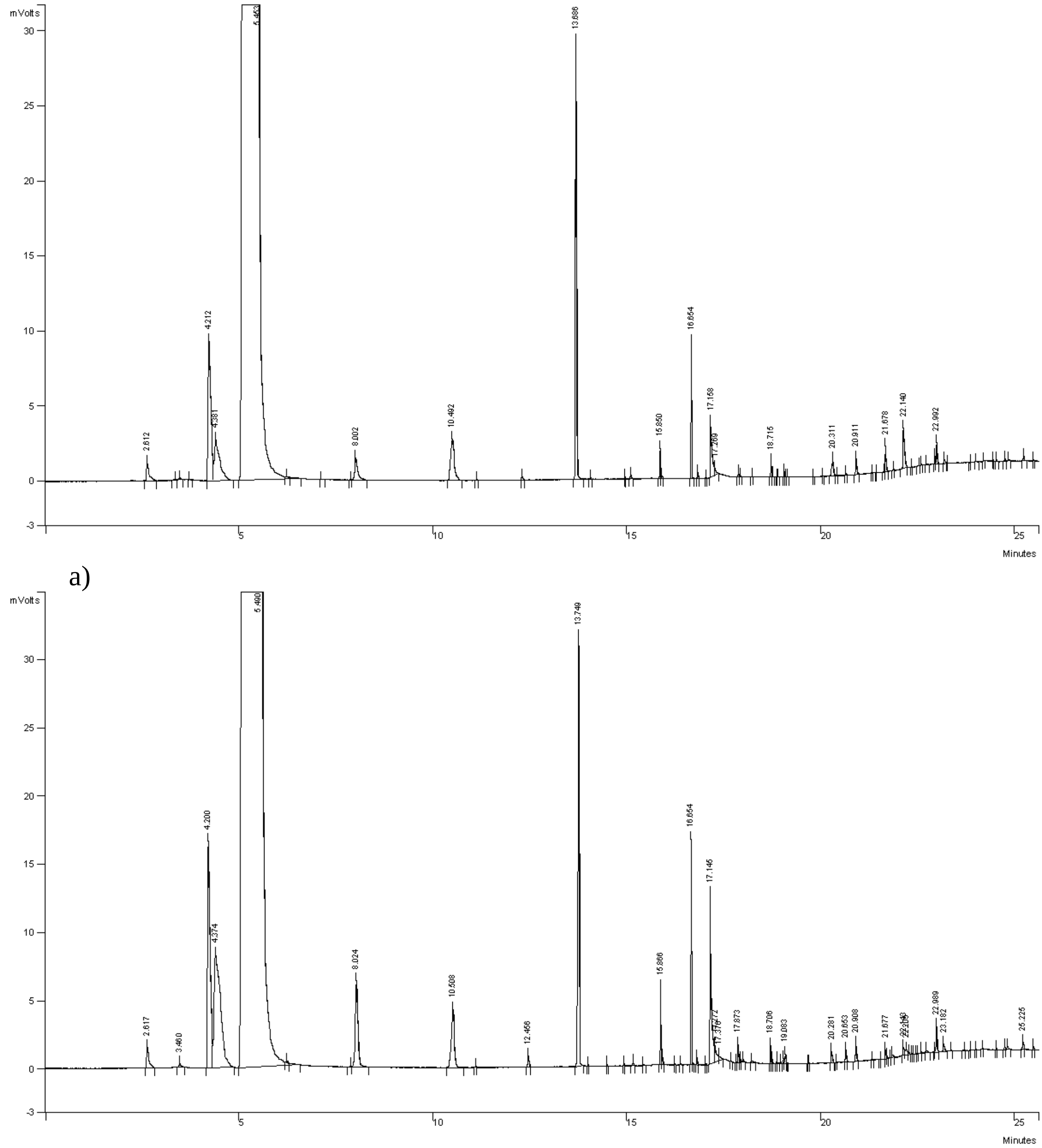


Figure 1. Chromatographic profile of standard solution



a) b)

Figure 2. Chromatographic profiles of the studied brandies: a) grape brandy; b) plum brandy

The established concentration for ethyl acetate is normal and contribute to its positive impact on the taste and aroma characteristics of studied grape brandy. The findings for the concentration level of this ester in our study correlated with the data of Apostolopolou et al. (2005), which indicate a wide range of variation - 7.66 - 692.1 mg/100ml (76.6 - 6920 mg/dm^3). The higher alcohols content is highly dependent of the availability of its precursor - the amino acids in fruits. Through metabolism of *Sacharomyces cerevisae* the amino acids are converted to higher alcohols, giving typical fruity and floral nuances of the distillates.

The total concentration of higher alcohols found in the grape brandy was 89.50 mg/dm^3. According to BNS (Bulgarian National Standard 394:51. Grape brandy, 1951) the grape brandy must contain higher alcohols up to 2.00 g/dm^3.

