

## Identification of Volatile Compounds in Hellenic Alcoholic Beverages from Native White Grape Varieties (*Vitis vinifera* L.)

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**Abstract:** The present study reports the identification of important volatiles compounds through the 1st and 2nd distillation in alcoholic beverages from Hellenic Native white grape varieties (*Vitis vinifera* L.). Grape marc alcoholic beverages, are produced in the winemaking industry after distillation of fermented grape marcs (under anaerobic conditions for a given period of time). Collected distillates from the 1st distillation were redistilled and analysed chromatographically. Assays were performed by gas/liquid chromatography for alcohols (methanol, 2-butanol, 1-propanol, 2-methyl-propanol, 1-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 1-hexanol and 2-phenyl-ethanol), carboxylic acids (hexanoic, octanoic, decanoic and dodecanoic acids), esters (ethyl acetate, ethyl hexanoate, ethyl octanoate), as well as for an aldehyde (ethanal) and its acetal (diethoxy-1-1-ethane). Changes of volatile compounds concentration during distillations 1st and 2nd were evaluated. The average values obtained for Hellenic grape marc distillates were compared with the corresponding values for grape marc alcoholic beverages produced in other countries (viz. Italy, Spain, Portugal etc.).

**Key words:** Marc distillates, gas chromatography, fermentation, grape varieties (*Vitis vinifera* L.), volatile compounds

### INTRODUCTION

Alcoholic beverages from fermented grape (*Vitis vinifera* L.) marcs are widely consumed in most European wine producing countries. Several names are used for this viticultural origin alcoholic beverages in different countries. In Italy it is named Grappa, in Portugal bagaceira, aguardente in Spanish, in Cyprus zivania, in Turkic raki, in Yugoslavia kormovica, in Georgia tshisanthis and in Greece tsipouro, tsikoudia etc. The winemaking sub-products (stalks, seeds and grape skins) a low value agricultural product, after alcoholic fermentation of the residual sugars, have been traditionally distilled to produce this alcoholic beverage. The aromatic profile of this alcoholic beverage is the product of a biochemical and technological parameters and depends by various factors such as environment, soil, climate, grape variety, degree of the grape ripeness, enological methods used for marcs and the aging of the distillate (Cole and Noble, 1997; Porto, 1998; Apostolopoulou *et al.*, 2005; Silva and Malcata, 1998, 1999; Soufleros and Bertrand, 1987; Fournaris, 1999;

Soufleros *et al.*, 2001, 2004, 2005; Gerogiannaki *et al.*, 2004, 2005, 2007). On the other hand, also very important factors for the qualified production of this alcoholic beverage are, the fermentation, the storage conditions and the distillation technique of the grape marcs (Silva and Malcata, 1998, 1999; Soufleros *et al.*, 2004, 2005; Porto, 1998; Cortes *et al.*, 2005; Fournaris, 1999).

Farmers in Mediterranean countries often store such marcs in closed plastic or wooden containers for several days to promote spontaneous anaerobic fermentation of the contained sugars (Silva and Malcata, 1999). Such fermentations are effected by the native microflora on the grapes and lead to the production of a variety of volatiles which play an important role in the flavour of spirits.

Grape marc volatiles belong to different chemical groups such as higher alcohols, esters, aldehydes, ketones, fatty acids, etc. Some of these compounds are volatile or highly volatile, while others exhibit lower volatility. These aroma compounds exist in a wide concentration range. Some of them are present at high concentrations (hundreds of mg L<sup>-1</sup>), but most of them

are found at a very low concentration range, from traces to 10 mg L<sup>-1</sup> (Porto, 1998; Apostolopoulou *et al.*, 2005; Silva and Malcata, 1996, 1998, 1999; Soufleros and Bertrand, 1987; Soufleros *et al.*, 2001, 2004, 2005).

Following this storage period, the grape marc is transferred to copper batch stills and distilled to release the volatiles. The first fraction (termed heads) is disposed of or redistilled. The correct separation of the distillation fractions (heads, hearts and tails) are very important for the sensory profile of the spirit (Porto, 1998; Apostolopoulou *et al.*, 2005; Silva and Malcata, 1998, 1999; Soufleros and Bertrand, 1987; Soufleros *et al.*, 2001, 2004, 2005).

The central fraction (termed heart) is a complex mixture, where most compounds are responsible for unique flavours of the grape pomace distillate. Due to its composition in unpleasant volatile compounds, the final fraction (termed tails) is disposed of or added and redistilled (Soufleros *et al.*, 2001, 2004, 2005).

Alcohols are major products of fermentation of sugars carried out primarily by yeasts. Besides ethanol, a number of alcohols are present in distilled beverages; these compounds are contributed by the grapes or are formed as a result of microbial action during anaerobic fermentation. Free fatty acids are normal components of distilled alcoholic beverages and are mainly produced via yeast metabolism of carbohydrates. Fatty acids are related to a large group of aroma compounds which includes esters. Some of the most important esters in grape pomace distillates are those of Hexanoic, octanoic, decanoic and dodecanoic acids. Aldehydes can be found in distilled alcoholic beverages and are thought to be an indicator of spontaneous oxidation or of the activity of unwanted contamination bacteria (Amerine, 1980). More than 90% of the total aldehyde content is accounted for by acetaldehyde and acetal (Cantagrel *et al.*, 1997; Boulton *et al.*, 1996; Cole and Noble, 1997; Cortes *et al.*, 2005).

The production procedures for all these alcoholic beverages are established by the increasingly strict food quality standards prevailing in most European countries (Official Journal of EEC, 1989).

In this study, the Identification of volatile compounds in Hellenic Alcoholic Beverages from Native White Grape Varieties (*Vitis vinifera* L.) is performed in order to achieve the following objectives.

- To establish the average concentration and range of variation for each compound determined in 1st and 2nd distillation.
- To know the behaviour of volatiles through the distillation of this in unique Hellenic Alcoholic Beverages from Native White Grape Varieties (*Vitis vinifera* L.)

## MATERIALS AND METHODS

**Samples:** Hellenic grape varieties (*Vitis vinifera* L.) samples, originating from different regions of Greece, were analysed for volatiles as detailed below. Four plots of four white grape varieties, Athiri from Cyclades, Malagouzia from Aitolocacania, Vilana from Crete and Lagorthi from the Ionian islands, cultivated in private vineyards of Greece were selected. Samples of 150 kg of grapes of each variety were collected (FAO, 1986). Sampling was performed by randomly collecting grapes from various places of the experimental plots. Grapes were crushed in a pilot horizontal press machine and the must was removed. Samples of 40 kg pomace from each variety were collected. Appropriate plastic containers were filled with the grape pomace, duly sealed to ensure anaerobic conditions and maintained at room temperature (20°C) for 12 weeks for fermentation by means of indigenous yeast flora. Distillations (1st and 2nd of the grape marcs) was followed.

**Distillation:** The distillation process of fermented grape pomace from the selected grape varieties realised with the small copper alembics of 130L, which traditionally used. Before the beginning of heating, the copper alembic is hermetically closed in order to prevent any vapour leakage. When the temperature reaches 80-90°C, the liquid spirit starts to run and collected in 4 equal cuts (1 L each) in glass bottles for each distillation.

