Original scientific paper
UDC 663.5:543.544.3

GAS-CHROMATOGRAPHIC ANALYSIS OF SOME VOLATILE CONGENERS IN DIFFERENT TYPES OF STRONG ALCOHOLIC FRUIT SPIRITS

Vesna Kostik[1*], Shaban Memeti[1], Biljana Bauer[2]

[1*]Institute of Public Health of Republic of Macedonia, 50 Divizija 6, 1000 Skopje, Republic of Macedonia
[2]Institute of Pharmacognosy, Faculty of Pharmacy, Ss. Cyril and Methodius University, Vodnjanska 17, 1000 Skopje, Republic of Macedonia

[*]e-mail: vesna2mk@yahoo.com

Abstract

Beside ethyl alcohol, the major active component of alcoholic beverages, almost all alcoholic drinks contain volatile and non-volatile substances called congeners. They are present in different concentrations depending on beverage type and manufacturing methods. In the current study, the major volatile compounds besides ethanol as: methanol, ethyl acetate, 1-propanol (n-propanol), 2-propanol (i-propanol), 1-butanol (n-butanol), i-butanol (2-methylpropan-1-ol), n-amyl alcohol (n-pentanol), i-amyl alcohol (3-methyl-1-butanol) were characterized by gas chromatography (GC-FID) on HP-Inowax column and Supecowax column, respectively. For that purpose, hundred samples of three types of grape brandies (lozova rakia, komova rakia and vinjak) and thirty samples of plum brandies (slivova rakia) from domestic producers were analyzed. In order to evaluate the difference in composition regarding the type of brandy it has been compared the mean value (MV) obtained for each volatile. When compared the mean values of volatiles in plum *vs* grape brandies, for methanol, ethyl acetate and n-propanol, the MV of plum brandies were significantly higher. Mean value for methanol in plum brandy was 1903 mg/100 mL anhydrous alcohol a.a, in grape brandy lozova was 464.7 mg/100 mL a.a, in grape brandy komova was 721 mg/100 mL a.a and in grape brandy vinjak was 169 mg/100 mL a.a. Mean value for ethyl acetate in plum brandy was 132.5 mg/100 mL a.a, which was 2.2 as high as mean value for ethyl acetate in grape brandy lozova (60.3 mg/100 ml a.a.). Mean value for n-propanol in plum brandy was 110.4 mg/100 mL a.a, in grape brandy lozova was 28.52 mg/100 mL a.a, in grape brandy komova was 42 mg/100 mL a.a., and in grape brandy vinjak was 33.2 mg/100 mL a.a. The highest mean value for i-amyl alcohol content was found in grape brandy komova rakia 176.6 mg/100 mL a.a.

The content and the type of volatile congeners in some strong spirits which are produced by process of fermentation of fruits and distillation could be considered as a marker of fermentation and (or) botanical origin. Ethyl acetate in fruit brandies is formed by enzymes' reactions during fermentation. Higher alcohols and fusel alcohols (1-propanol, 2-methylpropan-1-ol, 2-methyl-1-butanol, 3-methyl-1-butanol and phenyl ethyl alcohol) are formed in biochemical reactions by yeast on amino acids and carbohydrates. The amounts in different beverages vary considerably. Methanol represented the major volatile component, characteristic to fruit brandies which is released by enzymatic degradation of methoxylated pectin's and is not a by-product of yeast fermentation Therefore this molecule can be considered not only a parameter of distillate safety, but also as an indicator of natural origin of distillate.

***Key words**: Congeners, Methanol, Ethyl acetate, Higher alcohol, Fusel alcohol, Gas chromatography (GC-FID).*

1. Introduction

By definition, any drinkable liquid that contains from 0.5% (v/v) to 95% ethyl alcohol is an alcoholic beverage. Although the major physiologically active component of most alcoholic beverages is ethyl alcohol, there is a remaining fraction of compounds called congeners. Congeners may be highly volatile compounds, like alcohols, acids, aldehydes, ketones and esters. Other components include carbohydrates, tannins, phenols, metals, colouring agents, minerals, histamine and other pharmacologically active substances. Congener content of commercial alcoholic beverages differs significantly for each type of beverage, wine and beer having appreciably higher amounts than distilled spirits [1]. Even if quantitatively small, they play an important and often unnoticed role in the social use and of the alcohol abuse [2].

