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Head Space GC-MS Determination of Volatile Constituents in Wines (Appellation of Origin Controlled (AOC)) and Wine Distillates from Two Different Hellenic Native Grape Varieties (*Vitis vinifera* L.)

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Abstract: The volatile compounds are very important for the quality and acceptability of monovarietal wines and its distillates. The professional wine industries in the most areas are relatively young and little is known about those wines and its distillates volatile concentration. Those aromatic volatiles depend on many factors such as grape variety, alcoholic fermentation of must, microclima of cultivated area, vinification procedures employed, wine distillation etc. In this research the volatile constituents of two different Appellation of Origin Controlled (AOC) from Peloponissos (Island of The Ancient Kind Pelopos), Agiorgitiko and Morchofilero (Hellenic native grape varieties (*Vitis vinifera* L.) wines and its distillates were determined by head space GC-MS. The distillates are characterized by a high content of alcohols, acetates, ethyl esters and fatty acids whereas terpenes and norisoprenoids, alcohols, ethyl esters, acetates, fatty acids were the main constituents present in monovarietal wines(5μg L^{-1} for a-ionone to 268.4 mg L^{-1} for methanol). The results shows that the concentration and the complicity of terpenes, norisoprenoids, alcohols, ethyl esters and other volatiles in wine and distillate samples gives the unique character of those Hellenic alcoholic drinks and the complicity of compositional characteristics gives the profile of those native Hellenic varieties.

Key words: Grape varieties, *Vitis viniferous* L., volatiles, distillates, wine, principal component analysis

INTRODUCTION

Wines and distillates are a complex of many organic and inorganic compounds. The composition is very dependent on many factors. Among them, the grape cultivars and the geographical origin, certainly have a great influence. In certain viticulture areas the production of quality wines and distillates has important economical importance as in the island of Ancient King Pelopos (Poloponnisos). Varietal aroma is the main contributor and characteristic for every grape variety. The possibility of recognizing a wine and its distillates from its parameters received great attention in the last few years.

The compounds responsible for varietal aroma (terpenes, norisoprenoids, alcohols, ethyl esters and others and this compounds contribute the special character like rose like, fruity and nutty in wines and distillates. This compounds are at different concentrations (Amerine and Joslyn, 1970; Nelson *et al*., 1978; Nycanen, 1986; Laminkanra *et al*., 1996; Calleja and Elena, 2005; Gerogiannaki-Christopoulou *et al*., 2005, 2007). These compounds belongs to very heterogenous groups such as, terpenes, aldehydes, esters, volatile acids and the concentration of each of these is higly variable and range from hundreds of micrograms to milligrams per liter. It has been recognized that the aroma constituents of the wine have a leading contribution to the varietal character (Rapp, 1972; Noble *et al*., 1980). Those products have recently attracted international attention gained increasingly wide markets over the word. Their very fine taste and especially characteristic flavor have been probably the main reasons for their acceptance. Thus, it has to be expected that the aroma compounds of the products of this area would play an important role in their characterization and differentiation.

Several concentration methods have been used for the analysis of minor and major volatile compounds in wines and distillates, such as liquid-liqued extraction, simultaneous extraction and distillation, solid phase microextraction etc.

A serious number of surveys have been made for aroma volatiles compounds concentrations in wines and distillates from different grape varieties (Bayonone *et al*., 1984; Amerine and Joslin, 1970; Nelson *et al*., 1978; Nycanen, 1986; Laminkanra *et al*., 1996; Calleja and Elena, 2005; Gerogiannaki-Christopoulou *et al*., 2005, 2007).

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The purpose of the present study was to present experimental data clarifying the volatiles profile in wines and its distillates from two different Hellenic Appellation of Origin Controlled (AOC) from Peloponisso, Agiorgitiko and Morchofilero (Hellenic native grape varieties (*Vitis vinifera* L.).

MATERIALS AND METHODS

Wine samles: Six different monovarietal wines from local wineries of Nemea area and 6 from the area of Mantineia of Peloponissos investigated in triplicate in this study. Production techniques were uniform for all wines studied, and all wine samples were collected 6 months after winemaking and then analyzed.

The distillation of wine samples were realized with the small copper alambic of 130 L Which traditionally used before the beginning of heating, the copper alambic is hermetically closed in order to prevent any vapor leakage. When the temperature reaches 80-90°C, the liquid spirit starts to run and collected. The distillates were collected and analyzed for volatiles compounds by head space GC-MS. The mean results were used as a basis for this work.

