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# Characterization and safety evaluation of the traditional Greek fruit distillate ''Mouro'' by flavor compounds and mineral analysis

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#### Abstract

Mouro is the spirit beverage that comes from the distillation of fermented fruits of the mulberry tree (Morus nigra L.). Mouro is also the Greek common name of this fruit. Usually, it is used for the production of syrups, jams and jellies. In Greece it is also used for the production of the traditional aromatic *mouro* distillate. As far as we know it is the first time that such research has been made into mouro distillate. To gain a better perspective of this, we collected helpful information regarding the production process and we also analysed: (a) alcohols, esters and fatty acids by using gas chromatography according to the official method of O.I.V. Alcohols, acetaldehyde and ethyl acetate were analysed with direct injection of the diluted distillate, which contains the internal standard, on a Carbowax 400 + Hallcomid M. 1801 Classic column. The other volatile substances were determined after their extraction from the samples – along with the internal standard – by a mixture of solvents; the extract is injected in a capillary column CP Wax 57 CB. (b) mineral compounds, using atomic absorption spectrometry with flame for Fe, Ca and Cu and with graphite furnace for Pb and (c) the pH values throughout using standard methods.

From our point of view, it would be particularly valuable, firstly to provide data for the safety of *mouro* distillate for human consumption, specially regarding methanol and heavy metal (Cu and Pb) levels and secondly, to form conclusions from any essential results which might have an impact on the quality of this product.

The alcoholic title, even though it meets – except for one sample – the official minimum limit of 37.5% vol., it varies substantially from 35.48 to 45.59% vol. Methanol, acetaldehyde, ethyl acetate and ethyl lactate, which at high concentration can affect negatively the quality of the distillates, vary from 107 to 198, from 21 to 79.4, from 6.2 to 1031.7 and from traces to 51.21 g/hl AA, respectively. However, in most cases, these concentrations remain low comparing to the official limits or to qualitative thresholds. Mineral concentrations, mainly that of lead, presented significant fluctuations; the measured levels, however, do not pose a threat for consumer safety. On the other hand, the desirable concentrations of the higher alcohols (>140 g/hl AA), the favorable esters, such as ethyl octanoate (1.3 g/hl AA) and ethyl decanoate (1.4 g/hl AA) and, mainly, the relatively high concentrations of 2-phenylethanol (traces to 12.73 g/hl AA) seems to distinguish the mouro distillate. All these observed differences show that our goal should, also, be a better standardization process and a standard quality of this spirit. Areas, where a better quality control should be applied, are the fermentation and storage procedures, the distillation and the good condition of the alembics used. 2003 Elsevier Ltd. All rights reserved.

Keywords: Morus nigra; Blackberry tree; Mulberry tree; Distillation; Alcoholic beverages; Eau-de-vie; Volatile compounds; GC; Atomic absorption; Flavor; Minerals

# 1. Introduction

Mouro is the spirit beverage that comes from the distillation of fermented fruits of the mulberry tree. Mouro is also the Greek common name of the mulberry tree fruit from which this certain distillate takes its name.

In addition, Mouria, Mavromouria or Mavromournia are the Greek terms for mulberry tree or blackberry tree as it is otherwise commonly called (Gerasopoulos & Stavropoulos, 1997). Botanically, it is known as Morus nigra Linneus and belongs to the family of Moraceae (Vardavakis, 1993). Originated in Asia (Datta, 2001;

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Machii, Koyama, & Yamanouchi, 2000), it was introduced into Greece sometime between the 18th and 19th century and is found in several Greek regions (Cappellozza, 2000). Mavromouria was initially presented in Greece for sericulture, but it was abounded 35–40 years ago because of several cultural difficulties (Chitra Vijaya & Padmaja, 1999; Gerasopoulos & Stavropoulos, 1997; Machii et al., 2000).

Mouro is a sorosis fruit 1.5–2 cm or so long, made up of many bead-like single-seeded fruits. It turns from green to red or even to glossy deep purple as it ripens in mid-summer (July–August). It is a juicy, edible fruit, very delicious when fully matured, with a sugar content between 9% and 22.5% dw (Gerasopoulos & Stavropoulos, 1997) or according to Machii et al. (2000) from 12% to 20%. It is also rich in non-volatile organic acids and phenolic acids which contribute to the quality of its delicate taste and aroma (Gerasopoulos & Stavropoulos, 1997). Mouro is a very appealing fruit, but its soft flesh makes it susceptible to treatment and transportation as a fresh fruit. Nowadays, it is mostly processed into syrups, jam, fruit wine, distillates and liqueur and some times it is used for the extraction of colorants, contributing in this way to a total higher income for the country (Gerasopoulos & Stavropoulos, 1997; Machii et al., 2000). Also, it is used in medicine because its fruit and leaves contain gamma-aminobutyric acid and alanine used against high blood pressure, and 1-deoxynojirimycin amino-acids for lowering the blood-sugar levels (Machii et al., 2000). It contains morusin, a prenyflavonoid, which is used as antinociceptive or analgetic medicine (Souza et al., 2000) and lectins such as MornigaGII and MornigaMII with different specificity (Rabijns, Verbonnen, Damme, Peumans, & Ranter, 1999).

In Greece, local farmers developed a small venture with a topical character for the production of a traditional spirit named after mouro fruit; widely known in Greece as mouro distillate (region of Karpenissi) or mournoraki (region of Crete).

Up to 1989, the year that the European legislation 1576/89 about distilled beverages was issued, there were no legal restrictions concerning fruit distilled beverages in Greece. The National Law 971/1917 concerned only the production of industrial alcohol and distilled products of viticulture origin and among them tsipouro. Recently, the 2989/2001 law, which allowed the harmonization of Greece with the European Community under the 1576/89 legislation, encloses the distillation procedures and distillation products from grape pomace and pomace from other fruits such as mouro.

