

Article

Chemical characterization of craft *Filuferru* spirit from Sardinia, Italy

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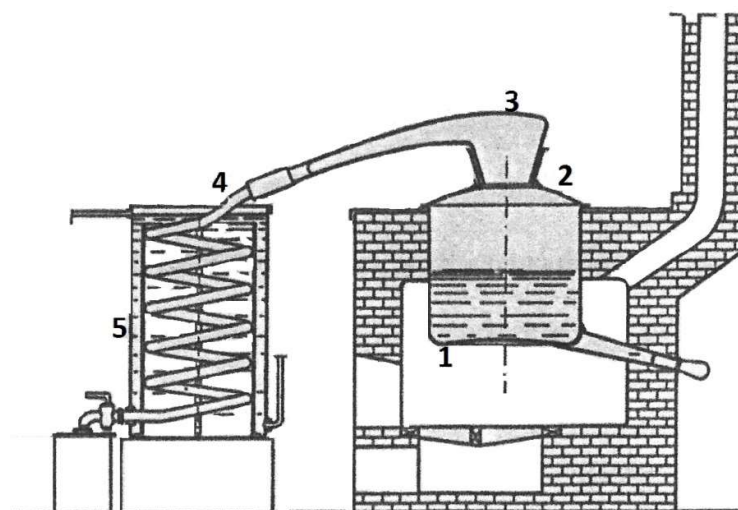
Abstract. Traditional *Filuferru* is an ancient spirit from Sardinia, Italy, usually obtained from the distillation of wine or grape marc. In this contribution, the results of the first chemical characterization of a wide number of craft *Filuferru* samples has been accomplished in terms of evaluation of the alcoholic strength, qualitative and quantitative GC-MS analysis of the volatile composition of the distillate, and its trace element composition by means a ICP-MS method. Both instrumental methods have been validated and applied on 21 craft samples of *Filuferru*, whereas one sample of commercial distillate has been analyzed for comparison purposes. Alcoholic strength ranged between 41.0 and 62.4% (v/v). Sixty volatile compounds were identified and ten of them have been quantified. Analogies and differences with Grappa (i.e. the Italian distilled spirit most close to *Filuferru*) have been highlighted in the qualitative and quantitative profile of this matrix. Often meaningful amounts of acetaldehyde, ethyl acetate, diethyl acetal and acetic acid were measured. Elemental analysis, performed on toxic, non-toxic elements and oligoelements, 18 in total, revealed a wide variability of concentrations in both analytes and samples. High concentrations of Cu are sometimes evidenced, likely caused by losses from the distillation apparatus. The principal components analysis (PCA) allowed the differentiation of the ten volatile compounds quantified in two groups: the former, described mainly by PC1, constituted by acetic acid, ethyl acetate, diethyl acetal and acetaldehyde, and the second, described by PC2, constituted by 1-propanol, 2-methyl-1-propanol, the two coeluting isomers 2-methyl-1-butanol and 3-methyl-1-butanol, 1-hexanol, 2-phenylethanol and 2,3-butanediol. Data obtained may be useful in order to establish a regulation for the production of high-quality traditional *Filuferru* from Sardinia.

Keywords: *Filuferru*; spirit; distillation; grape marc; volatile compounds; copper; trace elements; GC-MS; ICP-MS; PCA.

1. Introduction

Traditional *Filuferru* is an ancient, high alcoholic strength beverage from Sardinia, Italy. For centuries it is well-known and consumed by indigenous people not only as a beverage, but also as an ingredient in their traditional cuisine. Its artisanal manufacture took place in clandestine distilleries placed in domestic cellars or in the immediate neighbors of the vineyards, where usually wine (in the western side of Sardinia) or fermented grape marc (in the central or eastern side of the Isle) were distilled. Since unauthorized distillation was prohibited by law, the distillers buried the bottles of the product in order to occult them from inspections made by policemen, and only a wire (i.e. *filuferru* in the Sardinian language) tied at the neck of the bottle and emerging from the soil level for few centimeters indicated the point where the valuable spirit was left. Craft distillation of *Filuferru* is still today accomplished by using an internally tinned copper pot still. Figure 1 represents a typical apparatus for traditional batch distillation.

Figure 1. A typical pot still used for batch distillation of spirits.



1, Tinned copper pot; 2, still head; 3, swan's neck; 4, copper coil; 5, water cooling tank.