The first 0.5 L of the distilled product, corresponding to the beginning of the distillation procedure, is removed as "head". It usually presents a very high alcoholic title, 85% vol approximately. Then and for about 4.5 h, a pure spirit, distilled to levels lower than 85% vol. is collected into four glass bottles and analyzed immediately by gas chromatography. After the chromatographic analysis of the first distillation samples, the distillates led back to the alembic for a second distillation. The "tails" (distilled spirit below 5% vol.) used for the next first distillation process with the new lot of the fermented grape pomace. Distillation products of 2nd distillation fractions were collected also as four equal volume fractions. Analysis of volatiles of all samples from Hellenic native white grape varieties (*Vitis vinifera* L.) was followed.

**Chemicals analysis:** Compounds 2-methyl-propanol, 1-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 1-hexanol, 2-phenyl-ethanol, 3-octanol and 3-pentanol) and esters (ethyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate and ethyl dodecanoate) were purchased from Merck (Schuchardt, Switzerland). Standards of carboxylic acids (hexanoic, heptanoic,

octanoic, decanoic and dodecanoic acids), acetal (diethoxy-1-1-ethane) and ethanal (acetaldehyde) were purchased from Sigma (St Louis, Mo, U.S.A.). Ether, hexane and sulfuric acid were obtained from Fluca Chemie AC, (Buchs, Switzerland).

**Chromatographic assays:** For alcohol, acetal, ethyl acetate and ethanal assays, a 5 mL sample of each grape marc distillate was mixed with 50  $\mu\text{L}$  of an internal standard solution (50 g of 3-pentanol per liter of ethanol). The injector was maintained at 200°C and was operated in the split mode. Separation was achieved in a 50 m $\times$ 0.25 mm  $\times$  0.2  $\mu\text{m}$  capillary column CPWAX 57CB (Chrompack, Middelburg, The Netherlands). The oven temperature program was as follows: 40°C for 5 min, a linear ramp from 40 to 200°C at 3°C/min and 200°C for 20 min. Detection was by flame ionization at a temperature of 200°C. Helium was used as the carrier gas at a split ratio of 1:60. Chromatographic runs were carried out in triplicate and their average was used as a single data point in the result section. For assays of esters and fatty acids (which are in general present in relatively low concentrations) a 10- mL sample of each grape marc distillate was diluted with deionized water (in order to lower the total alcohol content to ca. 10% v v<sup>-1</sup>) and in the resultant solution were added 2 mL of a 40 mg L<sup>-1</sup> solution of 3-octanol and 2 mL of a 90 mg L<sup>-1</sup> solution of heptanoic acid (used here as internal standards). The pH was adjusted to 2.0 using a few drops of concentrated sulfuric acid. The mixture was extracted for 5 min with 4 mL of a 50% (v v<sup>-1</sup>) mixture of ether and hexane and this procedure was repeated twice with 2 mL of the same solvent (Bertrand, 1975). The injector was maintained at 250°C and was operated in the split mode. Separation was achieved in a 25 m $\times$ 0.32 mm $\times$ 0.3  $\mu\text{m}$  capillary column CB WAX 57 (Chrompack, Middelburg, The Netherlands). The oven temperature program was as follows: 40°C for 5 min, a linear ramp from 40 to 220°C at 37°C/min and 220°C for 20 min. Detection was by flame ionization at a temperature of 250°C. Helium was used as the carrier gas at a split ratio of 1:30.

In both cases, aliquots of 0.5  $\mu\text{L}$  were injected into a gas/liquid chromatograph.

The helium flow rate was 2 mL min<sup>-1</sup> and the gases required by the flame ionization detector were supplied at pressures of 90 kPa (H<sub>2</sub>) and 110 kPa (air). Analyses were carried out in triplicate and their average was used in the results section.

Qualitative and quantitative analyses of the peaks in the samples analysed were made by comparison of their areas with the internal standards).

In the calibration, the response factor of each compound of interest,

Rfi = was calculated by  $\text{RFi} = [\text{Ais}/\text{Asi}] \cdot [\text{Csi}/\text{Cis}]$ ,

Where Ais and Asi are the peak areas of the chromatographic internal standard and of the chromatographic standard of the compound of interest, respectively and Cis and Csi are the molar concentrations of the chromatographic internal standard and of the chromatographic standard of the compound of interest, respectively.

In the actual quantisation, the molar concentration of each compound of interest, Cis and Csi, was determined via  $\text{Csi} = [\text{Ais}/\text{Asi}] \cdot \text{Cis} \cdot \text{RFi}$ , where Ais and Asi is the area of peak of interest (EC regulation 2870/2003).

Measurements were made in triplicate. Relative standard deviations were between 0, 1-4.1.

## RESULTS AND DISCUSSION

The quantitative results for the composition of the various alcohols and aldehyde, carboxylic acids and esters are depicted in Table 1-4 and in Fig. 1 and 2. Aldehydes (acetaldehyde and acetal) are found in many alcoholic beverages (Porto, 1998; Apostolopoulou *et al.*, 2005; Silva and Malcata, 1996, 1998, 1999; Soufleros and Bertrand, 1987; Soufleros *et al.*, 2001, 2004, 2005). Are coming from the fermented grape marcs as a result of spontaneous or microbial mediated oxidation. Acetaldehyde is a direct alcoholic fermentation by-product and the sensory character range from nutty and sherry like to being reminiscent of overripe bruised apples (Fugersang, 1997). Acetaldehyde commonly increase during ageing of alcoholic beverages due to chemical oxidation of ethanol and further oxidation may result the formation of small amounts of acetic acid (Cole and Noble, 1997; Mangas *et al.*, 1996) reported that acetaldehyde decreased because it interacts with ethanol resulting in the production of acetal. Acetaldehyde content in all varietal grape marcs distillates was generally lower to that of Italian grappa and similar to bagaceira and cider brandies (Silva and Malcata, 1999; Cabras *et al.*, 1972; Versini, 1993; Varajao, 1991; MacNamara, 1984). The mean values of acetaldehyde concentration was found from 40.5-75 mg L<sup>-1</sup>. The above mean values are lower from the official limits adopted by the European Council (1579/89) (Official Journal of EEC, 1989) for fruit pomaces. That means that the production of this alcoholic beverages (fermentation, distillation and aging) was under conditions do not support the contamination from microorganisms (Silva and Malcata, 1998).

Ethyl acetate has significant effect on the organoleptic characteristics of alcoholic beverages with “finger polish remover” character and it derives from bacterial contamination of the marc alcoholic beverages (Silva and Malcata 1998, 1999). High

Table 1: Experimental data for concentration (mg L<sup>-1</sup>) of volatiles obtained from 1st and 2nd distillation of fermented grape marc from *Athiri* grape variety (*Vitis vinifera* L.)