Wine volatile compounds: Wine samples were extracted as follows: A sample of 100 mL of wine was adjusted to pH 7, by the addition of NaOH and 1 mL of 3-octanol (10 mg L^{-1}) was added as an internal standard. The sample was extracted three times (10, 5 and 5 mL) with diethyl ether-pentane (1:1,v/v). This organic extract was concentrated to 2 mL, under nitrogen (Calleja and Falquë, 2005).

Wine-distillates volatile compounds: Distillates samples analyzed by direct injection 5 mL of each distillates samples mixed with 50 μL of an internal standard solution of 50 g of 4-methyl-2-pentanol per liter of ethanol (Silva and Malcata, 1998).

Analysis of volatile compounds by head space CG/MS: The volatile components present in headspace fraction of extracted wine samples and distilled wine samples were isolated and identified by using a balance pressure headspace system Perkin-Elmer HS40 (Perkin-Elmer Analytical Instruments, Uberlingen, Germany) coupled to a GC/MS-Q 5050 system (Shimadzu Co, Kyoto, Japan). A 2 mL sample from each extracted wine and distillated wine samples was taken and introduced into a 22 mL round-bottomed vial; then, the vials were sealed with aluminium-rubber septa. The vials with samples were held at 80°C for 25 min, purged and pressurised with helium at a flow rate of 40 mL min^{-1}. The volatile compounds were driven through the transfer line which was held at 105°C to the injector of the Gas Chromatograph. The volatile compounds were separated on an HP Innowax capillary column (60 m length × 0.25 mm internal diameter, 0.25 μm film thickness) at the following conditions: injector temperature 200°C; carrier gas helium 0.6 mL min^{-1}; temperature program: 45-100°C at a rate of 4°C min^{-1}, held for 5 min and go to 200°C at a rate of 8°C min^{-1} and held for 12 min. The GC column was directly connected without splitting to the ion source of QP 5050 quadrupole mass spectrometric detector which was operating in the scan mode within a mass range of *m/z* 30-350 at 2 scans s^{-1}. The interface line to MS was set at 250°C. The MS was operating in an electron impact mode at electron energy of 70 eV and was calibrated by auto tuning. Identification of the compounds was carried out by computer-matching of their mass spectral data with those of known compounds in the Shimadzu NIST62 Mass spectral Database and by comparing their retention times and mass spectra to 3-pentanol as internal standards. Quantification was performed by integrating the peak areas of Total Ion Chromatograms (TIC) by the Shimadzu Class 500 software.

Quantitative results were obtained by calculating the average value of three samples.

Oven temperature programme, 50-260°C at a rate of 4°C min^{-1}; transfer line temperature, 270°C; carrier gas, helium at a linear velocity of 31.5 cm s^{-1}; inlet split ratio, 1: 60; MS source ionisation energy, 70 eV; scan time, was 1 sec, covering a mass range of 40-300 amu.

The constituents were identified by comparison of their mass spectra with those in a computer library (LIBR-TR and Wiley 5 Library) or with authentic compounds. The identifications were confirmed by comparison of their retention indices of volatiles either with those of authentic compounds or with data in the literature.

Measurements were made in triplicate. Relative standard deviations were between 0.1-3.7.

RESULTS AND DISCUSSION

Table 1 shows the average concentrations and the standard deviations of for both AOC varietal red wines. Totally 43 different aromatic volatiles identifided. Fifteen terpenes and norisoprenoids, 13 alcohols, 10 esters, 3 fatty acids and 3 other volatile compounds. The wines sampled from the red Agiorgitiko variety showed a similar volatile profile with red Moschofilero wine. The average concentration for the terpenes and norisoprenoids for Agiorgitiko variety was from 9-2012 μg L^{-1} and for the

Table 1: Experimental data for the concentration of volatiles constituents in wines (Appellation of Origin Controlled (AOC) from two different Hellenic native grape varieties (*Vitis vinifera* L.)