Higher alcohols and fusel alcohols (1 -propanol, 2-methylpropanol, 2-methylbutanol, 3-methylbutanol and phenylethyl alcohol) are formed in biochemical reactions by yeast on amino acids and carbohydrates. They are mildly toxic, and have a strong, disagreeable smell and taste [3].

While thousands of different volatile congeners may be found in various drinks at one time or another, several of them have been found to be constantly present: methyl alcohol, acetaldehyde, ethyl acetate, ethyl formate and the small aliphatic alcohols (n-propyl alcohol, isobutyl alcohol, n-butanol) make up the major volatile congener content of beers, wines and distilled spirits [4].

Macedonia has and old and rich tradition in fruit growing and production of distilled beverages. The national brands of distilled spirits are: rakia which is produced from grapes or grape pomace and plum brandy which is produced from plum. In the traditional way of home production, distillation process of fermented fruits is performed in special rakia production tanks. The brandy is boiled in a cauldron from which the fumes are drown through the copper tube into a coil. It is located in a large bowl with water and due to evaporation at the end of the chain, the final spirit is obtained. There is a risk of poisoning with methanol if somebody drinks freshly brewed rakia, so it should be avoided [5].

The storage and the maturing are achieved in wood barrels, stainless steel or glass recipients for at least three months. The yellow or gold-yellow colour of that traditional distillate can be obtained exclusively by maturing in wood barrels (mainly the oak barrels) without any colorants or pure alcohol of the industrial origin added [5].

The preparation and fermentation of raw material, distillation technology and maturation are main factors responsible for the specific bouquet of fruit brandies [6].

Some of volatiles found in fruit distillates, such as methanol, furfural, i-butanol and acetaldehyde have toxic potential. This is the reason why the European Commission established a maximum admissible value for methanol in fruit brandies being 10 g/L of pure ethanol (p.e.), equivalent to 1000 mg /100 mL anhydrous alcohol (a.a.). In the case of ethyl alcohol of agricultural origin, the limits of these toxic compounds are more restrictive (acetaldehyde maximum 0.5 mg/100 mL a.a.; methanol 30 mg/100 mL a.a.; furfural not detectable [7].

In the industrial production of rakia, distillation processes are well controlled, and therefore the risk for methanol presence in the final product is diminished.

The industrial production of different types of spirits in Macedonia has been changed and enlarged significantly in the last decade. The new producers have launched a new products with questionably quality. Due to the harmonization of national legislation, a new Regulation which defines the quality of alcoholic beverages has been adopted [8]. This replaced the old Regulation [9], where the quality demands for certain domestic brands of spirits were specified in more details.

Aim of our study was to identify and quantitatively analyze alcohol volatile congeners in different types of spirits from the domestic producers in order to evaluate their quality.

2. Materials and methods

2.1 Materials

Within the period from 2009 to 2013, a total of hundred samples of three types of grape brandies (lozova rakia, komova rakia and vinjak) and thirty samples of plum brandies (slivova rakia) were tested on the content on several volatile congeners as: methanol, ethyl acetate, 1-propanol (n-propanol), 2-propanol (i-propanol), 1-butanol (n-butanol), i-butanol (2-methylpropan-1-ol), n-amyl alcohol (n-pentanol) and i-amyl alcohol (3-methyl-1-butanol). The samples were provided by seven producers located in Macedonia.