Compound	1st Distillation fractions					2nd Distillation fractions				
	1st fract.	2nd fract.	3rd fract.	4th fract.	Mean	1st fract.	2nd fract.	3rd fract.	4th fract.	Mean
Methanol	1740(1.1)*	1566(1.3)	1360(1.1)	1160(1.4)	1456.5	1047(1.1)	896(1.5)	800(1.3)	726(1.1)	867.25
Acetaldehyde	38.2(2.3)	34.8(2.1)	49.9(2.2)	53.4(2.4)	53.4	45.5(2.6)	55.1(2.1)	38.4(2.1)	42.4(2.3)	45.35
Acetal	48.3(2.1)	57.7(2.4)	64.9(2.5)	68.4(2.7)	68.4	52.9(2.2)	67.3(2.3)	62.6(2.1)	69.9(2.2)	63.17
2-butanol	2.3(3.3)	2.1(2.3)	1.2(2.9)	0.9(3.1)	0.9	0.8(3.2)	2.5(2.2)	1.8(3.1)	1.9(2.9)	1.75
1-propanol	31.3(2.2)	36.2(2.5)	41.2(2.1)	47.2(2.7)	47.2	51.2(2.5)	59.2(2.6)	61.2(1.9)	33.3(4)	51.22
2-methyl-propanol	64(2.6)	25.1(2.3)	82.8(2.9)	45.9(3.1)	45.9	33.7(2.5)	36.5(2.6)	32.9(2.7)	37.3(2.5)	35.1
1-butanol	8.8(3.1)	8.4(2.9)	10.1(2.6)	10.5(1.9)	10.5	9.9(1.8)	7.2(2.4)	10.6(2.8)	11.4(2.3)	9.77
2-methyl-butanol	65.3(2.5)	52.5(2.1)	85.1(2.2)	67.7(2.7)	67.7	56.1(2.4)	55.7(2.6)	64.8(2.2)	61.3(1.9)	59.47
3-methyl-butanol	45.5(2.7)	46.9(2.8)	54.8(2.4)	45.3(2.7)	45.3	23.6(2.5)	30.7(2.6)	75.8(2.1)	74.9(2.4)	51.25
Ethyl-acetate	29.3(2.4)	25.6(2.5)	24.9(2.5)	28.3(2.6)	28.3	25.2(1.9)	28.4(1.5)	24.2(2.4)	25.5(2.1)	26.0
Hexanol-1	2.7(3.5)	2.6(3.2)	2.1(1.9)	2.5(1.8)	2.5	6.4(2.1)	7.6(2.4)	8.5(2.2)	8.9(2.3)	7.85
2-phenyl-ethanol	3.6(3.2)	3.6(3.3)	3.3(2.9)	3.5(2.8)	3.5	3.4(2.6)	3.6(1.9)	6.7(2.1)	6.4(2.6)	5.02
Hexanoic acid	1.2(3.6)	1.5(3.5)	1.5(2.4)	1.6(2.7)	1.6	1.9(1.1)	1.7(2.8)	2.2(2.9)	2.1(2.4)	1.97
Octanoic acid	0.9(3.9)	1.5(3.7)	3.6(2.1)	1.9(1.9)	1.9	0.9(3.2)	1.7(2.9)	2.4(2.5)	2.2(2.2)	1.8
Decanoic acid	1.1(3.3)	1.2(3.5)	1.9(2.1)	1.3(2.4)	1.3	1.6(2.1)	1.7(2.8)	2.1(2.6)	1.9(2.4)	1.82
Dodecanoic acid	1.4(3.6)	1.8(3.9)	1.2(2.3)	1.9(2.6)	1.9	1.9(2.9)	2.1(2.6)	2.4(2.7)	2.3(2.7)	2.17
Ethyl hexanoate	1.3(3.5)	1.4(3.2)	1.1(3.9)	0.9(3.7)	0.9	1.9(3.6)	1.5(3.2)	1.3(3.5)	1.1(3.7)	1.45
Ethyl octanoate	2.2(2.9)	2.4(2.7)	2.6(3.1)	0.5(3.8)	0.5	0.7(3.9)	1.6(3.1)	1.8(3.4)	1.6(3.6)	1.42

\* RSD

Table 2: Experimental data for concentration (mg L<sup>-1</sup>) of volatiles obtained from 1st and 2nd distillation of fermented grape marc from *Malagouzia* grape variety (*Vitis vinifera* L.)

Compound	1st Distillation fractions					2nd Distillation fractions				
	1st fract.	2nd fract.	3rd fract.	4th fract.	Mean	1st fract.	2nd fract.	3rd fract.	4th fract.	Mean
Methanol	1560(1.1)*	1342(1.4)	1274(1.5)	1053(1.3)	1307.25	942(1.2)	807(1.1)	696(1.7)	628(1.3)	768.25
Acetaldehyde	35.4(2.1)	31.4(2.6)	47(2.2)	51.8(1.9)	41.4	43(2.7)	50.9(2.2)	35.1(1.9)	40.2(2.1)	42.3
Acetal	47.2(2.7)	47.5(1.9)	47.5(2.4)	49.8(1.9)	48	51.7(2.5)	65.3(1.9)	60(2.7)	68.7(2.1)	61.42
2-butanol	2.1(2.3)	1.97(2.9)	1.16(3.1)	0.8(3.1)	1.52	0.7(3.1)	2.4(2.2)	1.7(2.4)	1.85(2.7)	1.66
1-propanol	28.4(2.1)	35(2.5)	40.3(2.9)	45.6(2.3)	37.3	50.2(2.9)	57.5(2.5)	60.4(2.6)	31.7(2.5)	49.95
2-methyl-propanol	60(2.7)	20.8(2.4)	78.5(2.8)	59(2.5)	54.575	28(2.6)	34.4(2.5)	27.6(2.9)	37.3(2.2)	31.82
1-butanol	8.49(2.4)	7.7(2.5)	9.8(2.4)	10.3(2.7)	9.07	9.45(2.5)	6.8(2.7)	10.2(2.9)	10.98(2.6)	9.355
2-methyl-butanol	62(2.9)	48(3.1)	80.2(2.7)	65.4(2.4)	63.9	54(2.8)	53.1(2.5)	63.7(2.9)	56(2.8)	56.7
3-methyl-butanol	42(2.3)	44.5(2.7)	52.5(2.9)	39.6(2.1)	44.65	23.6(2.1)	29.4(2.4)	71.3(2.1)	69.4(2.5)	48.42
Ethyl-acetate	28.4(2.3)	25.42(2.4)	24.59(2.1)	27.91(2.5)	26.58	25.2(2.5)	28.03(2.3)	24(2.5)	25.28(2.2)	25.62
Hexanol-1	2.4(2.9)	3.7(2.4)	4.43(2.6)	5.7(2.9)	4.05	6.4(2.3)	7.26(2.1)	8.25(3.1)	8.9(2.3)	7.7
2-phenyl-ethanol	3.4(2.1)	4.4(2.7)	5.43(2.6)	6.45(2.4)	4.92	2.98(2.9)	3.4(2.3)	6.34(1.8)	5.71(2.1)	4.6
Hexanoic acid	1.13(3.1)	1.45(3.2)	1.46(3.7)	1.14(3.1)	1.3	1.83(3.3)	1.63(3.5)	2.12(3.1)	1.98(3.3)	1.89
Octanoic acid	0.83(3.5)	1.8(2.9)	2.8(3.1)	3.76(2.4)	2.29	0.83(3.1)	1.7(3.7)	2.36(1.9)	2.06(2.9)	1.73
Decanoic acid	1.04(2.7)	1.97(2.1)	2.95(1.9)	3.95(2.2)	2.47	1.49(2.2)	1.61(2.5)	2(2.9)	1.68(2.1)	1.69
Dodecanoic acid	1.22(2.3)	1.71(2.7)	1.13(2.9)	1.83(2.1)	1.07	1.81(2.2)	2.02(2.6)	2.4(3.1)	2.18(2.9)	2.1
Ethyl hexanoate	1.18(2.5)	2.12(2.2)	3.04(2.9)	4.06(2.7)	2.6	1.78(2.1)	1.38(2.6)	1.15(2.4)	0.96(1.9)	1.31
Ethyl octanoate	1.98(3.1)	2.97(3.3)	3.85(2.9)	4.78(1.9)	3.39	0.45(2.4)	1.42(3.2)	1.75(2.9)	1.27(2.7)	1.22

