Advances in Biology & Earth Sciences
Vol.2, No.3, 2017, pp.263-270

CHARACTERIZATION OF TRADITIONAL FRUIT BRANDY PRODUCED IN AZERBAIJAN

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Abstract. Premium fruit spirits in Azerbaijan, are produced from autochthonous fruit-bearing species such as mulberry, cornelian cherry, fig, quince and feijoa. There are no chemical data on many of these and herein first time we performed the primary analysis of volatile compounds of these rare fruit distillates. Using gas chromatography the essential volatile compounds like light fractions: methanol, acetaldehyde, ethyl acetate, and heavy fractions like 1-propanol 2-methyl-1-propanol, 1-butanol, iso - amylol, 1-hexanol and 2-phenylethanol were identified and quantified. As the second goal in our research it was the study of antioxidant (AO) activity for presented samples. It was shown that all fruit distillates possessed different AO properties depends on the aging period as well as presence of decanoic, dodecanoic acids, their ethyl ethers and ethyl linoleate, ethyl oleate. Total phenol analysis shows the correlation to AO activity of distillates but for mulberry and cornelian cherry spirits there was unusual effect of AO despites low phenols concentrations.

Keywords: *distillation, alcoholic beverages, volatile compounds, gas chromatography, antioxidant activity, phenolic content.*

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Manuscript received*: 25 October 2017*

1. Introduction

Reactive oxygen species, including free radicals, such as superoxide anion radicals, hydroxyl radicals, and non-free-radical species, such as H_2O_2 and singlet oxygen, play a key role in the oxidation process that can damage cells. This process is considered to be one of the initial development stages of many chronic diseases, such as cancer, cardiovascular disease, atherosclerosis, and diabetes [1, 2, 7]. Over the last decade, considerable experimental evidence has confirmed the importance for health of following a diet rich in antioxidants, which can protect the organism against the damage caused by these radicals. Some of these antioxidants are well known, such as vitamins (particularly vitamins E and C) and carotenoids, including b-carotene. A healthy diet should provide an adequate and continuous supply of these antioxidants. Other antioxidants, like ubiquinols and antioxidant thiols, are produced in small amounts by the organism, but the levels of many of them can be increased by dietary supplements [11]. For these reasons, there is an increasing interest in characterizing foods and drinks in terms of antioxidant potential; numerous methods have been developed with this aim.

In regards to the traditional food and beverages from different regions of the world they can be considered as source of natural antioxidant species. Particularly, some of alcoholic beverages and wines contained polyphenolic compounds that act as scavengers of reactive species, preventing oxidation. Phenolic compounds are naturally occurring substances in fruits, vegetables, nuts, seeds, flowers, and some herb beverages, and are integral part of human diet. There a lot of publications about antioxidant activity of wines, especially red dry wines aging in oak barrels. Chromato-mass spectrometry determined variations of different phenolic compounds like cathehins, flavonoids, anthocyanins, stylbenes (reveratrol). It was revealed that aging process in oak barrels lead to increasing the antioxidant capacity which correlate with appearance in wine the additional species like gallic acid, caffeic acid, coumaric acid, roburins. Similar effect was determined for aged European brandy including Cognacs and Armagnacs manufactured from vine. But very little known about antioxidant activity of fruits brandy obtained from nonvine fruits and berries. Azery traditional brandy distilled from berries and other domestic fruits are the scope of our investigations. In present work, the antioxidant activities of Azery traditional brandy were measured and volatile components using gas chromatography were detected.

2. Materials and methods

Samples were obtained from artisan brandy available in local market. Different samples had different aging period but some of them were not aged at all. “Mulberry Club” fig brandy was aged in oak barrel 9 month as was marked by manufacturer.