Several countries produce traditional distilled alcoholic beverages from different raw materials. Very popular are those produced from grape marc, such as the Greek tsipouro and tsikoudia, the Italian grappa (Silva & Malcata, 1998, 1999), the Portuguese Bagaceiras (Silva, Malcata, & Revel (de), 1996; Silva & Malcata, 1998, 1999) and Aguardiente (wine or marc spirit) (Rogerson & Freitas, 2001) and lastly, the French marc grape or eau-de-vie de marc. Just as popular are other spirit beverages produced from different raw materials such as the Brazilian cachacas (sugar cane distillate) (Nascimento et al., 1999), the Japanese Sake (rise distillate), the Slivovicha (plum distillate) from ex-Yugoslavia and the *Williams* or *Poiré* (pear distillate) from France and some other countries. However, there is no available information or data on mouro distillate. From our point of view, it would be particularly valuable firstly to provide data on the safety of *mouro* distillate for the consumers without any repercussion to their health from methanol or heavy metal levels (Cu and Pb) and secondly to form conclusions from any essential results which might have negative consequences to the quality of this product (acetaldehyde, ethyl acetate, ethyl lactate, etc.). Furthermore, the purpose of this paper is to provide knowledge on the procedures followed for distilled product from mouro fruit, such as fermentation and distillation, as well as on its chemical characteristics (volatile and mineral composition). This knowledge should lead to a better standardization process and a more uniform quality of this spirit.

#### 2. Materials and methods

### 2.1. Processing and fermentation of mouro fruit

Mouro fruits are usually collected using big white hessians or sheets after shaking the trees (Gerasopoulos & Stavropoulos, 1997) or after smooth hits with big brooms. In most cases, the fruits are carried into small wooden caskets of 5–10 kg capacity, straight to the wooden barrels where, in a short time, the fermentation process will take place. Some times, if needed, small quantities of water are added progressively in order to assist the fermenting procedure and the fruit mass is frequently stirred with long wooden sticks.

## 2.2. Distillation

The distillation is realised with the traditional copper alembics of 130L, which is a simplified type of the Charentais alembic. The fermented raw material is transferred to the vessel up to the 3/4 of its capacity in order to be distilled. Before the beginning of heating, the alembic is hermetically closed with dough or cinder pulp in order to prevent any vapor leakage. When the temperature reaches 80–90  $\degree$ C, the liquid spirit starts to run from the funnel and is gathered in glass bottles, known as damitzanes, of varying capacity (30–100 l).

The first 0.5–1 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, 90% vol. approximately. Then, and for about 2.5 h, a pure spirit, distilled to levels lower than 86% vol., is poured into egg shaped glass bottles. This distillate is commonly called first distillation or first pitcher. The distillation product is subsequently obtained until 5% vol., and it is led back to the alembic for a second distillation or metavrasma. The distillation of the mouro pomace is being done without the use of any aromatic plants or seeds. In this case, the aroma of the spirit is deriving exclusively from the raw material, the fermentation process and the bouquet of the aging period divulge to the distillate. The ''tails'' (distilled spirit below 5% vol.) or – as it is commonly known – *aporaka*, ahamko or hamko are re-used for the next first distillation process with the new lot of the fermented mouro pomace. In the end, the various lots of the second distillation spirit are mixed and placed, before the dilution with water, into oak barrels and left to age one year before consumption.

# 2.3. Sampling

Ten samples of the mouro distillates from second distillation were obtained from local distillers of the mountainous regions of Evritania (Greece) for the needs of this research. All distilled samples were placed into glass bottles of 1/2 l capacity and stored in the dark at  $4^{\circ}$ C until they were analysed.

# 2.4. Analytical methods

The determination of the distilled sample composition involved of several analyses using classical, gas chromatographic and atomic absorption spectrometry methods. Studied parameters were pH values, 32 volatile compounds and four minerals.

Alcoholic title. The real alcoholometric title was determined after distillation, according to the official method (O.I.V., 1994).

 $pH$ . The pH evaluation was performed with a microprocessor pH/ion meter PMX2000WTW.

## 2.4.1. Volatile compounds

Volatile compounds were determined with gas chromatography. Acetaldehyde, ethyl acetate, methanol, 2 methyl-1-propanol, 1-butanol, 2-methyl-1-butanol and 3-methyl-1-butanol were analysed by the official method (O.I.V., 1994) with a few changes. According to this method, an amount of 5 ml of the sample was diluted 4 times with Milli-Q treated water (Millipore Corp., Bedford M.A.) and mixed with 0.3 ml of a 4-methylpentanol-1 solution (4.994 g/l in 50% vol.) as the internal standard. An amount of  $2 \mu$  of the above mixture was injected directly into a Hewlett Packard gas chromatograph equipped with a Carbowax 400 + Hallcomid M.18 01 (7.5 m  $\times$  2.3 mm) on a Chromosorb-W (0.177–0.250) mm) classic column and a flame ionisation detector (FID). Oven temperature was maintained to 80  $^{\circ}$ C and injector or detector to 180 °C. Nitrogen was used as carrier gas at a flow rate of 50 ml/min. For the flame ionisation detector, gases were supplied at a pressure of 40 psi for  $N_2$ , 12.5 psi for  $H_2$  and 25 psi for air. In order to identify and quantify all the above substances, a reference solution was prepared (Table 1). The integration of the peak area was accomplished with the use of the 3395 HP integrator.