\* RSD

concentration (150-250 mg L<sup>-1</sup> can add spoilage character to the alcoholic beverages and gives an acidic character (Ferreira *et al.*, 1999) when the concentration is higher than 180 g hL<sup>-1</sup> (Soufleros and Bertrant, 1987). At lower concentrations, ethyl acetate contribute to fruity character of the alcoholic beverages. Ethyl acetate concentrations of zivania are similar to those of the studied distillates (Fournaris, 1999). The mean values of the concentration of ethyl acetate for all the studied alcoholic beverages ranged from 18.7-96.7 mg L<sup>-1</sup> (Athiri has 24.9-29.3 mg L<sup>-1</sup>, Malagousia from 24-28.4 mg L<sup>-1</sup>, Vilana (67.7-85.3 mg L<sup>-1</sup>, Lagorthi 18.7-96.7 mg L<sup>-1</sup>).

Fused alcohols are quantitatively the largest group of flavour volatiles in alcoholic beverages and are the most abundant class of secondary compounds of grape marc

distillates (Silva and Malcata, 1999; Ferreira *et al.*, 1999; Soufleros *et al.*, 2001, 2004). The levels of higher alcohols are fixed by the European Council (1579/89) (Official Journal of EEC, 1989 at 225-600 g hL<sup>-1</sup> of 100% alcohol. 2-methyl-1-butanol and 3-methyl-1-butanol are formed during fermentation by deamination and decarboxylation reactions from iso-leukine and leucine (Aposolopoulou *et al.*, 2005; Kana *et al.*, 1988). It is known that the level of amyl alcohols is a predictor of sensory character (having an aromatic description of sweet, alcoholic and choking (Falque *et al.*, 2001); amyl alcohols (viz. 3-methyl-1-butanol and 2-methyl-1-butanol).

The amyl alcohol content of the analyzed samples was in the range from 59.47-67.7 mg L<sup>-1</sup> for 2-methyl-butanol and 45.3-51.25 mg L<sup>-1</sup> for 3-methyl-butanol

Table 3: Experimental data for concentration (mg L<sup>-1</sup>) of volatiles obtained from 1st and 2nd distillation of fermented grape marc from *Vilana* grape variety (*Vitis vinifera* L.)

Compound	1st Distillation fractions					2nd Distillation fractions				
	1st fract.	2nd fract.	3rd fract.	4th fract.	Mean	1st fract.	2nd fract.	3rd fract.	4th fract.	Mean
Methanol	1960(1.1)*	1030(1.4)	1716(1.2)	1475(1.1)	1545.2	1529(1.2)	1437(1.1)	1419(1.4)	1399(1.1)	1446
Acetaldehyde	65(1.8)	33.6(2.1)	95.4(1.9)	106.3(1.7)	75	64.8(1.9)	55.9(2.1)	49.4(2.3)	40.6(1.9)	52.7
Acetal	75(1.9)	56.6(2.1)	33.6(2.3)	36.8(1.9)	50.5	35.9(1.7)	33.1(1.9)	29.7(2.1)	22.2(1.4)	30.2
2-butanol	11.5(2.6)	11.2(2.1)	10.5(2.7)	7.9(2.1)	10.3	3.2(2.4)	15.6(2.6)	12.5(2.9)	10.5(2.1)	10.5
1-propanol	77(3.1)	63.1(2.5)	80.3(2.3)	59.2(2.6)	69.9	63.8(2.5)	56.1(2.6)	67.5(2.1)	75.3(2.4)	65.7
2-methyl-propanol	39.8(2.8)	26.5(2.1)	51.1(1.9)	32.9(2.3)	37.575	22.1	19.4	29.2	42.1	28.2
1-butanol	10.2(2.1)	10.6(2.7)	12.8(2.4)	12.5(2.1)	11.5	11.9(3.3)	9.3(3.1)	12.5(2.8)	11.7(2.9)	11.3
2-methyl-butanol	37.7(2.1)	24.8(2.9)	29.3(3.1)	10.6(1.9)	25.6	39.3(2.7)	38.8(3.1)	40.9(3.3)	29.6(2.9)	37.15
3-methyl-butanol	63.9(2.9)	53.8(3.1)	81.9(2.7)	62.8(3.5)	65.6	42.4(2.9)	18.9(3.1)	52.9(2.5)	72.1(2.8)	46.6
Ethyl-acetate	74.8(2.4)	70.4(2.7)	79.9(2.2)	82.3(2.1)	76.9	67.6(2.8)	70.2(2.4)	79.1(2.7)	85.3(2.9)	78.2
Hexanol-1	5.6(3.1)	4.5(3.5)	3.9(2.9)	5.5(3.4)	4.9	3.3(3.6)	4.6(3.7)	6.4(3.1)	3.8(3.6)	4.5
2-phenyl-ethanol	2.1(3.2)	2(3.5)	1.9(3.9)	1.8(3.6)	1.9	2.4(2.9)	2.7(3.3)	3(3.8)	3.1(2.9)	2.8
Hexanoic acid	2.3(2.1)	2.1(3.2)	2.4(2.7)	2.6(3.2)	2.35	1.8(2.9)	2.6(3.1)	2.9(2.7)	2.4(3.8)	2.4
Octanoic acid	1.3(3.4)	1.8(3.1)	3.9(2.9)	2.2(3.3)	2.3	1.2(3.6)	1.9(3.3)	2.6(3.7)	1.3(2.9)	1.75
Decanoic acid	0.3(3.9)	0.5(3.7)	0.9(3.3)	0.3(3.7)	0.5	0.6(3.5)	0.7(3.8)	1.1(3.7)	0.9(3.4)	0.8
Dodecanoic acid	1.7(3.9)	1.1(3.5)	2.1(3.2)	1.3(2.9)	1.5	2.4(3.7)	2.7(3.5)	2.1(2.9)	2.8(3.4)	2.5
Ethyl hexanoate	3.1(2.9)	2.8(3.2)	1.1(3.8)	2.5(2.9)	2.4	4.4(2.3)	5.7(2.9)	4.6(2.8)	3.4(2.8)	4.5
Ethyl octanoate	3.1(2.9)	4.8(3.3)	3.5(3.2)	4.6(3.9)	4	5.6(3.5)	6.2(3.2)	5.9(2.9)	4.2(3.10)	5.5

\* RSD

Table 4: Experimental data for concentration (mg L<sup>-1</sup>) of volatiles obtained from 1st and 2nd distillation of fermented grape marc from *Lagorthi* grape variety (*Vitis vinifera* L.)