	Varietal wine volatile compound			
	Agiortitiko terpenes and norisoprenoids (μg L)$^{-1}$		Moschofilero terpenes and norisoprenoids (μg L^{-1})	
	Average	SD	Average	SD
Linalool	157	26	117	31
Nerol	177	22	122	19
Geraniol	63	7	n.d.	
Citronellol	23	5	34	6
Citronellal	76	9	21	7
a-Pinene	201	19	231	22
b-Pinene	189	16	195	13
Theaspirane	37	5	42	6
a -Terpineol	33	4	n.d.	
a- Ionone	9	3	0.2	4
Terpinen-4-ol	11	5	19	3
b- Ionone	46	11	29	9
p-Menth-1-en-7.8-diol	34	4	45	3
3-Oxo-a-ionol	129	17	98	19
4-Oxo-a-ionol	19	5	9	7
Alcohols(mg L^{-1})				
Methanol	129	5.1	119	7.2
1-Propanol	41	3.6	39	2.9
1-Butanol	9	1.9	6	1.5
2-Butanol	n.d		n.d.	
trans -2-Hexenol	0.04	2	0.2	1.5
1-Hexanol	2.6	1.1	1.9	0.9
3-Hexanol	26	4.7	29	3.2
trans -3-Hexenol	0.9	2.8	1.1	1.9
cis -3-Hexenol	1.1	2	2.4	2.1
Isobutanol	55	14	41	9
Isoamyl alcohols	199	21	115	17
2-Phenyl-ethanol	28	6	31	7
Benzyl alcohol	9	2.3	7	2.1
Esters				
Ethyl lactate	48	11	51	9
Ethyl acetate	113	23	106	26
Isobutyl acetate	29	9	32	11
Ethyl butyrate	45	12	49	8
Butyl acetate	13	6	19	7
Ethyl hexanoate	4	2.1	12	2.6
Ethyl octanoate	3.8	1.9	5.5	1.4
Ethyl decanoate	1.6	2	2.1	1.9
Ethyl dodecanoate	0.9	1	3.2	1.1
Diethyl succinate	1.1	2.3	1.9	2.7
Fatty acids				
Hexanoic acid	4.5	1.2	5.1	1.9
Octanoic acid	2.1	1.1	2.9	1.5
Decanoic acid	1.2	4	2.1	2.9
Other compounds				
Acetaldehyde	57	6	51	5
γ-Butyrolactone	2.2	3	3.4	2

wines for Moschofilero variety was from 0, 2-231 μg L^{-1}. The alcohols concentration for Agiorgitiko wines was from 0,04 mg L^{-1} for trans-2-Hexenol to 199 mg L^{-1} for isoamyl alcohols. Two-phenyl ethanol with the aroma of roses was from almost the same in both wines.. The terpene content of a wine is considered to be a positive quality factor. This is because they contribute to its varietal aroma, serve to differentiate it from other varieties, and supply floral nuances to the wine (Marais, 1991). Figure 1 shows the different concentration of the total aromatic volatile compounds with higher in the wines from Agiorgitiko variety. Figure 2 shows also higher concentration in terpenes and norisoprenoids in AOC wines from Agiorgitiko grape variety. The esters and the fatty acids are 2 groups of compounds of undoubted importance in the wine aromas their nuances coincide with the fruity descriptors of the wines and, in the case of the red wines, play a modulating role (Ferreira *et al.*, 1995).

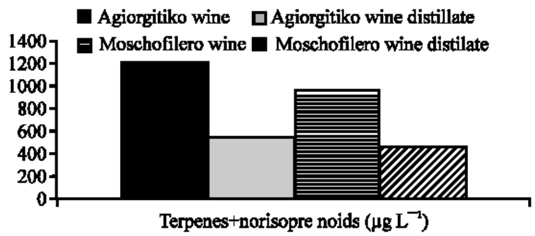

Fig. 1: Terpenes and norisoprenoids concentration in varietal (AOC) wines and wine distillates

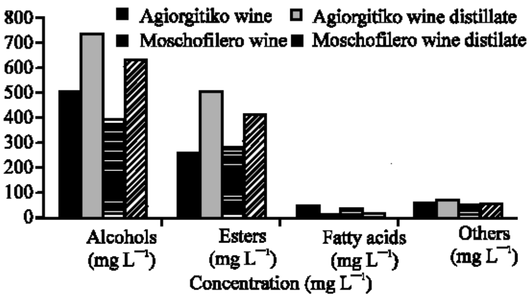

Fig. 2: Varietal concentration of alcohols, esters, fatty acids and others volatiles in varietal (AOC) wines and wine distillates

Table 2 shows the average concentrations and the standard deviations of for both varietal red wines distillates. Totally 35 different aromatic volatiles identified. 6 terpenes and norisoprenoids, 14 alcohols, 10 esters 3 fatty acids and 2 other volatile compounds.The average concentration for the terpenes and norisoprenoids for Agiorgitiko variety was from 19-189 μg L^{-1} and for the wines for Moschofilero variety was from 19-202 μg L^{-1}. The alcohols concentration for Agiorgitiko wines was from 0,4 mg L^{-1} for 3-Hexanol to 267 mg L^{-1} for 2-methyl butanol. Two-phenyl ethanol with the aroma of roses was from almost the same in both wines distillates too. The terpene s and norisoprenoids content of a wine distillates was only 6 (nero, citronellol, citronellal, a-pinene, b-pinene, a-terpineol).

