IDENTIFICATION AND QUANTIFICATION OF CERTAIN VOLATILE AROMATIC COMPONENTS IN GRAPE BRANDIES

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Abstract
The aromatic profile of three different grape brandies was examined by GC-FID. The standard solution (purity> 99.0%) based on which volatile aromatic compounds of brandies were identified includes: acetaldehyde, 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, ethylisovalerate, 1-pentanol, phenyl acetate, 1-hexanol, ethyl hexanoate, hexyl acetate, 1-heptanol, linalool oxide, phenyl acetate, ethyl caprylate, α-terpineol, nerol, β-citronellol, geraniol. As an internal standard 1-octanol was used. Seventeen volatile aromatic compounds have been identified. The highest total content of volatile compounds was established in grape brandy 2 (481.16 mg/dm^3). From the aldehydes group in all distillates the presence of acetaldehyde was found. The highest concentration of this compound was found in grape brandy 1 (113.73 mg/dm^3). The ester composition was diverse, with domination of ethyl acetate. The highest content of esters was found in grape brandy 2 (293.65 mg/dm^3). The total concentration of higher alcohols in brandies was low. 1-hexanol was found in all brandies tested. The presence of this compound indicates poor maturation of the grape raw material used. The highest terpenic content was found in grape brandy 1 (0.23 mg/dm^3). The brandies have been found to contain methyl alcohol. This is an indicator of their authenticity.

Keywords: Ofada rice, response surface methodology, OS6 processing conditions, optimisation

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1. INTRODUCTION

The brandy is a higher alcoholic beverage obtained from fermentation and subsequent distillation of grape pulp, fruit pulp, juices, by-products from wine-making (Marinov, 2005; Kostik et al., 2014).
The formation of a variety of volatile components giving typical aromatic characteristics of brandies is due to a number of interrelated factors: the quality and composition of the grape raw material used (including the aromatic potential of grapevine varieties), the technological conditions of the production process, the fermentation conditions, the yeast strains used, the technological and technical conditions of the distillation process, the aging process (using oak barrels or accelerated aging with the addition of oak extracts) (Marinov, 2005; Yankov Et al., 2000, Velkov, 1996, Marku et al., 2015, Coldea et al., 2011). Based on the combined effect of all these factors, a finished product with a strong aromatic potential and values is formed. In the case of improperly synchronized processes, however, formation of compounds giving negative flavor effects may occur.
The main groups of compounds forming the brandies aromatic matrix are esters, aldehydes, higher alcohols and terpenes (in particular, terpenic alcohols) (Velkov, 1996; Kostik et al., 2014).
The esters have the most significant aromatic effect. This is due to their low threshold of aromatic perception and their various aromatic nuances (Marku et al., 2015; Greizersten, 1981). Their presence in newly formed brandies is a consequence of their first mechanism of formation - direct formation through yeast metabolism (Chobanova, 2012). Their second formation mechanism is realized at the aging process. It is related to chemical transformation - esterification - the process of connecting the alcohols to the acids, resulting in the formation of various esters (Chobanova,

2012). According to Velkov (1996), the total ester content of grape brandies prodused from non-pressed marc ranges from 500-700 mg/dm^3. When using pressed marc, the amount increases and may reach 840 mg/dm^3. The dominant ester is ethyl acetate, which according to the author is in the range of 420 - 700 mg/dm^3. This ester contributes to fruit nuances when it is in normal concentration (Cortes et al., 2005). It is claimed that ethyl acetate exerts its positive influence on the fruit aroma of the beverage at concentration levels up to 150 mg/dm^3 (Apostolopoulou et al., 2005).
The aldehydes are another group of compounds that have a significant impact on the brandy aromatic matrix. Dominant from this group is acetaldehyde (Coldea et al., 2012; Tesevic et al., 2009). According to Velkov (1996), its concentration in grape brandies produced from non-pressed marc ranges from 50 to 300 mg/dm^3, and when pressed marc is used, it can reach 320 mg/dm^3. The total amount of aldehydes in grape brandies ranges from 55-390 mg/dm^3 (Velkov, 1996).
The higher alcohols are a group of compounds that also represent the part of brandy aromatic matrix. They are products of yeast amino acid metabolism (Tesevic et al., 2009; Kostik et al., 2014). They may also to be a result from bacterial viability (Yankov et al., 2000). The main representatives of this group in grape brandies are isoamylol, isobutanol and n-propanol. The total content of higher alcohols is ranging from 1400 to 2010 mg/dm^3 (Velkov, 1996). Their main role is as precursors to the esterification process, where they interact with acids to form esters with significant aromatic influence (Chobanova, 2012; Yankov et al., 2000).
Another group of compounds involved in the aromatic matrix of brandies are terpenes. They are not products of the fermentation process but are metabolites of the grapevine. From it they pass directly into the distillate. Quantitatively dominant in this group are terpenic alcohols linalool, geraniol, α-terpineol, nerol and β-citronellol (Lengyell, 2012). They are responsible for the fruit aromatic nuances, which is particularly true when fermentation is carried out on muscat grapevine varieties (Luan et al., 2006). Mark et al. (2015) detect the presence of terpenic alcohols in a Hamburg Muscat distillate obtained by two different distillation techniques. Mark et al. (2015) found the presence of α-terpineol in the amount of 0.40 mg /dm^3, geraniol - 0.08 mg/dm^3; linalool - 0.82 mg/dm^3 and citronellol - 0.27 mg/dm^3 in a Hamburg Muscat distillate obtained by direct steam distillation.
The purpose of this study is to identify and quantify some volatile aromatic components in grape brandies.