Compound	1st Distillation fractions					2nd Distillation fractions				
	1st fract.	2nd fract.	3rd fract.	4th fract.	Mean	1st fract.	2nd fract.	3rd fract.	4th fract.	Mean
Methanol	1650(1.3)*	1709(1.1)	1394(1.6)	1161(1.2)	1478.5	1523(1.5)	1425(1.3)	1231(1.1)	1401(1.3)	1395
Acetaldehyde	83.4(1.9)	52.9(2.4)	23.1(2.7)	25.2(2.2)	46.15	44.6(1.9)	28.5(2.4)	1.7(2.1)	86.1(2.2)	40.2
Acetal	50.6(2.1)	47.2(2.5)	36.7(2.2)	39.6(1.9)	43.5	33.6(2.1)	27.4(1.9)	23.7(2.2)	22.1(1.8)	26.7
2-butanol	33.5(2.2)	43.1(2.4)	39.1(1.9)	31.5(2.1)	36.8	25.3(2.8)	52.8(2.5)	11.9(2.1)	10.5(2.2)	25.1
1-propanol	83.4(2.7)	66.2(2.4)	86.8(2.6)	65.9(1.90)	75.6	69.4(1.9)	62.7(2.1)	73.4(1.6)	81.8(1.9)	71.8
2-methyl-propanol	66.7(0.9)	46.3(1.4)	53.9(2.1)	21.8(1.9)	47.1	26.4(1.7)	28.5(2.1)	25.6(1.6)	28.9(2.1)	27.3
1-butanol	6.1(3.3)	5.9(3.2)	7.9(2.9)	6.2(3.1)	6.5	7.7(2.8)	5.1(2.1)	8.3(2.1)	7.5(3.1)	7.15
2-methyl-butanol	93.3(1.9)	79.6(1.7)	83.5(2.1)	85.7(2.6)	85.5	74.3(3.1)	63.9(2.8)	51.8(2.1)	44.1(2.5)	58.5
3-methyl-butanol	55.9(0.9)	25.7(1.4)	73.6(2.1)	53.8(1.9)	52.2	33.9(2.1)	11.2(1.9)	64.9(2.2)	53.1(2.4)	40.7
Ethyl-acetate	43.4(0.8)	96.7(1.1)	79.2(1.9)	42.7(1.1)	65.5	78.9(1.7)	18.7(1.5)	22.9(1.9)	79.4(1.4)	49.9
Hexanol-1	-	-	-	-	-	-	-	-	-	-
2-phenyl-ethanol	-	-	-	-	-	-	-	-	-	-
Hexanoic acid	1.8(3.1)	1.9(3.7)	1.6(3.4)	1.7(2.9)	1.7	1.6(3.3)	3.1(3.9)	2.6(3.1)	1.7(3.6)	2.2
Octanoic acid	2.5(2.9)	2.8(3.1)	2.8(3.7)	2.9(3.6)	2.7	2.4(3.2)	2.6(2.9)	3.4(3.2)	2.8(3.7)	2.8
Decanoic acid	1.8(2.6)	2.3(2.1)	4.6(2.9)	2.9(3.4)	2.9	1.8(3.3)	1.7(3.9)	3.3(3.8)	1.7(3.1)	2.1
Dodecanoic acid	0.7(3.9)	0.9(4.0)	1.5(3.6)	0.9(3.8)	1	1.3(3.3)	1.7(3.8)	1.5(3.1)	1.2(3.6)	1.4
Ethyl hexanoate	1.8(3.9)	1.9(3.1)	1.5(3.5)	1.2(3.3)	1.6	1.1(3.2)	1.3(3.4)	1.4(3.1)	1.7(3.9)	1.4
Ethyl octanoate	4.9(2.9)	4.5(3.2)	3.6(2.8)	1.9(3.1)	3.7	2.1(3.2)	2.7(2.9)	2.3(3.6)	2.2(3.1)	7.05

\* RSD

in Athiri grape variety (*Vitis vinifera* L.), from 56.7-63.9 mg L<sup>-1</sup> for 2-methyl-butanol and 44.65-48.42 mg L<sup>-1</sup> for 3-methyl-butanol in Malagouzia grape variety (*Vitis vinifera* L.), 25.6-37.15 mg L<sup>-1</sup> for 2-methyl-butanol and 46.6-65.6 mg L<sup>-1</sup> for 3-methyl-butanol in Vilana grape variety (*Vitis vinifera* L.) and 58.5-85.5 mg L<sup>-1</sup> for 2-methyl-butanol and 40.7-52.2 mg L<sup>-1</sup> for 3-methyl-butanol in Lagorthi grape variety (*Vitis vinifera* L.) The mean values for amyl alcohols o those Hellenic grape marcs alcoholic beverages was similar to that of various distillates, such as grappa, wine brandies, zivania, bourbon and malt whisky (Bertrand,

1995; Cantagrel *et al.*, 1991; Fournaris, 1999; MacNamara, 1984; Versini, 1993; Vodret and Aquilino, 1972; Versini, and Odello, 1991).

2-methyl-1-butanol and 3-methyl-1-butanol are formed during fermentation by decarboxylation reactions from iso-leucine and leucine (Boulton *et al.*, 1996; Kana *et al.*, 1991). Increased concentration of amyl alcohols (having an aromatic description of “alcoholic”, “sweet” and “choking”) can contribute negatively to the aroma of the distillate (Falque *et al.*, 2001).

The lower levels of amyl alcohols indicate light-bodied grape pomace distillates.

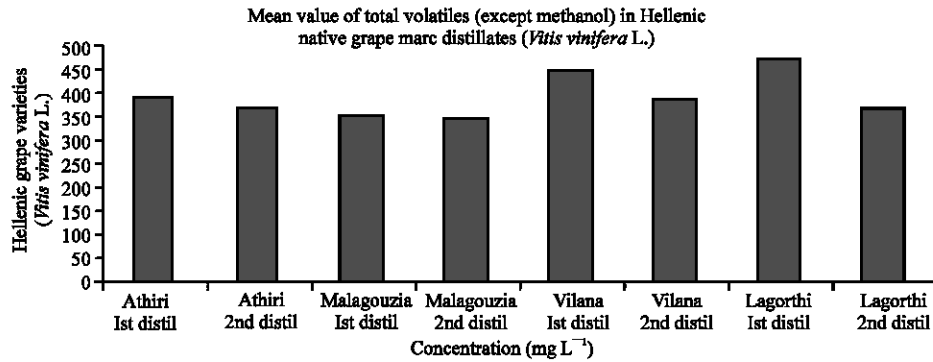


Fig. 1: Mean values of total volatiles concentration (mg L<sup>-1</sup>) in fermented grape marc distillates from Hellenic Native White grape varieties (*Vitis vinifera* L.)