Figure 1 and 2 show the different concentration of the total aromatic volatile compounds with higher in the

Table 2: Experimental data for the concentration of volatiles constituents in wine-distillates Appellation of Origin Controlled (AOC) from two different Hellenic native grape varieties (*Vitis vinifera* L.)

	Varietal wine distillates			
	Agiortitiko terpenes and norisoprenoids $(\mu g\ L)^{-1}$		Moschofilero terpenes and norisoprenoids $(\mu g\ L^{-1})$	
	Average	SD	Average	SD
Nerol	95	15	66	11
Citronellol	19	3	27	5
Citronellal	46	7	19	6
a-Pinene	188	16	202	11
b-Pinene	166	10	164	12
a Terpineol	31	5	n.d.	5
Alcohols(mg L^{-1})				
Methanol	211	5.7	198	6.9
1-Propanol	53	3.3	49	2.2
1-Butanol	11	1.1	9	1.4
2-methyl-propanol	n.d		n.d.	
2-Butanol	0.6	1.7	0.9	1.4
trans -2-Hexenol	2.9	1.9	3.1	1.1
1-Hexanol	44	3.9	28	2.9
3-Hexanol	0.4	3.2	0.9	1.5
trans -3-Hexenol	1.9	2.6	3.4	1.7
cis -3-Hexenol	67	13	55	12
2-methyl-butanol	267	55	194	32
3-methyl-butanol	54	21	52	5
2-Phenyl-ethanol	12	2	13	1.9
Benzyl alcohol	9	7	21	6
Esters				
Ethyl fornate	76	10	66	7
Ethyl acetate	245	21	142	19
Isobutyl acetate	41	11	44	8
Ethyl butyrate	88	14	56	11
Butyl acetate	34	4	52	13
Ethyl hexanoate	6	4	28	4
Ethyl octanoate	7.1	2	12	1.9
Ethyl decanoate	3.3	5	3.1	2.1
Ethyl dodecanoate	2.1	3	6.6	2.4
Diethyl succinate	2.9	2	2.9	1.9
Fatty acids				
Hexanoic acid	5.1	3	6.3	3
Octanoic acid	3.4	1	3.2	1.3
Decanoic acid	1.9	2	4.4	5
Other compounds				
Acetaldehyde	66	4	57	3
γ-Butyrolactone	1.9	1	1.5	2

wines distillates from Agiorgitiko variety. Figure 2 shows also the concentration in terpenes and norisoprenoids in wines distillates from both AOC wines.

The explanation for the differences in the distillation profile between major and minor volatile components is the following.

It is known from standard distillation theory (Atkins, 1986) that the concentration of different volatile compounds are continuously changing during distillation depending on their volatility and each volatile begins to distil when the solution temperature is near his boiling point temperature.

According to Raoult's law the vapor pressure (Pi) of a volatile component (i) above a solution is the product of the vapor pressure (Pi0) of the pure component and of the mole fraction (Xi) of the component (i) in the solution (Pi = Pi0.Xi). From Dalton's law it can be calculated that the Xi(gas phase) = Pi/Ptotal, where Ptotal is the sum of all partial pressures of volatile components of the alcoholic solution. Due to the very high concentrations of water and ethyl alcohol and to the very low concentration of all volatile components it can be calculated that Xi (gas phase) is 10-4 to 10-5 of Xi(solution). Due to this phenomenon volatile compounds of small or very small concentrations have very small mole fractions in the gas phase. Accordingly, during distillation these components will not follow the standard distillation pattern but instead they will distill at a slow and rather uniform rate throughout the whole distillation process.This attitude has important consequences for producers of distilled wines. It means that it is impossible to get rid of some minor unwanted component through distillation and some other way has to be devised.

Although those results from small number of wines, so a larger sample of wines should be examined in order to confirm this research.

CONCLUSION

The results obtained from the analysis of the red monovarietal Appelations of Origin Controlled Agiorgitiko and Moschofilero wines and its distillates, showed that these monovarietal samples present a high content of varietal compounds that can give typical aromatic connotations, which allows their differentiation from other red varieties from different geographical areas. Linaool, a-pinene, b-pinene and citronellol found at levels higher than their perception thresholds. Two-phenyl-ethanol, that provides an aroma of roses and benzyl alcohol that gives in wines a nuance of blackberry was detected. The presence of some other aromatic compounds from alcoholic fermentation such as alcohols, esters and fatty acids with was in good perception thresholds and therefore also contribute the bouquet of these wines and distillates. In order to classified the geographical origin of red wines must also study directed to the determination by a panel of wine-testers or with gas chromatography-olfactometry in order to confirm this research work.

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