2. MATERIALS AND METHODS

2.1. Samples

Three grape brandies obtained by the traditional production scheme were studied. The process of distillation was carried out at a licensed distillery. The brandy samples were analyzed in the “Chemical Laboratory” and “Scientific Laboratory of Chromatography” of 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, 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. Aromatic content determination by GC-FID

Gas chromatographic determination of the aromatic components in brandies was done. The content of major volatile aromatic 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 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, 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. As an internal standard 1-octanol was used.

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 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.

After determination of the retention times of aromatic compounds in the standard solution, we proceed to the identification and quantification of the volatile aromatic substances in the grape brandies. Prepared grape brandy samples were injected in an amount of 2 μl in a gas chromatograph and was carried out an identification and quantification of the volatile aromatic substances in each of them.

3. RESULTS AND DISCUSSION

Figures 1, 2 and 3 show the chromatographic profiles of the grape brandies tested. Table 1 presents the identified and quantified aromatic compounds of brandies.

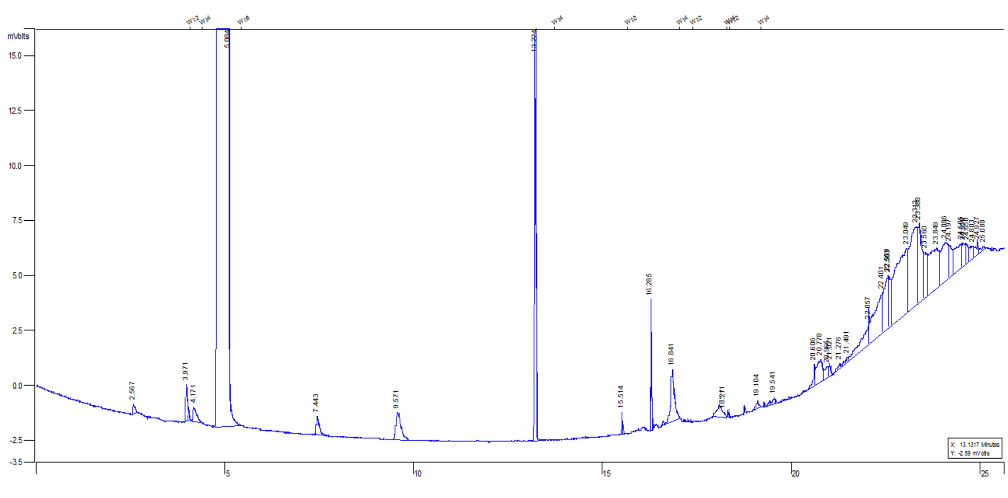

Figure 1. Chromatographic profile of grape brandy 1

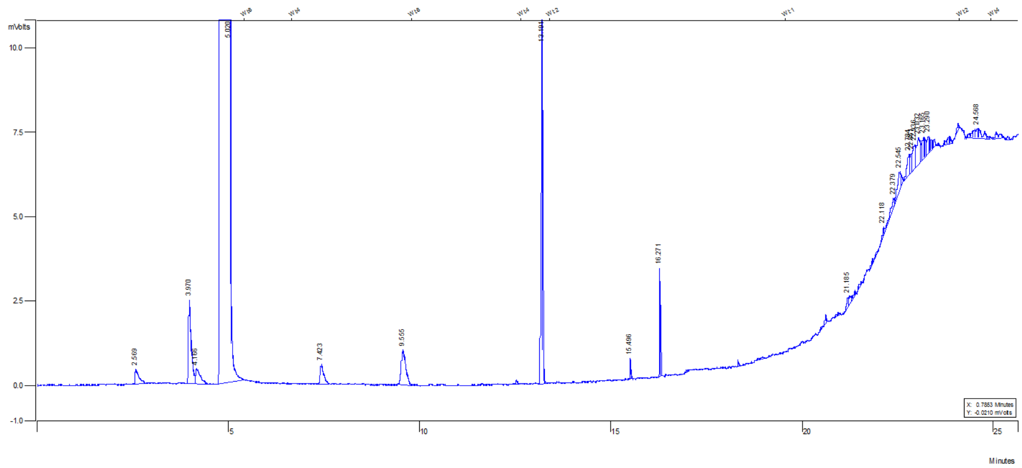

Figure 2. Chromatographic profile of grape brandy 2

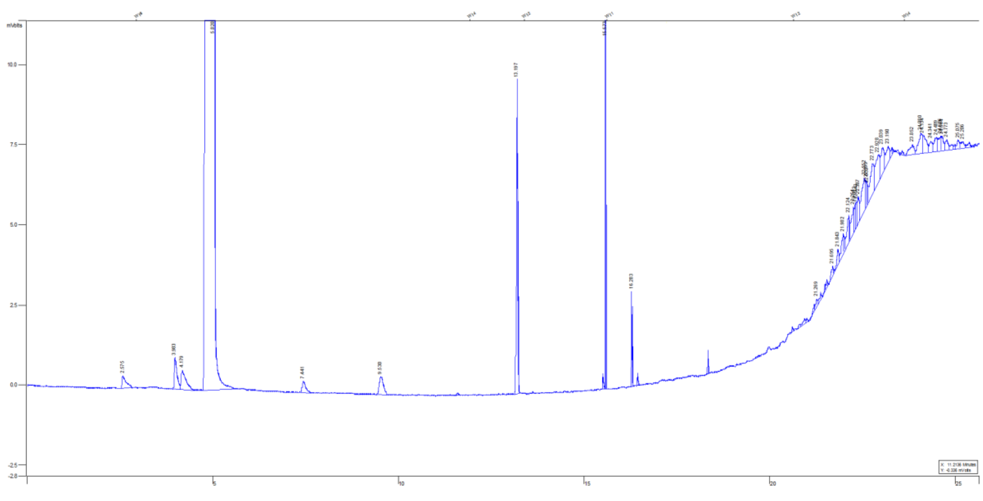

Figure 3. Chromatographic profile of grape brandy 3

In the studied distillates, a total of 17 volatile compounds were identified by GC-FID.

According to the results obtained, the highest alcohol content was found in grape brandy 3 (44.10 vol. %) and the lowest in grape brandy 1 (41.06 vol. %). Under the Law on Wine and Spirits of the Republic of Bulgaria the grape brandy must be characterized by a minimum alcoholic content of 40 vol. %. The three grape brandies tested met of this requirement.