Heating of pot was accomplished by the combustion of wood or (preferably) liquid petroleum gas. Wine (or a mixture of fermented grape marc and water) is first fed in the pot and then heated, and great care was kept in order to avoid overheating of the charge. The temperature of the vapors measured on the still head is representative of their chemical composition, and this information is used by the distillers in order to separate the heart of the distillate from the head and the tail, both containing toxic and unpleasant substances like methanol or high-boiling organic compounds, respectively. The heart of the distillate was hence condensed, giving rise to the final product. Traditional *Filuferru* is usually a colorless spirit, whose aromas recalled those of the wines (or the grape marc) of origin. Variants to the pure distillate are those aged or flavored with typical spontaneous essences of Sardinia like strawberry tree (*Arbutus Unedo L.*) fruits, seeds of wild fennel or hot pepper. Unlike Italian grappa (i.e. the distilled spirit by grape marc technologically most close to *Filuferru*) [1-6], at the best of our knowledge, no scientific publications concerning the chemical characterization of the main and the trace composition of traditional *Filuferru* are until now published, so the principal aim of the present contribution is to perform the qualitative and the quantitative determination of both principal and trace constituents of this ancient beverage. Hence, on a reliable sampling of *Filuferru* spirits distilled by grape marc collected by a number of craft producers from the Central Sardinia, the alcoholic strength, the chromatographic qualitative profile, the quantitative determination of ten of the principal volatile compounds beyond ethanol, and the amount of 18 trace elements (i.e. Al, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sb, Sn, Sr, W and Zn) have been measured and discussed.

2. Materials and Methods

2.1. Samples

21 *Filuferru* samples produced from craft distillation of grape marc in eleven towns of central Sardinia (Figure 2) have been considered in this study.

Figure 2. Geographical localization of the eleven towns of Central Sardinia where craft *Filuferru* samples considered in this study were produced.



A further commercial sample of *Filuferru* (sample 22) has been taken into account, just for comparison sake. The geographical origin of craft samples of *Filuferru* is reported in Table 1. Beyond differences in the geographic origin of many samples, the choice of a small number of them was planned to put in evidence both analogies and differences ascribable to the nature of the grape marc or to the vintage effect. As a matter of fact, samples 18 and 19, from Orotelli, were produced by the same distiller using grape marc from the same vineyard, but coming from two consecutive vintages, whereas samples 20 and 21, from Oliena, were produced in the same year by the same distiller using grape marc from two different vineyards. All samples were stored in glass bottles hermetically closed kept in the dark at +4°C. Before each determination, samples were allowed to reach the room temperature.

Table 1. Geographical origin of craft samples of *Filuferru* (1-21) considered in the study

Geographical origin	<i>Filuferru</i> sample
Galtelli	1
Atzara	2
Orgosolo	3
Orotelli	4,5,7,10,12,18 and 19
Silanus	6 and 9
Sarule	8 and 14
Arzana	11
Villagrande Strisaili	13
Fonni	15 and 17
Mamoiada	16
Oliena	20 and 21

2.2. Chemicals and Reagents

Ultrapure (Type 1) water (specific resistance $\geq 18 \text{ M}\Omega$) was used throughout the analyses. All reagents were at least of analytical grade. Absolute ethanol was purchased from Carlo Erba (Milan, Italy). Chromatographic standards (acetaldehyde, ethyl acetate, acetal, 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 1-hexanol, acetic acid, 2,3-butanediol and 2-phenylethanol) were from Sigma Aldrich (Milan, Italy). The elemental standard solutions were by: Carlo Erba (Milan, Italy) for B, K, Mg and Na (1000 mg dm^{-3} in 2% aqueous HNO_3) and for Cu, Mn, Pb, Cr, Zn, Cd and Ni (100 mg dm^{-3} in 2% aqueous HNO_3); Fluka (Milan, Italy) for Al and Zn (1000 mg dm^{-3} in 2% aqueous HNO_3); Sigma Aldrich (Milan, Italy) for Ca, W and Sb (1000 mg dm^{-3} in 2% aqueous HNO_3) and VWR International (Milan, Italy) for Fe and Sr (1000 mg dm^{-3} in 2% aqueous HNO_3). The 67% aqueous solution of HNO_3 was an Ultrapure Normatom reagent (VWR International, Milan, Italy). The NexION ICP-MS tuning solution (2% HNO_3 solution in water containing $1 \mu\text{g dm}^{-3}$ each of Be, Ce, Fe, In, Li, Mg, Pb and U, code N8145051) and the NexION ICP-MS KED tuning solution (1% HCl solution in water containing Co, $10 \mu\text{g dm}^{-3}$ and Ce, $1 \mu\text{g dm}^{-3}$, code N8145052) were both purchased from Perkin Elmer Italia (Monza, Italy).