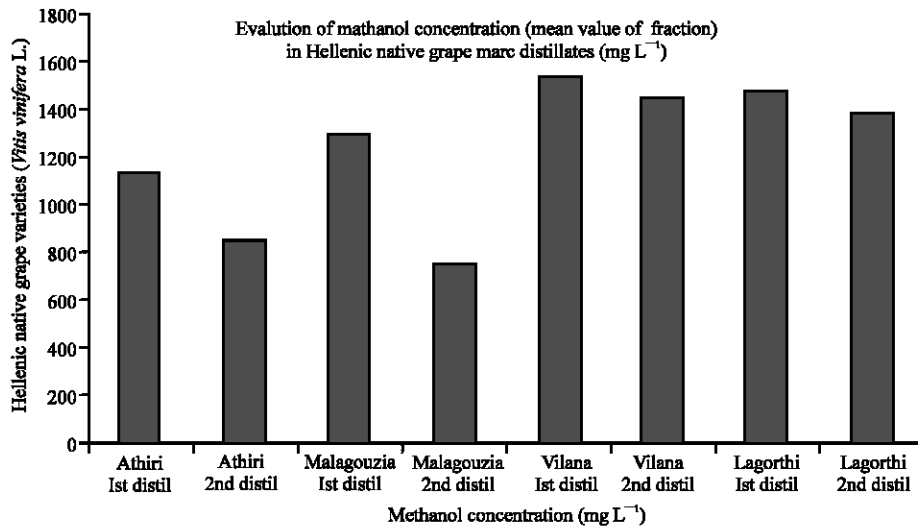


Fig. 2: Mean values of methanol concentration (mg L<sup>-1</sup>) in fermented grape marc distillates from Hellenic Native White grape varieties (*Vitis vinifera* L.)

The concentration of 1-Propanol which increased by the microbiological process that take place during the ensilage of the grape marc (Versini *et al.*, 1990; Vodret and Aquilino, 1972) ranged between 37.3-49.95 mg L<sup>-1</sup> for Malagouzia grape variety (*Vitis vinifera* L.), 71.8-75.6 mg L<sup>-1</sup> for Lagorthi grape variety (*Vitis vinifera* L.), 65.7-69.9 mg L<sup>-1</sup> for Vilana grape variety (*Vitis vinifera* L.), 47.2-51.2 mg L<sup>-1</sup> for Athiri grape variety (*Vitis vinifera* L.). Those values are slightly lower than those reported for grappa, bagaceira, whiskies and cider brandies (MacNamara, 1984; Mangas *et al.*, 1996; Silva and Malcata, 1999; Versini, 1993). 1-Propanol content in wine brandies and zivania is quite similar to that of the studied samples (Bertrand, 1995; Cantagrel and Vidal, 1991; Fourmaris, 1999; Versini, 1993).

The concentration of butanol-2 which originates from bacterial action (probably from 2, 3-butanediol) and with

a poor organoleptic character, (Silva *et al.*, 1998) range from 1.52-1.66 mg L<sup>-1</sup> for Malagouzia grape variety (*Vitis vinifera* L.), 25.1-36.8 mg L<sup>-1</sup> for Lagorthi grape variety (*Vitis vinifera* L.), 10.3-10.5 mg L<sup>-1</sup> for Vilana grape variety (*Vitis vinifera* L.), 0.9-1.75 mg L<sup>-1</sup> for Athiri grape variety (*Vitis vinifera* L.).

Another undesirable and toxic alcohol in alcoholic beverages is methyl alcohol which is generated via degradation catalyzed by native pectinesterase in grape pomace; the rate of such degradation is enhanced by microbial attack leading to increased methanol concentration in the final grape marc alcoholic beverages. Methanol is not one of the main flavour compounds and it has not specific odour. Methanol concentration of the studied samples of those grape varieties was lower compared to that of brandy, rum and whisky (Bertrand, 1975; Cantagrel *et al.*, 1997; MacNamara, 1984; Versini *et al.*, 1991; Postel and Adam, 1980).

The concentration of toxic methyl alcohol in Hellenic grape marc alcoholic beverages range from 867.25-1456.5 mg L<sup>-1</sup> for Athiri grape variety (*Vitis vinifera* L.), 768.25-1307.25 mg L<sup>-1</sup> for Malagouzia grape variety (*Vitis vinifera* L.), 1446-1545.2 mg L<sup>-1</sup> for Vilana grape variety (*Vitis vinifera* L.), 1395-1478.5 mg L<sup>-1</sup> for Lagorthi grape variety (*Vitis vinifera* L.). Methanol mean concentration in the studied samples was similar to that of Spanish, Portuguese, Cyprian, Italian alcoholic beverages (Fournaris, 1999; Silva and Malcata, 1999; Versini, 1993; Vodret and Aquilino, 1972; Kana *et al.*, 1991). Cider alcoholic beverages show similar methanol concentration which can be attributed to the pectin content of the row material (apples) (Mangas *et al.*, 1996).

The EC regulation 1576/89 established general manufacturing procedures of marc distillates and fixed common analytical composition of 1000 g hL<sup>-1</sup> of 100%vol. ethanol. The methanol contents of our samples are within the limits of acceptability of the European Regulation (EC 1576/98).

1-Hexanol is considered to have a positive influence on the aroma of the distillate when occurs in concentrations up to 20 mg L<sup>-1</sup>. On the contrary, increased concentration of 1-hexanol, having an aromatic description of "coconut-like", "harsh" and "pungent", can contribute negatively to the product aroma (Apostolopoulou *et al.*, 2005). At even higher 1-hexanol levels the organoleptic characteristics of the distillate are seriously impaired ("green" flavour) (Cantagrel *et al.*, 1997; Falque *et al.*, 2001). 1-Hexanol is not an alcoholic fermentation product and its origin is linked to the vine variety. When the grapes are not ripe enough, high 1-hexanol concentrations in spirits are observed (Cantagrel *et al.*, 1997).

1-Hexanol concentrations in our samples ranged between range from 2.5-7.85 mg L<sup>-1</sup> for Athiri grape variety (*Vitis vinifera* L.), 4.05-7.7 mg L<sup>-1</sup> for Malagouzia grape variety (*Vitis vinifera* L.), 4.5-4.9 mg L<sup>-1</sup> for Vilana grape variety (*Vitis vinifera* L.), zero concentration for Lagorthi grape variety (*Vitis vinifera* L.). It was found at lower concentrations compared to grappa and bagaceira (Silva and Malcata, 1999; Versini, 1993; Vodret and Aquilino, 1972). The low 1-hexanol concentrations in the studied samples are considered to affect positively the flavour of the product. Its concentration in the studied distillates was usually much lower than that of cider brandies while it was similar to that of wine brandies (Bertrand, 1995; Postel and Adam, 1985). Whiskies have very low concentrations of 1-hexanol (Postel and Adam, 1985).