The highest total content of volatile components responsible for the aromatic potential was found in grape brandy 2 (481.16 mg/dm^3), followed by grape brandy 3 (397.50 mg/dm^3). With the lowest total volatile content was grape brandy 1 (369.89 mg / dm3).

The determination of the aromatic profile of the distillates may serve as an indicator of their authenticity. This is due to the presence of components that must necessarily be present in the distillate. A typical such component is methyl alcohol. His formation is due to his precursor - pectin in the fruit. Pectin undergoes transformation due to the enzymatic pectolytic complex of the fruit, which hydrolyzes it to methyl alcohol (Lukic et al., 2011; Coldea et al., 2011). The quantitative variation of methanol in grape distillates is in the range of 0.40 - 2.00 g /dm^3 (Velkov, 1996). In the present study, the highest methanol content was found in grape brandy 3 (151.17 mg/dm^3), and the lowest in grape brandy 1 (71.35

mg/dm^3). These quantities are low and can not affect negatively the health status of the consumer. The identification of methyl alcohol in all three grape brandies is a reliable indicator of their authenticity.

A major component of the aldehyde fraction was acetaldehyde. It is a predominantly quantitative aldehyde, accounting for 90% of the total aldehyde content (Chobanova, 2012). The presence of acetaldehyde was found in the three grape brandies tested. The highest amount is found in grape brandy 1 (113.73 mg/dm^3). The lowest content was found in grape brandy 3 (73.14 mg/dm^3). The result obtained for acetaldehyde content in the grape brandies studied is correlated with the ranges of variation of this compound presented by Velkov (1996).

The ester fraction is the most important component of the brandy aromatic matrix. This is so because the esters have very low thresholds of aromatic perception. They add a variety of nuances to the aroma of the drink. The highest total ester content is found in grape brandy 2 (293.65 mg/dm^3), with a dominant ester ethyl acetate.

A grape brandy 3 with a total ester content of 145.82 mg/dm^3 and a predominant ester ethyl acetate was immediately followed. In grape brandy 1, the lowest total ester content (139.08 mg/dm^3) was established, with propyl acetate domination.