2.3. Instrumentation

Ultrapure water was obtained by a New Human Power II Scholar UV apparatus (Human Corporation, Seoul, Korea). GC-MS analysis was carried out using a gas-chromatograph model 7890, equipped with a VF-Wax $60 \text{ m} \times 0.25 \text{ mm i.d.}$, $0.5 \mu\text{m}$ film thickness column, and coupled with a model 7000C MSD detector, all from Agilent Italy (Cernusco S/N, Italy). A Gerstel MPS autosampler and sample preparation robot Gerstel Italy (Cernusco S/N, Italy) was also coupled to the GC unit, whereas data were analyzed using a MassHunter Workstation B.06.00 SP1 (Agilent Italy (Cernusco S/N, Italy) running under Windows 7 environment. Semiquantitative determination of methanol has been accomplished with a gas-chromatograph model 8310, equipped with a Carbowax $30 \text{ m} \times 1.0 \text{ mm i.d.}$, $1.0 \mu\text{m}$ film thickness column, and a LC-100 Laboratory Computing Integrator (Perkin Elmer, Monza, Italy). Elemental determinations were performed using an ICP-MS spectrometer model NexION 300X equipped with an autosampler model S10 (Perkin Elmer, Monza, Italy), running under the Windows 7 operating system.

2.4. Analytical methods

2.4.1. Alcoholic strength

Alcoholic strength was measured according the EC Commission Regulation 606/09 [7]. Each measurement has been performed twice (in physical duplicate). Repeatability was better than 2%, whereas average trueness, measured on six synthetic ethanol/water mixtures (i.e. 40, 45, 50, 55, 60 and 65% v/v) is always better than 3%.

2.4.2. GC-MS analysis

The chromatographic separation was accomplished using the following temperature program: 40°C hold for 4 min, then increased to 150°C at a rate of $5.0^\circ\text{C min}^{-1}$, held for 3 min, then increased to 240°C at a rate of $10^\circ\text{C min}^{-1}$, and finally held for 12 min. Helium was used as the carrier gas at a constant flow of $1 \text{ cm}^3 \text{ min}^{-1}$. The identification of the individual components was performed by comparison with the co-injected pure compounds and by matching the MS fragmentation patterns and retention indices with the built in libraries or literature data or commercial mass spectral libraries (NIST/EPA/NIH 2008; HP1607), both purchased from Agilent Technologies (Cernusco S/N, Italy). The retention indices were measured analyzing an hydrocarbon mixture of n-alkanes (between C9 and C22) under the same chromatographic conditions used for analyzing samples in order to calculate the retention indexes with the generalized equation by Van den Dool and Kartz [8]

$$I_x = 100[(t_x - t_n)/(t_{n+1} - t_n) + n]. \quad (1)$$

where t is the retention time, x is the analyte, n is the number of atoms of carbon of alkane that elutes before the analyte and $n+1$ is the number of atoms of carbon of the alkane that elutes after the analyte.

Quantification has been accomplished by means an external calibration on three levels of concentration. A stock solution of each standard was prepared by weighting it accurately into a 10 cm³ volumetric flask. The sample was first dissolved in ethanol/water mixture (70% v/v) and later the volume made up to the mark with the same mixture. The resulting stock solution was diluted with the same ethanol/water solution in order to obtain three reference solutions at increasing concentrations of all the external standards considered. For each compound the calibration curves were made by linear regression by plotting the peak area of external standard against their known concentrations. The mixture of isomers 2-methyl-1-butanol and 3-methyl-1-butanol, overlapped in the chromatograms, were quantified with a calibration curve of one isomer (i.e. the 3-methyl-1-butanol), and results are expressed in terms of 3-methyl-1-butanol equivalent. Ethyl acetate and diethyl acetal were quantified by a selected ion monitoring (SIM) method setting the quadrupole for filter the ions 88.0 and 103.0 for ethyl acetate and diethyl acetal, respectively. The MS detector has been disabled between RT of 10.3 and 13 minutes, in correspondence of the elution of ethanol, and this has caused the impossibility to evaluate the peak of methanol (RI = 907), very close to the ethanol's one (RI = 933). Each analysis was performed in triplicate, and results were expressed as mg dm⁻³.