Antioxidant activity was measured by DPPH assay according to the method Brand and Williams [4]. The absorption of DPPH-methanol solution at 5180 nm was adjusted to 0.5 that equal to 40 μM concentration. Absorbance changes were measured at the maximum on 518 nm within 25 minutes in a UV-Vis spectrophotometer (Jenway 7305). Concentrations were calculated from a calibration curve in the range between 1 and 10 μM of Trolox. All measurements were done in 10 mm optical path cuvette.

The total phenol content of extracts was determined according to the procedure described by Singleton and Rossi [12]. Distilled water (1.8 mL) was added to 0.2 mL of each extract. Folin-Ciocalteu reagent (0.2 mL) was then added and tubes were shaken vigorously. After 3 minutes, 0.4 mL sodium carbonate solution (35 % w/v) was added, along with 1.4 mL of distilled water. Samples were well mixed and left in the dark for 1 hour. The absorbance was measured at 725 nm using a UV-Vis spectrophotometer and the results were expressed in gallic acid equivalents, GAE mg/ml, using a gallic acid standard curve (0-0.2 mg/mL). Samples were further diluted if the absorbance value measured was above the linear range of the standard curve.

Gas Chromatography

Volatile components such as acetaldehyde, ethyl acetate, methanol, and higher alcohols (1-propanol, 1-butanol, iso-butanol, iso-amylol, hexanol) were detected by gas chromatography, using a FID detector and Agilent 6950 gas chromatograph supplied with HP-FFAP capillary column (length 50 m, I.D. 0.32 mm, film thickness 0.52 μm). Injector’s temperature was 150°C, detector ‘s temperature installed at 250°C.The oven temperatures were programmed as follows: starting at 45°C within 15 min and then increased to 75°C at the rate of 15°C/min. Nitrogen at the flow of 4.8 ml/min was used as a carrier gas. The standards from Sigma company were used for qualitative and quantitative calibrations. All determinations were executed by the internal standard method. Injected volume of the samples was 1 ml. The components contents were expressed in mg/l of absolute ethanol (A.E.).

3. Results

Total phenolic analysis was carried out over the six brandy samples from mulberry, cornelian cherry, black currant, fig, feijoa and quince fruits. Results shown at Fig.1. As shown in diagram total phenols concentration appeared highest value 0.0085 mg/ml in fig brandy sample which correlates to manufactures notes that brandy is matured in oak barrel within 9 month. In comparison to immatured mulberry and cornelian cherry brandy where the total phenols show lowest concentration among presented samples. Phenol concentrations in samples have good correlation with maturing period in oak barrels declared by manufactures. So, three month maturing in oak barrels for black current feijoa and quince brandy slightly increase total phenol concentration.

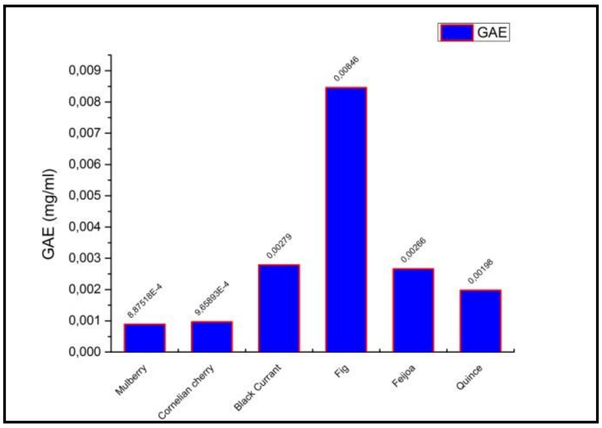

Fig.1 Total phenols concentration in different brandy expressed in gallic acid equivalents.

In order to evaluate the quality of brandy we got and assuming rather high phenols concentrations we evaluated the antioxidant capacity of that beverages using DPPH inhibition method as described in Materials and Methods. The value of 0.5 at DPPH inhibition scale corresponds to 6.8 µM of Trolox equivalent. Experiments results shown at the Fig.2. Highest activity was shown by fig brandy with reacting volume of 30 µl. The antioxidant activities of samples are strongly correlates to aging period (or its lack) in oak barrels and total phenols concentration in Fig.1. It was surprising observe some antioxidant activity in mulberry and cornelian cherry brandy despite that were not aged in oak barrels.