Table 1. Amounts of volatile compounds identified in grape brandies

Identified compounds	Amount of established compounds in the examined brandies, mg/dm^3		
	Grape brandy 1	Grape brandy 2	Grape brandy 3
Ethyl alcohol, vol.%	41.06	41.88	44.10
Acetaldehyde	113.73	77.36	73.14
Methanol	71.35	103.33	151.17
1.Esters			
Ethyl acetate	51.46	180.16	78.03
Propyl acetate	62.01	81.85	50.48
Ethyl butyrate	25.56	31.54	17.21
Hexyl acetate	≈ 0.05	ND	ND
Ethyl hexanoate	ND	≈ 0.05	ND
Phenyl acetate	ND	≈ 0.05	≈ 0.05
Ethyl caprylate	ND	ND	≈ 0.05
TOTAL	***139.08***	***293.65***	***145.82***
2. Higher alcohols			
2-methyl-1-butanol	34.05	ND	ND
3-methyl-1-butanol	≈ 0.05	ND	≈ 0.05
1-hexanol	5.75	6.62	27.27
1-heptanol	5.65	ND	ND
TOTAL	***45.50***	***6.62***	***27.32***
3. Terpenic alcohols			
Linalool oxide	ND	≈ 0.05	ND
α-terpineol	≈ 0.05	≈ 0.05	ND
Geraniol	0.18	≈ 0.05	ND
β-citronellol	ND	≈ 0.05	≈ 0.05
TOTAL	***0.23***	***0.20***	***0.05***
TOTAL CONTENT OF IDENTIFIED VOLATILE COMPOUNDS	***369.89***	***481.16***	***397.50***

It is worth noting that the esters of ethanol (ethyl esters) and esters of the acetic acid (acetates) are usually distinguished by the highest amounts in alcoholic beverages. This is because of the large amount of these two precursors to the esterification process.
The ester of ethanol and acetic acid - ethyl acetate was found in all three brandy samples. It is important for the fruit flavor when it is in normal concentrations (up to 150 mg/dm^3) (Apostolopolou et al., 2005). This concentration was exceeded in grape brandy 2. In it the presence of 180.16 mg/dm^3 of ethyl acetate was found, which did not correlate with the findings of Apostolopolou et al., 2005. In the remaining two brandies were found low amounts of ethyl acetate twice as low as the proposed concentration threshold for the positive aromatic influence of this ester.
The higher alcohols have been identified in very small quantities. The highest was their presence in grape brandy 1 (45.50 mg/dm^3) and in grape brandy 2 (6.62 mg/dm^3) was the lowest. 2-methyl-1-butanol (active amyl alcohol) was found in the highest concentration of all higher alcohols (34.05 mg/dm^3), but it was only identified in grape brandy 1. Interestingly was the presence of 1-hexanol. It is found in all tested samples. This compound give herbaceous ("green") aroma (Chobanova, 2012). Its presence may serve as an indicator of poor ripening of the grapes used for the production of distillate. 1-hexanol was found in a very high concentration in grape brandy 3 (27.27 mg/dm^3).
The higher alcohols have very high thresholds of aromatic perception. Therefore, their primary role in the aromatic potential of the beverage is as a precursors to the esterification process.
The terpenes are an important group of compounds from the aromatic matrix of distillates. They have very low thresholds of aromatic perception (Chobanova, 2012). This distinguishes them as a component with extremely strong aromatic potential. Dominant from this group in alcoholic beverages are terpenic alcohols. The highest total concentration of terpenic alcohols was found in grape brandy 1 (0.23 mg/dm^3). Grape brandy 3 (0.05 mg/dm^3) was with the lowest content. In it only one terpenic alcohol - β-citronellol in very low concentration was found. In grape brandy 1 two terpenic alcohols were identified - α-terpineol and geraniol. In grape brandy 2, linalool oxide, α-terpineol, geraniol and β-citronellol have been identified.

4. CONCLUSIONS

The gas chromatographic research of three grape brandies identified 17 aromatic compounds from different groups - esters, aldehydes, higher alcohols, terpenic alcohols. The presence of methyl alcohol has also been established. This is an indicator of the brandies authenticity.
2. Various esters have been identified, giving different fruity nuances to distillates. The presence of ethyl acetate was predominant.
3. Aldehydes are represented by acetaldehyde. It has been identified in all the samples tested.
4. Higher alcohols was found in very low concentrations. The presence of 1-hexanol was found in all samples, indicating poor maturation of the grape raw material.
5. A total of four terpenic alcohols have been identified in the brandies. Grape brandy 1 has the highest total terpenic content (0.23 mg/dm^3).

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