2.4.3. GC-FID semiquantitative determination of methanol

The semiquantitative GC-FID determination of methanol in *Filuferru* samples has been performed using the relevant temperature program: 35 °C hold for 5 min, then increased to 70 °C at a rate of 3.0 °C min⁻¹, and later increased to 180 °C at a rate of 15 °C min⁻¹. Nitrogen was used as the carrier gas at a constant flow of 10 cm³ min⁻¹. The temperatures of the injector and of the detector were 230°C and 300°C, respectively, whereas the volume of solution injected was 0.2 mm³. Quantification of methanol has been accomplished by means an external calibration on three levels of concentration.

2.4.4. ICP-MS analysis

The optimized parameters used during the ICP-MS measurements of the elemental amounts of Al, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sb, Sn, Sr, W and Zn are: RF generator power output: 1600 W; argon flows: plasma, 17.993 dm³ min⁻¹; nebulizer; 0.991 dm³ min⁻¹, auxiliary 1.203 dm³ min⁻¹; helium flow: 3.50 cm³ min⁻¹; optimization on masses of ⁷Li, ²⁴Mg, ¹¹⁵In and ²³⁸U; data acquisition: dwell time of 50 ms, acquisition time of 3 s; time to change modes: 60 s; analytical masses: ²⁷Al, ¹¹B, ⁴³Ca, ¹¹¹Cd, ⁵²Cr, ⁶³Cu, ⁵⁷Fe, ³⁹K, ²⁴Mg, ⁵⁵Mn, ²³Na, ⁶⁰Ni, ²⁰⁸Pb, ¹²¹Sb, ¹¹⁸Sn, ⁸⁸Sr, ¹⁸⁴W and ⁶⁶Zn. Al, Cr, K and Mg were always measured using the He-collision mode, whereas the remaining elements were analyzed in the normal mode. Before accurate quantification, the tentative amounts of each analyte in all samples were evaluated by a TotalQuant approach. Quantification was always performed using external calibration with a 1 mg dm⁻³ Rh solution as an internal standard. The external calibration line was obtained on three different concentration levels for each analyte, ranging between the relevant LoQ value and the highest concentration evaluated in the semiquantitative analysis of all samples. The multielemental solutions have been prepared in physical triplicate for each concentration level, and each solution is taken into account in the assessment of the external calibration line. These solutions were prepared daily for proper dilution of intermediate stock solutions containing 10 mg dm⁻³ of each analyte in a 98:2 (v:v) water- ethanol solution. The detection limits were evaluated on the bases of measurements performed on the samples and the blank solution (a 98:2 (v:v) water- ethanol solution), respectively. In order to check the reliability of the analytical data, each five samples a multielemental standard was reprocessed, and data obtained between this and the previous standard measurement were discarded if the last data obtained exceeded – for each analyte – the interval of confidence of $\bar{x} \pm 3s$. Prior ICP-MS analysis, each *Filuferru* sample was 1:50 diluted with ultrapure water, hence these solutions were filtered off in a 0.45 µm pore size polypropylene filter. Each sample was analyzed three times, and each analytical datum is the average of three replicated ICP-MS measurements.

2.5. Multivariate analysis

Principal Component Analysis (PCA) were performed using a R-based software developed by the Group of Chemometrics of the Division of Analytical Chemistry of the Italian Chemical Society, freely downloadable from the site gruppochemiometria.it.

3. Results and Discussion

3.1. Validation

3.1.1. GC-MS method

Validation of the quantitative GC-MS procedure of analysis of the volatile compounds has been accomplished mainly in terms of the definition of the interval of linearity and the precision, measured in terms of the repeatability. Linearity was evaluated for each analyte within the real experimental concentration range, usually spanning for at least two (usually three) orders of magnitude of concentration as a function of the different analytes considered. Typical correlation coefficients R^2 ranged between 0.994 and 0.998. The CV of five consecutive measurements performed on the same sample in the same analytical session has been calculated. Each precision measurement has been accomplished, for each analyte, in the real sample showing the experimental concentration close to the average value. Typical precision values ranged between 2.6 and 6.4%. Only acetic acid provided higher uncertainty values than those (i.e. 30%). It is however interesting to note that, in a different *Filuferru* sample (i.e. sample 7, 1482 ± 7 mg dm⁻³) showing a concentration of this analyte not too far from the average value (1021 mg dm⁻³), the precision for the same analyte (measured on three replications) was less than 1%. Table 2 report the validation data for GC-MS quantitative measurements.