Gas chromatography was applied in order to determine volatile compounds and their concentrations in samples. The main components of the distillates were ethanol and water, with a series of volatile substances that distil together and comprise a smaller portion of the spirit. These volatile substances, together with the components that are present in higher proportions, give distinctive flavor characteristics. The nature and composition of these components depend on the characteristics of the raw material, and on the fermentation and distillation processes. A typical chromatogram for the samples is shown in Fig. 3. The chromatograms of the other samples showed the same pattern as those in Fig. 3, although the peak heights were different in each case. The retention times for the major compounds and their concentrations are summarized in Table I. Some unidentified minor peaks were detected with retention time 1 and 17 minutes but calibration wasn't perform and further mass spectrometry analysis must be done for their identification.

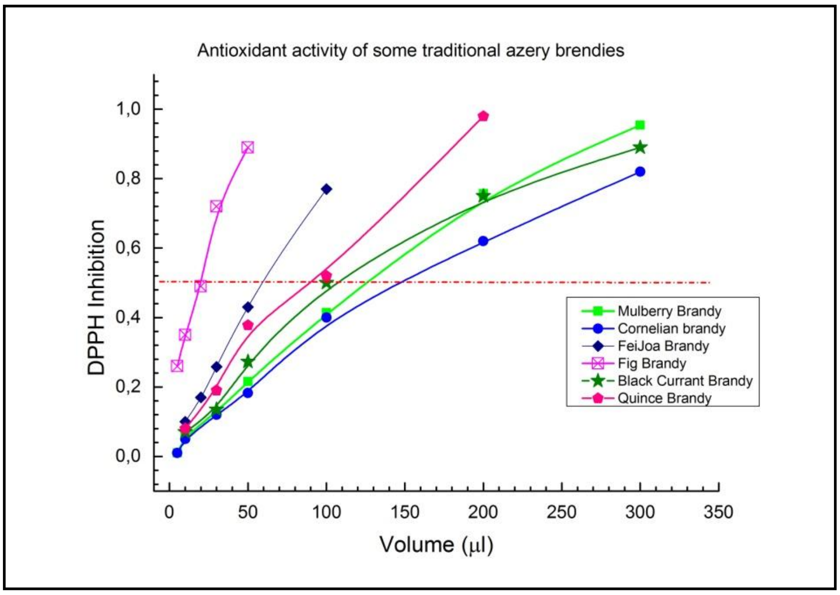

Fig.2. Dependence of DPPH$^{\bullet}$ radical quenching upon the volume of different samples brandy. Dashed line represents of IC50 for 6.8 μM of Trolox equivalent

As seen from chromatogram methanol and acetates are pass immediately before main fraction of ethanol and in artisan distillation process it is very hard to separate them from each other.