Higher esters (ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl decatetranoate, isoamyl acetate, hexyl acetate, ethyl acetate, phenyl-ethyl acetate, ethyl lactate and diethyl succinate), fatty acids (butyric, isobutyric,





isovaleric, hexanoic, octanoic, decanoic and dodecanoic), and higher alcohols (1-hexanol, trans-3-hexen-1-ol, cis-3-hexen-1-ol, trans-2-hexen-1-ol and 2-phenylethanol) were analysed by the official method (O.I.V., 1994) with minor modifications, after their extraction by a mixture of solvents. Their determination was achieved by the use of a capillary column CP Wax 57 CB  $(d<sub>I</sub> = 0.22$  mm  $\times$  50 m) and they were detected by a flame ionisation detector (FID). These compounds were quantified by an appropriate 50 ml distillate sample diluted as many times as necessary to get a sample with an alcoholic title of approximately 10% vol. The diluted sample was mixed with 2 ml of a 3-octanol solution (39.36 mg/l in 60% ethanol) used as an internal standard. The pH value was adjusted to 2.0 with HCl (Silva et al., 1996; Silva & Malcata, 1998, 1999; Ferreira, Hernandez-Orte, Escudero, Lopez, & Cacho, 1999). This mixture was extracted three times successive for 5 min each time with 4, 2 and 2 ml, respectively, with a mixture of diethyl ether/hexane (1:1 v/v). An amount of 2  $\mu$  of this extract was injected into the capillary column. Conditions hold for the gas chromatograph are as given below: The temperature program employed was set to start at 50  $^{\circ}\textrm{C}$ hold for 1 min, gradually increasing to 230  $\degree$ C at 3  $\degree$ C/ min rate and holding at 230  $\degree$ C for 10 min. The injector was maintained at 220  $\rm{^{\circ}C}$  under split mode of 50 ml/min rate. The flame ionisation detector was set at 250  $\degree$ C with gas pressure of 80 kPa for He, 60 kPa for  $H_2$  and 150 kPa for air.

#### 2.4.2. Mineral profile

The inorganic elements Fe, Ca and Cu of the distilled spirits were analysed by flame atomic absorption spectrometry (Camean, Moreno, Lopez-Artiquez, Repetto, & Gonzalez, 2001; Muntean, Laslo, Chitulescu, & Muntean, 1998; Nascimento et al., 1999) and Pb by graphite furnace atomic absorption spectrometry (Akrida-Demertzi, 1999; Camean et al., 2001; Chen, Chen, Cheng, & Chou, 1999; Nascimento et al., 1999). Spectrometer standardization was done with standard solutions prepared with metals purchased from Merck-Schuchardt, Switzerland (Table 1).

For assays of Fe, Ca, Cu and Pb 10 ml of each distilled sample were diluted five times with Milli-Q treated water into a 50 ml volumetric flask and allowed to evaporate in a 250 ml boiling glass to a final volume of the 10 ml. The condensed sample was transferred in a 20 ml volumetric flask by carefully rinsing the boiling glass with Milli-Q treated water and was adjusted to 20 ml. For Ca analysis, a ten times dilution of the condensed samples was indispensable. For the determination of these three metals, we used a Perkin–Elmer/403 spectrometer with a hollow cathode lamp, air–acetylene flame atomisator and slit width of 0.2 nm.

For the determination of Pb, a 30 µl quantity, taken from the 20 ml volumetric flasks, was injected to a Perkin–Elmer/AA800 flameless spectrometer with graphite furnace (an Align lamp, slit width  $= 0.7$  nm, injector programmed at 20  $^{\circ}$ C). The quantity used was exposed to a five stage treatment through the flameless spectrometer; the first drying temperature was ramped from 80 to 110  $\degree$ C in 30 s and held for 20 s, then increased from 110 to 140  $\degree$ C in 15 s and held for another 10 s. The initial ashing temperature was set at 850  $\degree$ C and held at this point for 20 s. Next, the atomising temperature was set at 1600  $\degree$ C for 5 s. The cleaning stage was performed at 3000  $^{\circ}$ C for 3 s (Akrida-Demertzi, 1999; Camean et al., 2001; Muntean et al., 1998; Nascimento et al., 1999; Rodushkin, Odman, & Appelblad, 1999; Vilaykumar & Ashwini, 2001).

#### 3. Results and discussion

Our results are given in Table 2 and further discussed in relation to distillates from wine, grape pomace, pomace of other fruits, etc., because until now no other studies have been found relating to the mouro distillates.

## 3.1. Volatile compounds

Their concentrations were compared with those from other distilled alcoholic beverages such as tsipouro, bagaceiras, aguardiente, cachacas, whiskey, brandy, rum, marc, plum, cherry, pear and apple, given by Soufleros and Bertrand (1987), Silva et al. (1996), Bauer-Christoph, Wachter, Christoph, Roßmann, and Ludwig (1997), Silva and Malcata (1998, 1999), Lehtonen, Keller LaDena, and Ali-Matila (1999), Fitzgerald, James, Macnamara, and Stack (2000) and Rogerson et al. (2001). For an easier comparison, we expressed all results of these other authors as ours (g/hl AA, Alcohol Absolute), by making the necessary changes according to the alcoholic title of each distilled alcoholic beverage.

#### 3.1.1. Ethanol

Table 2 shows that the alcoholic title of our samples was found to be higher – except one sample – than the official lower limit of 37.5% vol. given by the European Council (Reg. 1576/89). Therefore, the variation of the alcoholic title shows that mouro distillate needs a more uniform treatment and systematic production in order to ensure a more qualitative product with a standard profile.

#### 3.1.2. Methanol

Methanol is formed by pectinolytic enzymes that split the methoxyl group from pectin present in crushed fruits; for this reason the concentration of methanol in the wine or in the final distillate increases with the kind,





the processing and the extraction time of the pomace (Silva et al., 1996; Silva & Malcata, 1998, 1999).