Table 2. Linearity and precision data for the GC-MS determination of volatile compounds in *Filuferru* samples

Compound	Linearity		Precision (n = 5)
	Range (mg dm ⁻³)	R^2	CV repeatability (sample)
Acetaldehyde	10 - 10000	0.997	3.0 (16)
Diethyl acetal	1 - 10000	0.994	5.3 (4)
Ethyl acetate	10 - 10000	0.995	30 (7)
1- propanol	10 - 1000	0.998	4.8 (13)
2-methyl-1-propanol	10 - 1000	0.997	6.4 (20)
2-methyl-1-butanol + 3-methyl-1-butanol ¹	50 - 5000	0.996	5.7 (4)
1-hexanol	1 - 1000	0.995	4.0 (9)
2-phenylethanol	1 - 250	0.996	2.6 (17)
2,3-butanediol ²	5 - 500	0.995	3.1 (18)
Acetic acid	10 - 10000	0.998	4.3 (16)

¹ both isomers coelute, ² expressed as the sum of all isomers identified

3.1.2. ICP-MS method

For each element considered, validation of the method has taken into account the limit of detection (LoD), limit of quantification (LoQ), the linearity, the precision (expressed on a real *Filuferru* sample as both repeatability and intermediate precision) and the bias (measured by recovery tests of each analyte on a real sample). LoD was calculated according to the Upper Limit Approach (ULA1) approved by IUPAC [9], whereas LoQ values have been established as 3.3 times the LoD value. Linearity was usually checked on at least two orders of magnitude of concentration, spanning in an interval from the relevant LoQ to 900 µg dm⁻³ as a function of the different analytes considered. Typical correlation coefficients R^2 ranged between 0.992 and 0.999. Precision was evaluated in terms of both repeatability and intermediate precision, whereas the bias was measured by means of recovery tests. Precision and trueness measurements have been accomplished on *Filuferru* real samples (i.e. samples 13, 18 and 20), fortified for Cr measurements with known amounts of analyte. Precision was evaluated in terms of both repeatability and intermediate precision. The CV of five consecutive measurements performed a) on the same sample in the same analytical session, for repeatability

evaluation, and b) on the same sample on different analytical sessions across three weeks, for intermediate precision measurements, have been calculated. The acceptability of all precision figures was successfully checked according Horwitz's theory [10]. Trueness was measured in duplicate by means of recovery tests. Whereas Ca, Cr, Mg, Mn and Pb provided recoveries not statistically different from 100% (criteria: t-test, $p=0.95$), the bias in the recovery amounts substantiated by the remaining elements was largely within the limits posed by AOAC [11] for the concentration ranges considered. Table 3 reports the validation figures for the parameters above considered.

3.2. Alcoholic strength

Table 4 reports the alcoholic strength of the samples considered. The average ethanol amount measured in all the samples analyzed is $49.9\pm0.2\%$ (v/v), being the range of the craft products between 41.0 ± 0.2 and $62.4\pm0.4\%$ (v/v). The measurements obtained confirm the reputation of high-alcoholic spirit of craft *Filuferru*: 10 samples on 21 showed alcoholic strength higher than 50%, and one of them (i.e. the sample 13, from Villagrande Strisaili) was characterized by an ethanol amount even higher than 60% (v/v). It is interesting to note that samples 18 and 19, from the same producer, but related to the distillation of grape marc obtained by the same vineyard in two consecutive vintages, show only a low difference between the relevant alcoholic strength, whereas even smaller differences were measured by the alcoholic strength of *Filuferru* 20 and 21 (same producer, same vintage, different vineyards). Finally, the commercial sample 22, from an industrial distillery, shows one of the lowest alcoholic strengths (i.e. $41.8\pm0.6\%$) measured in this study (higher only of that of the sample 1, $41.0\pm0.2\%$).