2.2 Methods

The major volatile components in spirits were analyzed using gas chromatography (GC) with a flame-ionization detector (FID) [10]. Measurements were performed in the Food Control Department in the Institute of Public Health, using HP model 5890 series II (plus) gas chromatograph equipped with an HP automatic liquid sampler and a flame-ionization detector (FID). Separation of the analytes was made on a polar fused silica capillary column (30 m x 0.32 mm id. x 0.25 μm film thickness) coated with bonded; poly(ethylene glycol)), commercially available as Supelcowax obtained from Supelco (USA). The carrier gas (nitrogen) flow rate was 1.5 $mL \cdot min^{-1}$ and the split ratio was 1:10. The injection port was maintained at 250 ^{0}C and the FID at 280 ^{0}C. Oven temperature was set at 60 ^{0}C (5 minutes) increasing for 10 $^0C \cdot min^{-1}$. The final oven temperature was maintained at 200 ^{0}C (10 minutes). Qualitative identification on the analytes was based on retention time in the column and appearance of the peak signal as compared with the standard solution with known substances. For quantitative evaluation the internal standard method was applied, with a known amount of 4-methyl-1-pentanol as the internal standard (IS). The concentration of each volatile was determined with respect to the internal standard from the relative response factors (RRF), which were obtained during calibration under the same chromatographic conditions as those of the sample analysis.

Samples of spirits were filtered through Milipore filters with the pore diameter of 0.45 μm. 1 μl of the sample was directly injected into the column, followed by the injection of the standard solution. Retention times of tested congeners are given in Table 1.

Table 1. Retention times in minutes for some volatile congeners in spirits

Type of congener	Retention time (min)	SD (min)
Ethyl acetate	3.492	0.0082
Methanol	3.691	0.0063
i-propanol	5.94	0.0048
n-propanol	6.271	0.0053
i-butanol	7.366	0.0044
n-butanol	7.538	0.0065
i-amyl alcohol	10.316	0.0069
n-amyl alcohol	10.583	0.0078

The determination of ethanol concentration in the samples was performed by the same gas chromatographic method, after sample dilution (1:1000) in order to comply with the calibration curve.

2.3 Statistical analysis

The statistical analysis was performed using Origin software package version 7.0. The statistical significance of the difference between the data pairs for i-amyl alcohol content was evaluated by analysis of variance (One-way ANOVA) followed by the Tukey test. Statistical differences were considered significant at $p < 0.05$.

3. Results and Discussion

The results for the determination of ethanol concentrations in fruit spirits are outlined in Table 2.

Table 2. Concentrations (minimum - maximum) of ethanol in fruit spirits, determined by GC-FID (%v/v; n=number of samples; MV)

Type of fruit brandy	Concentration of ethanol (% v/v)
Grape brandy lozova (n=45)	40.5 – 50.1 (45.8)
Grape brandy komova (n=35)	40.7 – 50.3 (47.8)
Grape brandy vinjak (n=20)	37.8 – 39.2 (38.5)
Plum brandy (n=30)	46.7 – 50.8 (48.3)

The amount of ethanol in the samples varied from 37.8% (v/v) for grape brandy vinjak to 50.8% (v/v) for plum brandy (Table 2). Ethanol is present in alcoholic beverages as a consequence of the fermentation of carbohydrates with yeast and is responsible for the beverage's body [6]. The ethanol concentration in different types of spirits is defined by Official Regulation [8] which is harmonized wit the International regulation [7]. Its determination is part of the quality control of spirit drinks. Following this regulation, the authentic spirits made from grapes should meet the minimum limit approved for the ethanol concentration within the range from 36% to 37.5% (v/v). In all tested samples, ethanol content was found to be in compliance with proposing Regulations [7, 8].

The results for the determination of volatile composition of fruit spirits are shown in Table 3.