2-phenyl ethanol, which is described as a rose-like positive influence and can be generated from amino-acids, in our samples was in low concentrations from

1.9-4.92 mg L<sup>-1</sup>. The comparison of the results, demonstrated in Table 1-4, show that 2-phenyl-ethanol has similar low levels for bagaceira and lower than aguardiente and grappa.

Second to acetic acid, the most abundant acids in the grape marc alcoholic beverages were Hexanoic, octanoic, decanoic and dodecanoic acids. The concentrations of those acids in our samples was at an average level of from 1.6-1.97 mg L<sup>-1</sup> for Hexanoic acid, 1.9-1.8 mg L<sup>-1</sup> for octanoic acid, 1.3-1.82 mg L<sup>-1</sup> for decanoic acid, 1.9-2.17 mg L<sup>-1</sup> for dodecanoic acid for Athiri grape variety (*Vitis vinifera* L.), 1.3-1.89 mg L<sup>-1</sup> for Hexanoic acid, 1.73-2.29 mg L<sup>-1</sup> for octanoic acid, 1.69-2.47 mg L<sup>-1</sup> for decanoic acid, 1.07-2.1 mg L<sup>-1</sup> for dodecanoic acid for Malagouzia grape variety (*Vitis vinifera* L.), 2.35-2.4 mg L<sup>-1</sup> for Hexanoic acid, 1.75-2.3 mg L<sup>-1</sup> for octanoic acid, 0.5-0.8 mg L<sup>-1</sup> for decanoic acid, 1.5-2.5 mg L<sup>-1</sup> for dodecanoic acid for Vilana grape variety (*Vitis vinifera* L.), 1.7-2.2 mg L<sup>-1</sup> for Hexanoic acid, 2.7-2.8 mg L<sup>-1</sup> for octanoic acid, 2.1-2.9 mg L<sup>-1</sup> for decanoic acid, 1-1.4 mg L<sup>-1</sup> for dodecanoic acid for Lagorthi grape variety (*Vitis vinifera* L.). It can be seen that octanoic and decanoic acids were the most important components of this family, except in what concerns one of the grape marc distillates.

The fatty acid ethyl ester content of the Hellenic grape marc alcoholic beverages were studied ranged from 0.9-1.45 mg L<sup>-1</sup> for ethyl hexanoate and 0.5-1.42 mg L<sup>-1</sup> for ethyl octanoate for Athiri grape variety, 1.31-2.6 mg L<sup>-1</sup> for ethyl hexanoate and 1.22-3.39 mg L<sup>-1</sup> for ethyl octanoate for Malagouzia grape variety, 2.4-4.5 mg L<sup>-1</sup> for ethyl hexanoate and 4-5.5 mg L<sup>-1</sup> for ethyl octanoate for Vilana grape variety and 1.4-1.6 mg L<sup>-1</sup> for ethyl hexanoate and 3.7-7.05 mg L<sup>-1</sup> for ethyl octanoate for Lagorthi grape variety.

Ethyl acetate, one of the most important esters due to its unpleasant flavour, ranged from 26-28.3 mg L<sup>-1</sup> for Athiri, 25.62-26.58 mg L<sup>-1</sup> for Malagouzia, 76.9-78.2 mg L<sup>-1</sup> for Vilana and 49.9-65.5 mg L<sup>-1</sup> for Lagorthi grape variety. These values can, in general, be considered to lay on the low side for spirits.

Results were compared with those obtained for Spanish grape pomace distillates, called aguardiente, with analogous raw material and distillation technique (Orriols *et al.*, 1991). It can be concluded that the major differences were those concerning acetal and ethyl acetate, with higher contents in aguardiente (ca. 800 mg L<sup>-1</sup> for acetal and ca. 1300 mg L<sup>-1</sup> for ethyl acetate). Regarding changes of grape pomace volatile (Table 1-4) during distillation it can be seen that their concentration exhibit relatively small changes. The explanation of this phenomenon is as follow. According to Raoult's law the vapor pressure (Pi) of a volatile

component (i) above a solution is the product of the vapor pressure ( $P_i^0$ ) of the pure component and of the mole fraction ( $X_i$ ) of the component (i) in the solution ( $P_i = P_i^0 \cdot X_i$ ). From Dalton's law it can be calculated that the  $X_i$  (gas phase) =  $P_i/P_{total}$ , where  $P_{total}$  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 the other volatiles it can be calculated that  $X_i$  (gas phase) is  $10^{-4}$  to  $10^{-5}$  of  $X_i$  (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. Instead they will distill at a slow and rather uniform rate throughout the whole distillation process as it can be actually seen from Table 1-4. This attitude has important consequences for producers of distilled alcoholic beverages. It means that it is impossible to get rid of some minor unwanted component through distillation cut and some other way has to be devised.

### CONCLUSION

In general, it can be concluded that the studied grape marc alcoholic beverages from native white Hellenic grape varieties (*Vitis vinifera* L.) were characterised by high complicity of different congeners, high levels of aldehydes and volatile esters and a considerable variation in the levels of amyl alcohols and 2-phenyl-ethanol.

This research shows that the alcoholic beverages which produced from native grape varieties (*Vitis vinifera* L.) has a variety of different volatiles and chemical complicity. The volatile compounds that can pose health hazards, methanol, acetaldehyde and ethyl acetate are recovered at levels inferior to those reported by the European Council and the threshold perception. These results are present at those levels because the good vinification technique of grapes and the satisfactory maintenance of the raw material which are important for the quality of the spirit. In all grape marc distillates from the Hellenic native white grape varieties (*Vitis vinifera* L.), some fatty acid esters that contribute to fruity and flowery aroma are present in satisfactory levels. 2-phenyl ethanol, that contribute to the typical flavour of rose in distillates, is found also in satisfactory concentrations in comparison to levels reported for other alcoholic beverages.

This study for those alcoholic beverages from Hellenic native white grape varieties (*Vitis vinifera* L.) never studied before. Consequently, this report on the

composition of grape marc alcoholic beverages from selected monovarietal grape varieties, is a first approach on a subject that requires and is already under further investigation.