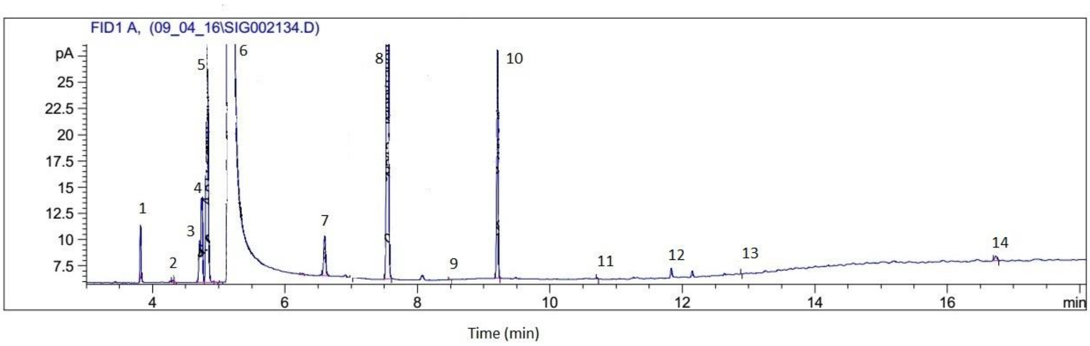

Fig.3. One of gas chromatograms obtained after direct injection of cornelian cherry distillate sample: 1: acetaldehyde; 2: acetone; 3: methyl acetate 4: ethyl acetate; 5: methanol; 6: ethanol; 7: 1-propanol; 8: isobutanol; 9: 1- butanol; 10: iso-amylol; 11: hexanol; 12:acetic acid; 13: benzyl aldehyde; 14: 2-phenylethanol.

Table 1. Concentration of volatile compounds in samples in mg/l of A.E.

Compound	Mulberry	Cornelian cherry	Black currant	Quince	Feijoa	Fig
acetaldehyde	102	136	30	176,3	59,7	68,3
acetone	9	4,8	5,7	5,7	2,37	4,19
methyl acetate	7	1,8	0	2,2	1,13	16,98
ethyl acetate	536	303	39	541	121,29	695
methanol (g/l)	3.16	0,76	4.07	1,3	0,617	6,65
2-butanon	3,4	1,15	5,26	1,5	0	0
2-propanol	6,2	1,19	3,98	2,53	1,087	11,61
iso buthyl acetate	0	3,4	0	0	0	1,03
2-butanol	0	0	0	0	0	0,885
1-propanol	173	40	142	53	56,72	81,76
ethyl butirate	0	0	0	0	0	0
croton aldehyde	0	1,87	0	1,24	0	0
iso butanol	199	604	450	45	130,9	171,6
1-butanol	2	0,272	1,18	6,4	0	0,99
iso amylol	122	136	50	117	16,17	189,8
1-pentanol	0	0	0	0	0	0,107
hexanol	3	0,587	0,358	2,46	0,276	40
benzaldehyde	0	0,057	0	0	0	4,53
benzyl alcohol	0	0	0	0	0	0
phenyl alcohol	2,6	2,477	0,5	0,744	0,247	1,79
acetic acid	18,8	13,5	9	18,5	7,5	372

4. Discussion

Fermentation process relies on transformation of fruits sugars to ethyl alcohol by yeasts. This process pass through the degrading the sugars to acetaldehyde with further reduction it to ethanol. The concentration of ethanol strongly depends upon sugars concentration. The ethanol concentration in presented samples varied from 55 to 75 % v/v. According to European standards such class of brandy usually has ethanol concentration between 40-45 % v/v. Therefore, the variation of the alcoholic title shows that obtained fruits distillates are need more uniform treatment and systematic production in order to ensure a more qualitative product with a standard profile.

As a byproduct during fermentation the methanol appears by reaction of pectolytic enzymes which are splitting methoxyl group from pectin always presented in fruit mash. According to the European legislation (EEC no.1576/89), the distillate must have a methanol concentration lower than 10 g/l of A.E.. The levels for investigated samples were found to be lower than the European limits, varying from 0.76 to 6.65 g/l

AE (Table 1). This means that the manipulation of the raw material was the proper one and was managed with great sensitivity and also very good distillation procedures were performed (Silva et al., 1996). At the same time distillation procedure allow to save the natural aromatic ethers specific for that fruits.