Table 3. Concentrations (minimum - maximum) of volatile components of fruit spirits, determined by GC-FID (mg/100 mL a.a; n=number of samples; MV)

Type of volatile congener	Grape brandy lozova (n=45)	Grape brandy komova (n=35)	Grape brandy vinjak (n=20)	Plum brandy (n=30)
ethyl acetate	3.6 – 248.1 (60.3)	71.8 – 145 (104.4)	38 – 223.2 (90)	51 – 404 (132.5)
methanol	2.24 – 1210 (464.7)	42.6 – 1276 (721)	45 – 375 (169)	245 – 1903 (895)
i-propanol	9.5 – 15.6 (11.3)	10.3 – 17.8 (16.3)	6.7 – 10.3 (8.80	11.5 – 20.6 (14.8)
n-propanol	3.6 – 86.9 (28.52)	27.5 – 51.8 (42)	28.9 – 47.4 (33.2)	11 – 250 (110.4)
i-butanol	6.1 – 57 (34.76)	1.0 – 108.7 (36.7)	32 – 64.3 (40.8)	11.5 – 57 (35.2)
n-butanol	1.1 – 4.4 (4.83)	3.0 – 4.0 (3.68)	1.2 – 2.2 (1.4)	4.1 – 11.2 (6.7)
i-amyl alcohol	46.8 – 273.8 (172.7)	49.4 – 276.3 (176.6)	114 – 137 (128)	98.8 – 135.6 (112.8)
n-amyl alcohol	0.87 – 3.2 (2.25)	1.1 – 5.5 (3.92)	0.55 – 2.2 (1.88)	9.5 – 12.4 (10.7)

Methanol is a constituent arising from the enzymatic degradation of pectin contained in fruits. Generally, its quantity is related to the amount of pectin present in fruits used for fermentation. The methanol concentration is suitable for proving the authenticity of fruit spirits [11].

The amounts of methanol in the samples (Table 3) varied from 2.24 mg/100 mL a.a. for grape brandy lozova to 1903 mg/100 mL a.a. for plum brandy. The obtained results for methanol content in plum brandy are lower compared with those reported by Jung *et al.* (55mg/100 mL a.a. - 403 mg/100 mL a.a.) [1], but are in compliance with those of Winterova *et al.* (288 mg/100 mL a.a - 1141 mg/100 mL a.a.) [12].

Limits are posed by the Official Regulations on the methanol content in many spirits [7, 8]. Its determination is part of the quality control of spirit drinks. Following this regulation, the authentic fruit spirits should meet the maximum limit approved for the methanol concentration, i.e. 1000 mg/100 mL a.a. for grape brandy (lozova and komova) as well as for plum brandy and

200 mg/100 mL a.a. for grape brandy vinjak. These limits were exceeded in 12 samples (2 samples of grape brandy lozova, 3 samples of grape brandy komova, 3 samples of grape brandy vinjak and 4 samples of plum brandies).

Besides methanol, the main volatile compounds detected in the currents spirits samples were ethyl acetate, i-propanol, n-propanol, i-butanol, n-butanol, i-amyl alcohol and n-amyl alcohol.

Esters are very important compounds due to their particular contribution to flavour and aroma, since they have the lowest organoleptic threshold [13]. The quantity of this compound presented in the final product can vary widely, since it is synthesized from acetic acid and ethanol [14]. The mean values of the concentration of ethyl acetate for studied samples ranged from 3.6 mg/100 mL a.a. for grape brandy lozova to 404 mg/100 mL a.a. for plum brandy (Table 3). High concentrations of ethyl acetate are indicative of prolonged storage of the row material and probable acetic bacteria spoilage. Concerning ethyl acetate, many authors have documented high variability [15, 16]. The results obtained for ethyl acetate content in plum brandy were in correlation with those of Winterova *et al.* [12], where the values of ethyl acetate in plum brandy ranged from 56.3 mg/100 mL a.a to 236/100 mL a.a.

Higher alcohols are characteristic components which are metabolised from amino acids by yeasts during alcoholic fermentation of fruits and other raw materials. The amounts of these compounds depend on the quantity of amino acids in fruits. Higher alcohols have a significant impact on the flavour of alcoholic beverages [1, 2]. But, except having a significant impact on the flavour of alcoholic beverages higher alcohols are mildly toxic. Wencker *et al.* showed that n-butanol is a strongly discriminating parameter for the fruit spirits [3]. In their investigations of Australian and imported brandies Hogben and Mular found out that the i-amyl alcohol *vs* i-butanol content and the i-butanol *vs* n-propanol content is one of the several criteria for characterization the authenticity of brandies [11].