According to the European legislation (EEC no. 1576/89), the distillate must have a methanol concentration lower than 1000 g/hl AA. The levels for our samples were found to be much lower than the European limit, varying from 106.38 to 196.65 ( $x = 145.74$ ) g/ hl AA (Table 2). 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).

Soufleros and Bertrand (1987) demonstrated lower values of methanol in tsipouro (Greek grape pomace distillate) ranging from 50.4 to 84 g/hl AA. Silva et al. (1996) and Silva and Malcata (1998, 1999) presented for bagaceiras a much more higher concentration of methanol with a mean value equal to 755 g/hl AA or higher than the European limit, ranging from 1021 to 1031 g/hl AA and from 346.8 to 3828 g/hl AA, respectively. These values are dependent mainly on the applied technique of the grape treatment and the distillation and secondly from the grape variety. In other reports, the concentrations of methanol in grape pomace distillates range between 530 and 1590 g/hl AA (Cordonnier, 1971), from 39 to 2860 g/hl AA (Amerine, Berg, & Cruess, 1972), from 205 to 1157 g/hl AA (Bertrand, 1975), while Bauer-Christoph et al. (1997) reported a mean of 537 g/hl AA. The last authors also gave mean values of methanol for distillate from apple 359 g/hl AA, from cherry 457 g/hl AA, from pear 796 g/hl AA and from plum 866 g/hl AA. Last, Lehtonen et al. (1999) for whiskey introduced significantly low mean concentrations of 0.077 g/hl AA and for rum even lower of 0.023 g/hl AA. After the above comparison, it can be said that mouro distillate has a very low levels of methanol in relation to other fruit pomace distillates. The relative similarity between tsipouro (marc distillate) and mouro distillate, even though they are derived from different raw materials, indicates the high effect that the use of the same technique of distillation and the type of alembic has on the chemical composition of the distillate.

#### 3.1.3. Higher alcohols

Higher alcohols constitute the group with the highest concentration in distillates, which gives to them a flavouring aroma and an essential character (Ferreira et al., 1999; Silva & Malcata, 1999). For this reason, the European legislation demands minimum requirements for these aromatic substances higher than 140 g/hl AA. However, some alcohols (e.g. the amylic) in very high concentrations are responsible for certain toxicity. The levels of these compounds are influenced by grape variety, fermentation conditions and distillation, and are concentrated mainly in the first fraction, named ''head'', of the distillates (Silva & Malcata, 1998).

The most important higher alcohols of mouro distillate (Table 2) are 2-methyl-1-propanol, 1-butanol, 2 methyl-1-butanol and 3-methyl-1-butanol. Silva et al. (1996) reported for bagaceiras mean values equal to 80 g/hl AA for 2-methyl-1-propanol, three times higher than our results, and 5.1 g/hl AA for 1-butanol. Also for bagaceiras, Silva and Malcata (1998), depending on grape variety, demonstrated two mean values for each compound: 77.1 and 85.1 g/hl AA for the first and 0.56 and 2.27 g/hl AA for the second. In addition, for 1999, they recorded mean values ranging, respectively, between 28 and 131 and from traces to 6.16 g/hl AA according to grape variety and extraction time. In comparison to these, Bauer-Christoph et al. (1997), for 2-methyl-1-butanol, presented the following concentrations for distillates coming from apple 67 g/hl AA, from pear 67 g/hl AA, from plum 53 g/hl AA, from cherry 48 g/hl AA and finally from grape marc 66 g/hl AA. For 1-butanol, they gave mean values of 11.4, 12.4, 11.8, 1.9 and 2.5 g/hl AA for the aforementioned distillates. Soufleros and Bertrand (1987) for tsipouro reported that 2-methyl-1-propanol varies from 29.2 to 65.2 g/hl AA. This means that mouro distillate has a concentration of 1-butanol more similar to tsipouro than other pomace distilled products and the least of all others. In contrary to the above, Fitzgerald et al. (2000), for whiskey, reported values as low as 0.96 g/hl AA for 2-methyl-1-propanol and 1.09 g/hl AA for 1-butanol.

The amylic alcohols (2-methyl-1-butanol and 3 methyl-1-butanol) constitute quantitatively the biggest part of the higher alcohols and are considered to be predictors of sensory character in the distilled product (Silva et al., 1996). For these two compounds, Silva et al. (1996) gave for Portuguese bagaceiras mean values of 62.2 and 204.4 g/hl AA, higher than mouro distillate (Table 2), but Silva and Malcata (1998) presented concentrations similar to ours for 2-methyl-1-butanol, ranging from 39.5 to 50.3 g/hl AA, and slightly lower values between 86.8 and 103.56 g/hl AA for 3-methyl-1 butanol. The same authors, in 1999 also, reported for bagaceiras mean values varying from 11.1 to 56.2 and from 35.2 to 201.6 g/hl AA, respectively, for these two alcohols and depending on the grape variety and extraction time. Similar concentrations were evaluated from Soufleros and Bertrand (1987) for tsipouro. Fitzgerald et al. (2000) demonstrated a little lower concentrations for whiskey, ranging from 7.5 to 20 and from 21.4 to 49.8 g/hl AA. On the other hand, Lehtonen et al. (1999) defined levels, respectively, from 0.19 to 0.20 and from 0.56 to 0.59 g/hl AA for whiskey, 0.03 to 0.07 and 0.19 to 0.28 g/hl AA for rum and equal to 0.49 and from traces to 2.32 g/hl AA for brandy. Generally, all our distillate samples satisfy the minimum limits of 140 g/hl AA that the European legislation demands.