### REFERENCES

- Amerine, O., 1980. Methods for Analysis of Musts and Wines, Wiley, New York, pp: 74-152.
- Apostolopoulou, A.A., A.I. Flouros, P.G. Demertzis and K. Akrida-Demertzi. 2005. Differences in concentration of principal volatiles constituents in traditionnel Greek distillates. Food Control, 16: 157-164.
- Bertrand, A., 1975. Recherches sur l'analyse des vins par chromatographie en phase gazeuse. Thèse Docteur en Sciences. Université de Bordeaux II, France.
- Bertrand, A., 1995. Armagnac and wine-spirits. In: Lea, A.G.H. and J.R. Piggott (Eds.). Fermented beverage production (1st Edn.), London: Blackie Academic and Professional, pp: 229-246.
- Boulton, R.B., V.L. Singleton, L.F. Bisson and R.E. Kunkee, 1996. Principles and practices of winemaking. New York: Chapman and Hall.
- Cabras, P., P. Petruso and A. Vodret, 1972. La composizione dei distillati di vinaccia in Sardegna (Composition of Sardinian grape pomace distillates). Industria Agraria, 10: 376-381.
- Cantagrel, R., L. Lurton, J.P. Vidal and B. Galy, 1997. From vine to Cognac. In: Lea, A.G.H. and J.R. Piggott (Eds.). Fermented beverage production (1st Edn.), London: Blackie Academic and Professional, pp: 208-228.
- Cole, V.C. and Noble, 1997. Flavour chemistry and assessment. In: Lea, A.G.H. and J.R. Piggott (Eds.). Fermented beverage production (1st Edn.). London: Blackie Academic and Professional, pp: 361-385
- Cortes, S., M.L. Gil, E. Fernandez, 2005. Volatile composition of traditional and industrial Orujio spirits. Food Control, 16: 383-388.
- Da Porto, C., 1998. Grappa and grape-spirits production. Criti. Rev. Biotech., 18: 13-24.
- FAO/WHO Recommendations methods of sampling. 1986. Codex Alimentarius Commission of the joint FAO/WHO Food Standards Program: Food and Agricultural Organization/World Health Organization, Rome.
- Falque, E., E. Fernandez and D. Dubourdieu, 2001. Differentiation of white wines by their aromatic index. Talanta, 54: 271-281.



- Ferreira, V., P. Hernandez-Orte, A. Escudero, R. Lopez and J. Cacho, 1999. Semipreparative reversed-phase liquid chromatographic fractionation of aroma extract from wine and other alcoholic beverages. *J. Chromatography, A* 864: 77-88.
- Fournaris, K., 1999. Zivania, the traditional Cyprus alcoholic beverage. In Greek Chemists' Association (Ed.), Proceedings of the 6th Chemistry Conference Greece-Cyprus. Rhodes: Greek Chemists' Association, pp: 260-265.
- Fugelsang, K.C., 1997. Wine microbiology. New York: Chapman and Hall.
- Gerogiannaki-Christopoulou M., Kyriakidis N.V., P.E. Athanasopoulos, 2005. The evaluation of grape pomace distillates from selected red grape varieties. *Int. J. Food Sci. Tech.*, 40: 1-7.
- Gerogiannaki-Christopoulou M., N.V. Kyriakidis and P.E. Athanasopoulos, 2004. Effect of grape variety (*Vitis vinifera* L.) and grape pomace fermentation conditions on some volatile compounds of the produced grape pomace distillates. *Int. J. Vine Wine Sci. (Vigne Vin)*, 38: 155-162.
- Kana, K., M. Kanellaki, A. Papadimitriou and A.A. Koutinas, 1991. Cause of and methods to reduce methanol content of Tsikoudia, Tsipouro and Ouzo. *Int. J. Food Sci. Tech.*, 26: 241-247.
- MacNamara, K., 1984. Rapid determination of major congeners in distilled spirits by direct analysis on bonded capillary columns. *J. High Resol. Chromatography and Chromatography Commun.*, 7: 641-643.
- Mangas, J., R. Rodríguez, J. Moreno and D. Blanco, 1996. Changes in the Major Volatile Compounds of Cider Distillates During Maturation. *Lebensmittel-Wissenschaft und-Technologie*, 29: 357-364.
- Official Journal of EEC, 1989. No L.160, 12/6/89.
- Orriols Fernandez, I., B. Rodriguez and J.F. Perez Fernandez, 1991. L'eau-de-vie de marc (aguardente) d'albarino: Influence de trois systemes de distillation sur les teneurs en composés volatiles. In *Les eaux-de-vie traditionnelles d'origine viticole* (Bertrend, A., Ed.), Lavoisier, Paris, pp: 43-49.
- Postel, W. and L. Adam, 1980. Gas chromatographic characterization of brandy, cognac and armagnac. II. Content of volatile substances. *Die Brautweinwirtschaft*, 120: 154-164.
- Silva, M.L., X.F. Malcata, 1998 Relations Between Storage Conditions of Grape Pomace and Volatile Composition of Spirits Obtained Therefrom. *Am. J. Enol. Viticul.*, 49: 6-64.
- Silva, M.L. and F.X. Malcata, 1999. Effects of time of grape pomace fermentation and distillation cuts on the chemical composition of grape marcs. *Zeitschrift für Lebensmittel Untersuchung und Forschung A*, 208, 134-143. Silva, M.L., Malcata, F.X., and G. Revel (de), (1996). Volatile Contents of Grape Marcs in Portugal. *J. Food Comparative Anal.*, 9: 72-80.
- Soufleros, E and A. Bertrand, 1987. Etude sur le "Tsipouro", eau-de-vie de marc traditionnelle de Grèce, précurseur de l'ouzo. *Conn Vigne Vin*, 21: 93-111.
- Soufleros, E., I. Pissa, D. Petridis, M. Lygerakis and K. Mermelas, 2001. Instrumental analysis of volatile and other compounds of Greek kiwi wine; sensory evaluation and optimization of its composition. *Food Chem.*, 75: 487-800.
- Soufleros, E., A. Bertrand, 1987. Etude sur le "Tsipouro", eau-de-vie de marc traditionnelle de Grèce, précurseur de l'ouzo. *Connaissance de la Vigne et du Vin*, 21: 93-111.
- Soufleros, E.H., S.A. Mygdalia and P. Natskoulis, 2004. Characterization and safety evaluation of the traditional Greek fruit distillate "Mouro" by flavor compounds and mineral analysis. *Food Chem.*, 86: 625-636.
- Soufleros, E.H., S.A. Mygdalia and P. Natskoulis, 2005. Production process and characterization of the traditional Greek fruit distillate "Koumaro" by aromatic and mineral composition. *J. Food Comp. Anal.*, 18: 699-716.
- Varajao, G.A., 1991. Les eaux-de-vie de marc du Portugal. In *Les eaux-de-vie traditionnelles d'origine viticole* (Bertrand, A., Ed.), Lavoisier, Paris, pp: 38-41.
- Versini, G. and L. Odello, 1991. La grappa: Considerations sur la distillation traditionnelle en Italie. In 'Les eaux-de-vie traditionnelles d'origine viticole' (Bertrend, A., Ed.), Lavoisier, Paris, pp: 32-37.
- Versini, G., A. Monetti, Dalla A. Serra and S. Inama, 1990. Analytical and statistical characterization of grappa from different Italian regions. In: *Proc. first symposium international sur les eaux-de-vie traditionnelles d'origine viticole*, Bordeaux, France; Lavoisier-Tec and DOC, pp: 137-150.
- Versini, G., 1993. Volatile compounds of spirits. In: Doneche, B. (Ed.), *Les Acquisitions Recentes en Chromatographie du Vin Cours Europeen de Formation Continue*; 31 Mars, 1, 2 et 3 Avril 1992; Porto Paris: Tec and Doc-Lavoisier, pp: 189-213.
- Vodret, A. and A. Aquilino, 1972. Il componenti minori dei distillati di vinaccia sarda (Minor components of Sardinian grape pomace). *Industria Agraria*, 10: 406-411, 420.