The most important higher alcohols of grape and plum brandies (Table 3) were found to be i-propanol, n-propanol, i-butanol, n-butanol, i-amyl alcohol and n-amyl alcohol. Our investigations showed that i-amyl alcohol was the most abundant higher alcohol in all tested samples of grape and plum spirits. The highest mean value for i-amyl alcohol content was found in grape brandy komova rakia 176.6 mg/100 mL a.a.

The statistical significance of the difference between the data pairs for i-amyl alcohol content was evaluated by analysis of variance (One-way ANOVA) followed by the Tukey test. The evaluated statistical data are shown in Table 4.

Table 4. Statistical significance of differences between data pairs, evaluated by one-way ANOVA followed by the Turkey test

Type of brandy	i-amyl alcohol mg/100 mL a.a			
	Grape brandy lozova	Grape brandy komova	Grape brandy vinjak	Plum brandy
Grape brandy lozova		n.s.	$p < 0.01$	$p < 0.01$
Grape brandy komova	n.s.		$p < 0.01$	$p < 0.01$
Grape brandy vinjak	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
Plum brandy	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$

n.s. - not statistical difference ($p > 0.05$)

At the 0.05 level of significance, the analysis of variance showed that the population means for i-amyl alcohol were not significantly different between the analyzed samples grape brandy lozova – grape brandy komova, which means that there were not differences observed during alcoholic fermentation of fruits, since the row material used was similar between the varieties.

Among the investigated higher alcohols, the lowest content in all tested samples were found for n-amyl alcohol with mean values ranged from 1.88 mg/100 mL a.a for grape brandy vinjak to 10.7 mg/100 mL a.a. for plum brandy (Table 3). The content of n-butanol was also low in all tested varieties ranged from 1.4 mg/100 mL a.a. for grape brandy vinjak to 6.7 mg/100 mL a.a for plum brandy. According to Pietruszka *et al.*

in the spirits obtained from ray mashes, i-amyl alcohol has the highest contribution, and n-propanol the lowest [17]. According to the investigations of Winterove *et al.*, the higher alcohols most frequently found in low concentrations in fruit spirits were n-butanol and i-butanol [12]. They found the lowest values measured for n-butanol in sweet cherry and sour cherry brandies (0.5 - 3.1 mg 100 mL a.a.). Comparing these results, it can be concluded that the concentrations of higher alcohols found in our samples of grape and plum brandies are commonly acceptable.

4. Conclusions

- The requirements for quality food products have been increasing in recent years and the interest in the quality and purity of fruit spirits has grown in this connection as well. The aim of our study was to identify and quantitatively analyze alcohol volatile congeners in different types of spirits from the domestic producers in order to evaluate their quality according to the Official Regulation [8]. For that purpose, a total of 100 samples of three different types of grape brandies (lozova rakia, komova rakia and vinjak) and 30 samples of plum brandies obtained from seven domestic producers were analyzed on the content of ethanol, ethyl acetate, methanol, i-propanol, n-propanol, i-butanol, n-butanol, i-amyl alcohol and n-amyl alcohol.

- Maximum admissible limits were exceeded for methanol content in twelve of tested samples (2 samples of grape brandy lozova, 3 samples of grape brandy komova, 3 samples of grape brandy vinjak and 4 samples of plum brandies). In general, the compounds identified in major quantities in grape and plum spirits are similar to those present in other alcoholic beverages.
- The major higher alcohol present in all tested samples was i-amyl alcohol. The content of i-amyl alcohol in grape brandy lozova rakia and grape brandy komova rakia didn't show significant statistical difference, since the row material used was similar between the varieties.