#### 3.1.4. 1-Hexanol

1-Hexanol is an alcohol originating only from raw material (Soufleros & Bertrand, 1987). It is considered to be a favorable compound if its concentration is above 0.5 g/hl AA but not higher than 10 g/hl AA; otherwise, a grassy flavor is imposed, making the distillate product unpleasant both in aroma and taste (Tourlière, 1977). Ferreira et al. (1999) estimated that the presence of 1 hexanol in the above mentioned concentrations imparts to wines and distillates a fruity, liquorice and even a toothpaste flavor profile. 1-hexanol concentrations are presented in Table 2. According to Soufleros and Bertrand (1987) the concentrations of 1-hexanol in tsipouro ranges from 1.6 to 4.3 g/hl AA and were higher than those in mouro. Silva et al. (1996) observed for bagaceiras a mean value of 13.3 g/hl AA, while Silva and Malcata (1998) found concentrations from 11.4 to 21.89 g/hl AA and from 6.36 to 31.56 g/hl AA in 1999. Bauer-Christoph et al. (1997) demonstrated values of 10.2 g/hl AA for apple, 10.3 g/hl AA for pear, 3.2 g/hl AA for plum, 1.6 g/ hl AA for *cherry* and 15.4 g/hl AA for *marc* distillates. Generally, mouro distillate has a low content of 1-hexanol in relation to all other alcoholic products. This is probably due to the weak grassy character of mouro fruit.

# 3.1.5. 2-Phenyl-ethanol

2-Phenyl-ethanol introduces a pleasant aroma to distillates, resembling to rose (Stark, Munch, Sonnleitner, Marison, & von Stocker, 1998), and derives from Lphenylalanine through metabolic reaction of Saccharomyces cerevisiae during carbonic anaerobiosis (Silva & Malcata, 1998, 1999; Stark et al., 1998).

Table 2 shows a mean value of 6.39 g/hl AA, ranging between traces and 12.73 g/hl AA. Silva et al. (1996) gave for bagaceiras a mean value up to 2.22 g/hl AA. Silva and Malcata (1998) gave two mean values ranging between 1.1 and 1.31 g/hl AA according to the grape variety, while the same authors in 1999, also for bagaceiras, reported mean values from 0.54 to 4.08 g/hl AA directly related to grape variety and extraction time. Fitzgerald et al. (2000), for whiskey, presented a mean concentration of 1.44 g/hl AA and Rogerson et al. (2001) revealed values ranging from 0.55 to 1.8 g/hl AA for aguardiente. These amounts are more or less inferior to our results. Higher concentrations, for 2-phenylethanol, were demonstrated by Soufleros and Bertrand (1987) for the Greek grape distillate named tsipouro and varying from 2.8 to 23.4 g/hl AA. Probably, the high content of 2-phenylethanol in to our samples can be explained from the fact that the mulberry tree is relatively rich in amino acids and particularly in phenylalanine with a mean of 12.3 mg/g dw in the leaves (Machii et al., 2000). The high levels of 2-phenylethanol for tsipouro, too, mean that the distillation technique and the type of alembic used, which are the same for these two spirits, seem to play a significant role.

# 3.1.6. Acetaldehyde

Acetaldehyde is originating from the fermented raw materials and increases during distillation and aging of spirit (Silva & Malcata, 1998). It is also considered to be mainly the result of spontaneous or microbial mediated oxidation (Silva et al., 1996; Silva & Malcata, 1999). The mean concentration of acetaldehyde is found to be 44.8 g/hl AA (Table 2), value much lower than the official limits (73–500 g/hl AA) adopted by the European Council (Reg. 1576/89) for fruit distillates. This means that, generally, the above spirits have been fermented, distilled and aged under favorable conditions and without intervention of unwanted bacteria (Silva et al., 1996; Silva & Malcata, 1998). Silva et al. (1996) showed for bagaceiras a higher mean value of 133 g/hl AA, although for the same distillate, Silva and Malcata (1998) demonstrated much lower mean values down to 69.9 and 86.3 g/hl AA depending on the grape variety, while in 1999 they indicated even inferior values ranging between 3.9 and 135 g/hl AA. Even smaller were the results given by Soufleros and Bertrand (1987) and varying from 4.7 to 40.8 g/hl AA, in contrast to Lafon, Couillaud, and Gay-Bellile (1973) who reported significantly higher values from 80 to 400 g/hl AA.

#### 3.1.7. Esters

The fatty acid esters contribute to the distillates' flavor with a pleasant fruity and flowery smell (Karagiannis & Lanaridis, 2002), indicative of the quality of the spirit (Silva & Malcata, 1999; Soufleros, Pissa, Petridis, Lygerakis, & Mermelas, 2001). Beyond these, ethyl hexanoate is the most abundant of all esters (Bartley & Schwede, 1989). The ethyl esters hexanoate, octanoate and decanoate, which are produced during the raw materials' fermentation (Silva  $&$  Malcata, 1998), pass to the spirits and increases during aging (Silva & Malcata, 1999; Soufleros et al., 2001). Through the distillation process, the heat releases a significant amount of these esters from the yeast cells where they remain bond after fermentation (Caumeil, 1983). These three compounds have quantitatively a small participation compared to the other volatile compounds (e.g. higher alcohols) and determine a vastly profiled aromatic character for the spirits (Ferreira et al., 1999).