Soufleros and Bertrand [16] demonstrated lower values of methanol in greek grape pomace distillate ranging from 0.5 to 0.84 g/l AE. Some authors [13] and [14, 15] presented for bagaceiras a much more higher concentration of methanol with a mean value equal to 7.55 g/l AE or higher than the European limit, ranging from 10.21 to 10.31 g/l AE and from 0.346 to 3.828 g/l AE, respectively. These values are dependent mainly on the applied technique of the fruits treatment and distillation and, secondly, from the fruits variety. In other reports, the concentrations of methanol in grape pomace distillates range between 0.53 and 1.59 g/l AE (5), from 0.39 to 2.86 g/l AE [3]. The last authors also gave mean values of methanol for distillate from apple 0.35 g/l AE, from cherry 4.57 g/l AE, from pear 7.96 g/l AE and from plum 8.66 g/l AE. Last, [8] for whiskey introduced significantly low mean concentrations of 0.077 g/l AE and for rum even lower of 0.0023 g/hl AE. It can be explained with the low concentration of pectins and even of their lack in starting grain must for whiskey and sugar for rum. We conducted some experiments with sucrose distillates and confirmed very low concentration of methanol in double distilled product (data not published).

It should be mentioned detailed investigation of traditional Serbian distillate from cornelian cherry pomace carried by authors [18] that shown the methanol values ranged between 2.42 to 7.72 g/l AE. Considering above mentioned results it can be said that studied domestic distillates has a low enough levels of methanol in relation to other fruit pomace distillates.

In our study we also evaluated the antioxidant activity (AO) of the samples using standard technique of DPPH free radical quenching. Because distillates have different aging period in oak barrels it was expected that AO activity will be different and for that purpose we tested total phenols concentration in samples. It is well correlates with antioxidant activity of all distillate even with mulberry and cornelian cherry despite they were not aged in oak barrels. We would explain that phenomenon with the presence of phenolic volatile compounds like phenyl alcohol with concentration 2.6 and 2.5 mg per liter of AE. Also it was detected several unidentified peaks in chromatogram with retention times 11.5 minutes, 12.2 and 12.5 minutes, 13.5 minutes, 16 minutes. The lack of calibration for that retention times doesn't allow obtain any information about the nature of that compounds and mass spectrometry must applied for elucidation. Earlier investigations of authors (cornelian cherry) determined the compounds that contribute to the typical flavor characteristics of the cornelian cherry distillate, such as 2-phenylethanol, limonene and 4-ethylphenol, are desirable for this specific distillate. Also high concentrations of decanoic, dodecanoic acids, their ethyl ethers and ethyl linoleate, ethyl oleate impact to the individual flavor and taste of cornelian cherry distillates that was established before (cornelen cherry distillates) and that compounds also can reveal AO activity [10]. The same analysis was performed by authors [17] for mulberry brandy samples where non saturated lipid acids like hexanoic, octanoic, decanoic, dodecanoic acids were discovered. The presence of these compounds can explain AO activity of mulberry brandy's samples we studied.

We couldn't find in available literature detailed study of chemical composition for fig, feijoa and quince brandy which would explain strong AO effects apart of presence of some polyphenols extracted from oak barrels during aging process. The study of chemical compositions of fig extracts shows significant content of palmitic, oleic, stearic, linoleic acids and their ethyl ether [9]. Feijoa peel extract have significant amount of sesquiterpenes like humelene, β-caryophyllene, ledene, bicyclogermacrene [6] but authors didn't detect any signs for nonsaturated lipid acids and their ethers. Probably it is related with preparation procedure of peel extract and absence of fermentation of whole fruits.

5. Conclusion

To our knowledge our study is the first analysis of chemical composition of that traditional azery brandy. In studied distillates, volatile compounds that pose health hazards or organoleptic faults, such as methanol or acetaldehyde, were found at levels lower than those established by the EU standards. The higher alcohols, especially iso-amylol also are the largest group of the volatile composition distillates but still under the established standards. The ethyl esters and fatty acids formed enzymatically during the fermentation process constitute important groups of aroma compounds that contribute to the "fruity" note to the sensory properties of distillates. It seems that the complex and luxurious aroma of "Drenja" spirits depends on the subtle balance of the various mentioned functionalized compounds. Our samples reveal AO activities which we rely to the presence ethyl ethers of lipid acids and presence of polyphenols that extracted during brandy's aging in oak barrels.

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