3.3 Analysis of volatile organic compounds

3.3.1. Qualitative analysis

Many tens of volatile organic compounds coming from contributions of different production steps characterize the *Filuferru* aroma. Firstly, a meaningful contribution is due to compounds derived from the grape(s) variety: usually they are free compounds, easily transferred to the spirit during distillation. Another contribution originates from compounds, like alcohols, acetates and ethyl (or methyl) esters, formed during fermentation by metabolism of yeasts and bacteria. Finally, the last contribution is from compounds, like acetals, formed as a consequence of high temperature and alcohol concentration during the distillation process. Table 5 reports the qualitative attribution of GC-MS peaks obtained in the total ion current (TIC) chromatogram (reported in Figure 3) for the samples analyzed.

60 compounds were identified, belonging to seven different chemical classes. In particular, five aldehydes, four among ketones and hydroxyketones, six acetals, eight esters and diesters, thirty among alcohols, enols and polyols, six carboxylic acids and one linear alkene have been identified. Aldehydes and ketones are species normally formed during the alcoholic fermentation of grapes [12], and for this reason they are present in the grape marc. Five of the six aldehydes are almost ubiquitous in the samples, whereas the nonanal was identified only in one (i.e. the sample 5) of the seven samples distilled in Orotelli. Among the ketones group, the acetoin and the 1-hydroxy-2-propanone were identified in almost all the samples, whereas the 2-hydroxy-2-cyclopenten-1-one and the pyranone were contemporarily recognized only in samples 1,5,7,21 and the commercial sample 22 (i.e. less than 25% of the total of the samples analyzed). Unlike aldehydes and ketones, acetals are a class of volatile compounds usually not present in the grape marc, but the reaction of acetalization occurs significantly during distillation due the concomitant effect of higher temperatures and concentration ethanol [13]. For these reasons the acetaldehyde diethyl acetal (commonly termed "acetal") is always present in *Filuferru* samples. Only slightly less frequent is the qualitative identification in the samples of the acetaldehyde ethyl methyl acetal, of the hexanal diethyl acetal and of the isovaleraldehyde diethyl acetal, all recognized in more than 70% of the samples.

Table 3. Validation data for the ICP-MS determination of elements in *Filuferru* samples

Element	Sensitivity (n = 3)		Linearity		Precision (n = 5)		Bias (n = 2)
	LoD ($\mu\text{g dm}^{-3}$)	LoQ ($\mu\text{g dm}^{-3}$)	Concentration range ($\mu\text{g dm}^{-3}$)	R ²	Repeatability (CV)	Intermediate precision (CV)	Recovery (%±SD)
Al ^a	0.08	0.26	0.26 - 26	0.999	4.2 ^b	3.2 ^b	107±6 ^b
B	0.004	0.013	0.013 – 1.3	0.998	2.1 ^b	4.0 ^b	112±4 ^b
Ca	0.60	2.0	2 - 200	0.997	0.4 ^c	0.8 ^c	98±4 ^c
Cd	0.002	0.006	0.006 – 6	0.999	10 ^c	15 ^c	110±1 ^c
Cr ^a	0.04	0.14	0.14 - 14	0.992	15 ^d	15 ^d	102±7 ^d
Cu	0.05	0.16	0.16 - 300	0.998	2.9 ^b	8.2 ^b	90±5 ^b
Fe	0.13	0.44	0.44 - 44	0.996	0.3 ^e	0.7 ^e	93±4 ^e
K ^a	0.002	0.007	0.007 - 900	0.997	0.7 ^c	0.2 ^c	92±3 ^c
Mg ^a	0.021	0.069	0.07 - 70	0.994	1.8 ^b	2.4 ^b	98±4 ^b
Mn	0.012	0.040	0.04 - 4	0.998	3.2 ^b	2.5 ^b	100±4 ^b
Na	0.16	0.53	0.6 - 300	0.997	5.3 ^b	9.0 ^b	90±3 ^b
Ni	0.019	0.065	0.065- 6.5	0.997	30 ^e	35 ^e	95±3 ^e
Pb	0.002	0.008	0.008 - 80	0.996	0.4 ^b	21 ^b	98±1 ^b
Sb	0.005	0.018	0.018 – 1.8	0.996	33 ^b	30 ^b	111±3 ^b
Sn	0.056	0.18	0.18 - 18	0.997	13 ^b	17 ^b	110±2 ^b
Sr	0.004	0.013	0.013 – 1.3	0.998	6.7 ^b	12 ^b	96.8±0.3 ^b
W	0.008	0.025	0.025 – 2.5	0.998	13 ^b	20 ^b	86±4 ^b
Zn	0.019	0.062	0.062 - 62	0.998	7.0 ^c	2.2 ^c	93±6 ^b