According to Table 2, ethyl hexanoate, ethyl octanoate and ethyl decanoate are given at mean values of 0.39, 1.29 and 1.4 g/hl AA, respectively. For the same esters, concerning the bagaceiras samples, Silva et al. (1996) indicated mean values of 0.89, 21.44 and 2.44 g/hl AA correspondingly. Moreover, Silva and Malcata (1998) presented lower concentrations with mean values varying from 0.18 to 0.24, from 0.10 to 0.45 and from 0.16 to 0.4 g/hl AA, respectively, while in 1999, they presented almost double values, varying from 0.22 to 2.25, from 0.25 to 1.84 and from 0.08 to 1.04 g/hl AA for the aforementioned esters, depending on grape variety

and extraction time. Rogerson et al. (2001), for aguardiente, demonstrated values for ethyl hexanoate from 101.5 to 326 g/hl AA, extremely high compared to our results. However, for ethyl octanoate and ethyl decanoate, they gave concentrations varying from 0.69 to 1.57 and from 0.6 to 2.4 g/hl AA, respectively. Aside from the above, Fitzgerald et al. (2000) determined for whiskey values ranging between 0.04 to 0.22, 0.03 to 0.13 and 0.02 to 0.13 g/hl AA for these three esters respectively, which are lower compared to our data (Table 2). From these two articles it is concluded that both Rogerson et al. (2001) and Fitzgerald et al. (2000) gave such values due to the influence of the initial raw materials being used for the distillates. Furthermore, Soufleros and Bertrand (1987) reported for tsipouro, for these ethyl esters, values ranging respectively from 0.4 to 1.2, from 0.8 to 4.0 and from 0.3 to 4.1 g/hl AA, almost twice than ours.

Isoamyl acetate, hexyl acetate and phenyl-ethyl acetate constitute the acetic acid ester group, which are mostly responsible for the flowery and fruity aroma of the distillates (Ferreira et al., 1999; Silva & Malcata, 1999). Table 2 shows that isoamyl acetate has the highest concentration among these three acetates.

According to Silva et al. (1996) the mean value of isoamyl acetate for bagaceiras was 1.33 g/hl AA. In 1998, Silva and Malcata reported concentrations from 0.4 to 0.96 g/hl AA for isoamyl acetate and, in 1999, values slightly higher ranging from traces to 0.91 g/hl AA, depending mostly on the two grape varieties used and the extraction time. Rogerson et al. (2001) reported for aguardiente very surprising values of isoamyl acetate, varying from 183.5 to 398 g/hl AA, while Soufleros and Bertrand (1987) gave for *tsipouro* values lower than 10 g/ hl AA. For *whiskey*, much lower mean concentrations were given from Fitzgerald et al. (2000) for this same compound, ranging from 1.43 to 4.92 g/hl AA. Ethyl acetate, ethyl lactate and diethyl succinate derive mainly from bacterial spoilage of the distilled marc (Silva & Malcata, 1998, 1999; Soufleros & Bertrand, 1987). Ethyl acetate is the ester with the higher concentration, which above the perception threshold of 180 g/hl AA gives to the spirit an acidic character (Ferreira et al., 1999). Table 2 revealed that only two of our samples present concentrations beyond this limit. Ethyl lactate presents a yeast extract, wet and bakery character to the distillate, whereas, diethyl succinate a fusel-like (Ferreira et al., 1999) and camphor-like character (Karagiannis & Lanaridis, 2002). On the other hand, Soufleros and Bertrand (1987) note that ethyl lactate, at relatively low amounts, stabilize the odor and smoothens the firm character of certain substances.

For ethyl acetate, ethyl lactate and diethyl succinate, Silva et al. (1996) presented for bagaceiras mean values of 44.4, 42.2 and 1.11 g/hl AA, respectively. In addition, Silva and Malcata (1998) gave two mean values of 314.7 and 494 g/hl AA for ethyl acetate, of 10.5 and 47.9 g/hl AA for ethyl lactate and two others of 0.4 and 0.5 g/hl AA for diethyl succinate, related to the grape variety used for the distillates. In 1999, Silva and Malcata gave, for the same spirit, two mean values of 45.1 and 853.8 g/ hl AA for ethyl acetate, of 16.58 and 221.2 g/hl AA for ethyl lactate and of 0.25 and 12.2 g/hl AA for diethyl succinate, depending on the grape variety and the extraction time. Soufleros and Bertrand (1987), for tsipouro, demonstrated concentrations up to 58 g/hl AA for ethyl acetate lower than ours, very high values ranging from 28 to 408 g/hl AA for ethyl lactate and from 1.6 to 8.6 g/hl AA for diethyl succinate, similar to ours. Fitzgerald et al. (2000), for whiskey, gave concentrations for ethyl lactate ranging between 1.58 and 3.17 g/hl AA and Rogerson et al. (2001) showed values between 8 and 33.5 g/hl AA. The last authors, for aguardiente, gave values ranging between 0.56 and 1.46 g/hl AA. About ethyl acetate, many other authors gave results with a high diversity. Lafon et al. (1973) and Cordonnier (1971) gave for marc values of 100–280 g/hl AA and 230–330 g/ hl AA, respectively, almost twice as much as ours. For a different distillate, whiskey, Fitzgerald et al. (2000) and Lehtonen et al. (1999) gave significantly lower values, ranging from 1.59 to 20.6 g/hl AA and from traces to 0.19 g/hl AA, respectively. On the other hand, Lehtonen et al. (1999) recorded for rum concentrations with a lower mean value ranging between 0.06 and 0.12 g/hl AA and for brandy slightly higher up to 0.41 g/hl AA. About ethyl acetate, comparing all the above results we conclude that in our samples, with two exceptions, he is present at concentrations commonly acceptable. Comparing all above mentioned values we conclude that among the pomace distillates, ours samples have the lowest concentrations of these three acetates. This means that the maintenance of the pomace was satisfactory and that no undesirable lactic and acetic fermentations took place.