5. References

[1] Jung A., Jung. H., Auwarter W., Pollak S., Farr A. M., Hescer L. and Schiopu A. (2010). *Volatile congeners in alcoholic beverages: analysis and forensic significance.* Romanian Society of Legal Medicine 18, pp. 265 - 270.

[2] Bonte W. (1987). *Begleitstoffe alkoholischer Getranke: Biogenese, Vorkommen, Pharmakologie, Physiologie und Begutachtung.* Verlag Max Schmidt – Romhild, Lubeck.

[3] Wencker D., Louis M., Nomura G.and Hasselmann M. (1981). *Etude de la Fraction"Alcools Superieurs Methanol" de diverses Eaux-de-vie II –Distribution des differents alcohols en function de l'origine botanique des fruits.* Annales des Falsifications et de l' Expertise Chimique 74, pp. 487 - 499.

[4] Kucuk Z. and Ceylan K. (1998). *Potential Utilization of Fusel Oil: A Kinetic Approach for Production of Fusel Oil Esters through Chemical Reaction.* Turk. J. Chem., 22, pp. 289 - 300.

[5] Stanimirović S., and Stanimirović D. (1982). *Bromatolgija*. Zavod za Udzbenike i Nastavna Sredstva, Beograd, SFRJ.

[6] Scanavini H. F. A., Ceriani R., and Meirelles A. J. A. (2012). *Cachaça distillation investigated on the basis of model systems.* Braz. J. Chem. Eng. 29 (2), pp. 429 - 440.

[7] EEC, Council Regulation No. 110/2008 (2008). *Laying down general rules on the definition, description and presentation of spirit drinks.* Off. J. Eur. Union, L 39/16.

[8] Sluzben Vesnik na RM 82/2010 (2005*). Pravilnik za posebnite baranja za bezbednosta na žestokite alkoholni pijaloci*.

[9] Sluzben Vesnik na SFRJ 16/88 (1988). *Pravilnik za kvalitetot na žestokite alkoholni pijalaci*.

[10] Coldea R., Sosaciu C.T.E., Parv. M. and Vodbar D. (2010). *Gas-chromatographic analysis of Major Volatile Compounds Found in Traditional Fruit Brandies from Transylvania, Romania.* Not. Bot. Horti. Agrobo, 39 (2), pp. 109 - 116.

[11] Hogben R and Mular M. (1976). *Major congeners of Australian and imported brandies and other spirits as indicator of authenticity.* Journal of the Science of Food and Agriculture 27 (12), pp.1108 - 1114.

[12] Winterove R., Mikulhkove R., Maceč J. and Havelec P. (2008). *Assessment of the Authenticity of Fruit Spirits by Gas-Chromatography and Stable Isotope ratio Analysis.* Czech J. Food Sci., 26 (5), pp. 368 - 375.

[13] Ramsay C. M.and D. R. Berry. (1984). *The effect of temperature and pH on the formation of higher alcohols, fatty acids and esters in malt whisky fermentation*, Food Microbiology 1, pp. 117 - 125.

[14] Silva M. L., Macedo A.C. and Malcata F.X G. de Revel. (2000). *Steam distilled spirits from fermented grape pomace,* Food Sci. Technol. Int. 6, pp. 285 - 300.

[15] Silva M. L., Malcata F. X. and De Revel G. (1996) *Volatile content of grape marcs in Portugal.* J. Food Compos. Anal. 9, pp. 72 - 80.

[16] Apostolopoulou A. A.., Flouros I., Demertzis P. G. and Akrida-Demertzi K.(2005).*Differences in concentration of principal volatile constituents in traditional Greek distillates*. Food Cont. 16, pp. 157 - 164.

[17] Pietruszka M., Pielech-Przybylska K. and Szopa J.S. (2010). *Synthesis of higher alcohol during alcoholic fermentation of rye mashes.* Scientific Bulletin of the Technical University of Lodz, Food Chemistry and Biochemistry, 1081 (74), pp. 51-64.