Table 1. Quantity of volatile aromatic compounds identified in the studied brandies

Identified compounds	Quantity of identified compounds, mg/dm^3	
	Grape brandy	Fruit (plum) brandy
Acetaldehyde	162.00	106.70
Methanol	453.00	973.00
1.Esters		
Ethyl acetate	170.80	155.90
Propyl acetate	Traces	120.70
Isobutyl acetate	52.20	39.70
Ethyl butyrate	ND	5.60
Pentyl acetate	Traces	Traces
Hexyl acetate	71.10	Traces
TOTAL	***294.10***	***321.90***
2. Higher alcohols		
Isobutanol	57.90	ND
1-pentanol	16.80	31.90
2-methyl-1-butanol	ND	Traces
1-heptanol	14.80	32.10
TOTAL	***89.50***	***64.00***
3. Terpene alcohols		
Linalool oxide	0.29	0.21
α-terpineol	ND	Traces
Geraniol	2.60	0.60
β-citronellol	0.60	0.20
TOTAL	***3.49***	***1.01***

Established concentration in our study was less than half of that threshold. With the highest concentrations of the group of higher alcohols was identified isobutanol (57.90 mg/dm^3). It is one of the most common higher alcohols in alcoholic drinks. The data for its established concentrations are correlated with the results of Kostik et al. (2014) and Tesevič et al. (2009).

It is important to note that excessive concentration levels of higher alcohols lead to deterioration of the taste and aroma characteristics of the resulting spirits. Established in our study total concentration of higher alcohols satisfies the expression of their positive effect on the brandy flavoring matrix.

Proof for the authenticity of brandies is the presence of methyl alcohol in them. Its presence is due to the enzymatic transformation of the pectin in the fruit (Marinov, 2005). The final products of this transformation are polygalacturonic acid and methanol (Coldea et al., 2011; Lukic et al., 2011). The methyl alcohol is a compound with a toxic effect on the human body, due to its final metabolic products - formaldehyde and formic acid (Cleland and Kingsbury, 1977; Skrzydlewska, 2003). Therefore, there are requirements for strict and regulated control for the presence of this component in the brandies. The Bulgarian legislation (Law on Wine and Alcohol Beverages,2014), in line with the EU legislation (Regulation (EC) No. 110/2008), sets maximum permitted level of methanol in grape brandy up to 10 g/dm^3. Established concentration of methanol in our study was 453.00 mg/dm^3 (0.453 g/dm^3). It is in times lower than the threshold. The established presence and concentration of methyl alcohol in the test grape brandy prove its authenticity and safety for consumption.

A number of researchers found the presence of terpenes in various types brandies (Tešević et al., 2005; Zhao et al., 2010; Miljić et al., 2013). The presence of terpenes can be an indicator for the identity of the used fruit raw material, because they pass directly from the fruits without metabolized by the yeasts. The terpenes are accumulate at low concentrations in the resulting brandies and have a low threshold of flavor perception (strong aromatic effect at low concentrations). The total content of terpenes identified in the studied grape brandy was 3.49 mg/dm^3, with domination of geraniol (2.60 mg/dm^3).

In the plum brandy (alcohol - 45.38 vol. %) were identified fifteen volatile aromatic compounds.

The results for the ester content of the fruit (plum) brandy showed significant content of ethyl acetate (155.90 mg/dm^3). Compared with the grape brandy, however, its concentration level in plum brandy was lower. The total content of detected esters in plum brandy (321.90 mg/dm^3), however, was higher as compared to grape brandy.

The total content of higher alcohols in the plum brandy (64.00 mg/dm^3) was lower as compared with the established concentration of higher alcohols in the grape brandy.

The total content of the terpenes (1.01 mg/dm^3) in the plum brandy was lower than that established in the grape brandy. With the highest content was established geraniol (0.60 mg/dm^3).

Regarding the content of methyl alcohol is apparent that in the plum brandy its concentration is almost twice as high - 973 mg/dm^3 (0.973 g/dm^3), compared with the grape brandy. This is explained by the normal higher content of pectin (a precursor for the formation of methanol) in plum fruit. Established concentration of methyl alcohol in plum brandy meets the requirements of the Bulgarian and European legislation and proves authenticity and safety for brandy consumption.

4. CONCLUSIONS

From the conducted research for the determination of the aromatic profile of traditional Bulgarian brandies - grape and fruit (plum) can be concluded:

1) Fruit (plum) brandy shows a higher total concentration of esters in comparison with grape brandy - 321.90 mg/dm^3 versus 294.10 mg/dm^3. The dominant ester in both types brandies was ethyl acetate.

2) The grape brandy shows a higher total concentration of higher alcohols, compared with fruit (plum) brandy - 89.50 mg/dm^3 against 64.00 mg/dm^3.

3) The identification and the difference in the concentrations of methyl alcohol proves the authenticity of brandies and confirm that brandies were produced from their corresponding fruit raw material. The findings for the methyl alcohol content meets the safety criteria of the Bulgarian and European legislation.

4) From the terpene alcohols in both types of spirits dominate geraniol. In grape brandy was found three times higher concentration of terpenes in comparison with fruit (plum) brandy.

5) The conducted study proves rich aromatic picture, with presence of all major groups of compounds responsible for the well-balanced aromatic profile of traditional Bulgarian brandies.

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