#### 3.1.8. Volatile acids

Short chain fatty acids (butyric, isobutyric and isovaleric) are usually found at low concentrations but with an odor similar in strength string smell equal to acetic acid (Silva & Malcata, 1999; Soufleros et al., 2001) and consequently, with an important contribution to the aromatic character of the distillates (Ferreira et al., 1999; Silva & Malcata, 1999). Karagiannis and Lanaridis (2002) note that there are probably released as intermediate products of long chain fatty acids via yeast metabolism of carbohydrates and can be influenced by insoluble grape solids during fermentation presenting a soapy odor.

The results in Table 2 show that, from these three fatty acids, isobutyric and isovaleric acid are quantitatively the most important. Rogerson et al. (2001) for aguardiente demonstrated values from traces to 379.5 g/ hl AA for isobutyric acid and from not detectable concentrations to 556.0 g/hl AA for isovaleric acid. Silva et al. (1996) presented, for bagaceiras, a total mean concentration of 0.44 g/hl AA for isobutyric acid. Furthermore, Silva and Malcata (1998) evaluated isodutyric acid and gave two mean values of 0.26 and 0.41 g/hl AA relating to the grape variety used for the distillates. Later on, the same authors (1999), added that this compound was present in bagaceiras at mean concentrations from traces to 1.4 g/hl AA, depending on the grape variety and extraction time. Soufleros and Bertrand (1987), for tsipouro, gave values ranging between 0.1 and 0.6 g/hl AA for isovaleric acid, and from traces to 0.5 g/hl AA for isobutyric acid. On the other hand, Cortes et al. (2000), for the initial spirit sample of aguardiente, gave high concentrations for butyric acid (4196.6 g/hl AA) and relatively low for isobutyric (2.25 g/hl AA) and isovaleric acid (2.71 g/hl AA). After comparing all above results it is concluded that mouro distillate presents low values for isobutric acid close to those mentioned by Silva and Malcata (1998, 1999) and Soufleros and Bertrand (1987) and much more lower than Rogerson et al. (2001).

Long chain fatty acids, hexanoic, octanoic, decanoic and dodecanoic acid, are of smaller flavor effect to the distillates (Silva & Malcata, 1999; Soufleros et al., 2001). Table 2 shows that hexanoic acid has the highest mean value of all these acids, followed by dodecanoic acid, decanoic acid and octanoic acid. According to Silva et al. (1996) the mean concentrations for bagaceiras corresponding to the pre-mentioned four acids were 0.44, 0.89, 0.89 and 0.22 g/hl AA, respectively. Silva and Malcata (1998) determined mean values ranging from 0.21 to 0.36, from 0.16 to 0.36, from 0.17 to 0.36 and from 0.08 to 0.14 g/hl AA, respectively, while Silva and Malcata (1999) defined results from 0.12 to 3.0, from 0.09 to 1.06, from 0.04 to 1.6 and from 0.02 to 0.37 g/hl AA, respectively for these four fatty acids, directly related to grape variety and extraction time. Rogerson et al. (2001) from the aguardiente evaluation gave concentrations for hexanoic, octanoic and dodecanoic acid from traces to 518 g/hl AA, from 45.5 to 1662.5 g/hl AA and finally from 0 to 116.5 g/hl AA, respectively. Soufleros and Bertrand (1987), for tsipouro, estimated values ranging from 0.3 to 1.2 g/hl AA for octanoic, from 0.2 to 1.4 g/hl AA for decanoic and from 0.1 to 0.4 g/hl AA for dodecanoic acid, concentrations similar to those given from other authors and more or less close to those gathered at Table 2.

# 3.2. pH

The pH values were given in Table 2. Cortes et al. (2000) gave for aguardiente pH mean value similar to ours (4.34). According to Caumeil (1983) and Lafon et al. (1973) this pH usually characterizes wine and grape pomace distillates of 1–2 years old. Soufleros and Bertrand (1987) presented for tsipouro similar pH values varying from 4.15 to 7.0, while Lehtonen et al. (1999) gave for brandy much lower pH mean values, equal to 3.50. These last authors gave for whiskey and rum intermediary values of 3.95 and 3.89, respectively.

# 3.3. Minerals

The importance of the mineral analysis is due to the toxicity of Cu, Pb that mostly derives from the coppermade alembics and lead welding repairs. These metals might also originate both in food and drinks from fertilizers that are being used polluting the soil, the equipment containers and utensils used for food processing, storage or cooking, and lastly from massive industrial activity (Karadjova, Zachariadis, Boskou, & Stratis, 1998; Muntean et al., 1998). Some times the metals might also originate from polluted water, which is used for dilution of the distillates.

#### 3.3.1. Fe

The results (Table 2) showed very low iron concentrations  $(x = 0.15 \text{ mg/l})$ . This amount is in no position to cause any damaging effects to the consumers (Muntean et al., 1998), so these spirits are free for consumption. Such values indicate that a careful manipulation of the mouro fruits and distillates took place and the raw material was collected from regions without pollution problems with iron. The values demonstrated (Table 2) are close to the results reported from Nascimento et al. (1999), though even lower. They have found concentrations varying from 0.009 to 2.24 mg/l for cachacas, the Brazilian sugar cane spirit, and from 0.013 to 1.28  $(x = 0.19)$  mg/l for other international spirits. Their results resemble those introduced by Camean et al. (2001) for brandy and determined values from not detectable to 2.30 mg/l. In contrast to those, Rodushkin et al. (1999) demonstrated for whiskey concentrations of 0.068 mg/l and for flavored strong aperitifs an even lower mean value equal to 0.0048 mg/l, which are most likely lower than ours.

#### 3.3.2. Ca

Its presence in the distillate is due to possible dilution with water. The values presented at Table 2, according to Muntean et al. (1998), are without any problems for the consumption of these distillates. Results, similar to ours, are demonstrated by Rizzon, Rosa, Salvador, and Zucco (1992), who gave for cognac a concentration of 11.04 mg/l. In addition, Nascimento et al. (1999) reported for cachacas spirit mean values of 9.2 and 12.6 mg/l (varying from 1.36 to 44.6 mg/l). Also, according to the same authors, the values for international beverages vary between 9.6 and 27.2 ( $x = 14.2$ ) mg/l, which are twice as much as our concentration. On the other hand, a smaller variation is issued by Lehtonen et al. (1999) for brandy, ranging between not detectable concentrations to 14.8 mg/l.

#### 3.3.3. Cu

Copper, found in grapes and wines, originates from treatments with  $CuSO<sub>4</sub>$  and from grapes' and wine's contact with copper made surfaces. Furthermore, its presence in the distillates is due to copper alembics and the funnel of the freezer. A more careful and frequent cleaning of the alembics will certainly help to eliminate concentrations of this element to the distillates.

The values of Cu concentration, in the distillates, as given in Table 2. According to Brazilian legislation, Cu limits are set at 5.0 mg/l (Portaria L371, 18/09/74). The same limit for Cu was also adopted from the Latvia Republic (Reg. L101, 6/03/2001). The daily intake of Cu for a normal adults' diet is between 1 and 3 mg, roughly corresponding to the intake levels recommended by most authorities (Muntean et al., 1998; World health Organization, 1973, 1984). Nascimento et al. (1999) claims that for cachacas spirit the content of copper varies from traces to 14.3 mg/l with mean values equal to 1.67 and 5.01 mg/l corresponding to the category of the sample. For international alcoholic beverages, the same authors reported mean values of 1.64 mg/l, while for cognac they presented a mean value of 1.58 mg/l, levels most agreeable to those mentioned by Camean et al. (2001) for brandy and ranging between 0.30 and 5.31 mg/l, but very low compared to ours. Rodushkin et al. (1999) stated that this mineral is detected in whiskey at concentrations equal to 0.23 mg/l and to even smaller amounts in flavored strong aperitifs. Muntean et al. (1998) indicated a mean value equal to 5 mg/l for distilled alcoholic beverages, and lower than 1 mg/l for industrial alcoholic beverages. Last, Vilaykumar and Ashwini (2001) gave for Cu values ranging from 0.103 to 0.214 mg/l in country liquor. In conclusion, our values for copper are much lower than the daily intake allowed (Muntean et al., 1998) indicating firstly that the alembics being used, in most cases, are properly cleaned before distillation (Nascimento et al., 1999) and secondly, that the consumption of the *mouro* distillate is not dangerous for the consumer's health regarding to the fact that the daily intake amounts do not overcome 50–100 ml.

# 3.3.4. Pb

Lead pollution is caused by factors like toxic rain, fertilizers and factory residues that pollute initially the air and the water (Muntean et al., 1998), but, most importantly by the repairing of the alembics with lead. For the samples tested (Table 2) the mean  $(x = 64.89 \text{ µg/l})$ given is far below the limit of 300 ug, that is considered as the maximum daily consumption for an adult (Latvia Republic Reg. L101, 6/03/2001), and even lower than the usual intakes from several food and beverages consumed per person in an industrial country, equal to  $250-300 \mu g$ / kg/day (Muntean et al., 1998). Nascimento et al. (1999) presented for cachacas spirit mean values of 36 and 92  $\mu$ g/l (varying from traces to 421  $\mu$ g/l) according to the sample category, though for international alcoholic beverages, the mean values are coming up to  $250 \mu g/l$ (varying from traces to  $600 \mu g/l$ ) and for *cognac* to  $420$ lg/l. Similar were the values given by Vilaykumar and Ashwini (2001), about country liquor, with a range from 295 to 486 lg/l. In addition to these, Camean et al. (2001) presented for brandy a variation between 8 and 224 ug/l. Aside from all the above, Rodushkin et al. (1999) mentioned that the mean value for whiskey is extremely low and comes up to  $0.0034 \mu g/l$  and to  $0.0011$ lg/l for flavored strong aperitifs.

# 4. Conclusions

In *mouro* distillate, the volatile compounds that can pose health hazards or organoleptic issues, 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 of the good vinification technique, the satisfactory maintenance of the raw material and the double distillation. The results show that the alcoholic title varies substantially, which means that the distillation process needs a better standardization, in order to eliminate the variations of ethanol and mainly those being lower than 37.5% vol. In all cases, higher alcohols respect the authorized limits of 140 g/hl AA reported from the European Community, while, some short and long chain fatty acid esters that contribute to fruity and flowery aroma are present in satisfactory levels. Moreover, 2-phenyl ethanol, that contribute to the typical flavor characteristics of *mouro* fruit to its distillate, is found at higher concentrations in comparison to levels reported for other distilled beverages. On the other hand, ethyl lactate that stabilize the odor and smoothens the firm character of certain substances is found generally in desirable levels. The pH values indicate that this distillate is at least one year old, establishing in this way the positive effect of the above aromatic compounds.

The relative similarity in volatile composition between the two Greek distillates tsipouro (from grape pomace) and mouro (fruit pomace), even though they are products of different raw materials, indicate the high effect that the use of the same technique of distillation and same type of alembic has on their chemical composition.

Furthermore, its very low mineral profile indicates that the consumption of mouro distillate does not constitute any dangers for the consumer's health and also shows the sensitivity of the distillers for the quality of this product and the safety of the consumers.

Mouro is a traditionally made Greek distillate produced in small scale, but with good quality, from local distillers of many Greek regions, Karpenisi and Crete and mostly for domestic use. For this reason, a more systematic approach of the production procedures, aside from a better standardization of the product would offer an opportunity to improve the distillation process and to maintain a more qualitative distillate. The importance of the mouro distillate leads to the necessity of a larger scale production and further to the refreshment of the old mouro cultures and trees.

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