Prior to ICP-MS determination all *Filuferru* samples were 1:50 diluted with ultrapure water ^a elements measured in He-collision mode; measured on sample 20; ^c measured on sample 18; ^d CV measured after spiking *Filuferru* sample of a known amount of analyte up to reaching the lowest limit of the relevant interval of calibration; ^e measured on sample 13.

Table 4. Alcoholic strength (n = 2) and GC-MS quantification (n = 3) of the principal volatile compounds in *Filuferru* samples

Sample	Alcoholic strength	Acetaldehyde	Acetaldehyde diethyl acetal	Ethyl acetate	1-propanol	2-methyl-1-propanol	3-methyl-1-butanol ^a	1-hexanol	2-phenylethanol	2,3-butanediol ^a	Acetic acid
1	41.0±0.2	900±100	250±10	410±20	275±20	160±50	950±60	32±2	31±0	80±40	485±40
2	50.2±0.2	1710±90	890±30	900±30	112±7	330±20	1546±4	38±1	58±1	65±35	555±15
3	58.2±0.2	52±0	5±0	14±5	55±8	220±20	390±60	21±2	20±2	35±9	110±10
4	46.8±0.0	3650±150	1530±80	1230±40	175±0	530±10	1220±70	38±1	27±0	40±1	450±50
5	54.0±0.0	8000±1000	3900±900	4000±100	370±60	430±20	1950±300	70±10	50±8	100±100	2600±300
6	51.2±0.0	150±10	51±7	150±20	43±3	380±20	350±30	11±0	11±0	26±1	134±7
7	52.2±0.2	2900±500	1400±400	2550±800	350±80	220±40	1310±20	115±10	27±0	150±100	1482±7
8	46.2±0.2	1600±400	700±100	1100±200	160±30	340±30	1600±300	28±5	70±20	110±40	700±200
9	47.6±0.4	2230±70	670±20	890±20	350±30	450±30	1740±90	75±3	66±5	200±100	1000±200
10	47.6±0.4	1500±80	610±80	530±70	170±50	410±40	800±200	70±10	28±0	50±20	360±50
11	52.2±0.2	2300±100	980±90	1500±100	350±40	300±100	1500±150	39±5	31±3	50±20	1140±50
12	49.0±0.2	1800±500	650±100	700±100	160±20	240±30	650±100	23±3	24±5	80±30	400±100
13	62.4±0.4	6300±200	630±500	1400±100	210±10	220±20	1200±300	42±4	29±5	220±10	540±60
14	50.0±0.0	1000±300	500±100	1050±200	190±40	400±40	1650±350	26±5	42±9	55±20	500±200
15	46.2±0.2	1050±100	340±10	1520±40	136±8	358±7	1640±80	54±3	50±10	90±10	1200±200
16	46.6±0.2	490±6	182±9	290±10	145±20	400±60	2100±100	27±2	71±6	60±10	180±20
17	45.8±1.0	510±20	124±7	1170±60	215±20	200±100	900±1200	50±4	38±1	113±8	1180±50
18	59.0±0.2	1970±60	1900±300	1700±300	180±20	400±100	1500±200	50±5	65±4	99±3	885±30
19	55.2±0.0	1500±300	800±80	470±40	210±30	170±50	670±90	54±7	27±8	85±30	400±100
20	49.2±0.4	1210±70	3000±300	9000±1000	400±20	310±20	1010±70	7±0	35±2	80±9	2530±60
21	46.2±0.2	190±7	42±1	122±2	187±4	160±50	600±20	590±60	80±20	250±50	5300±800
22 ^c	41.8±0.6	240±20	55±3	57±4	260±60	230±60	400±100	57±1	15±9	255±9	310±20
Average ^d	49.9±0.2	1875	873	1398	214	312	1167	69	41	104	1020
Range	41.0÷62.4	52 - 8000	5 - 6300	14 - 9000	43 - 400	160 - 530	350 - 2100	7 - 590	11 - 80	26 - 250	110

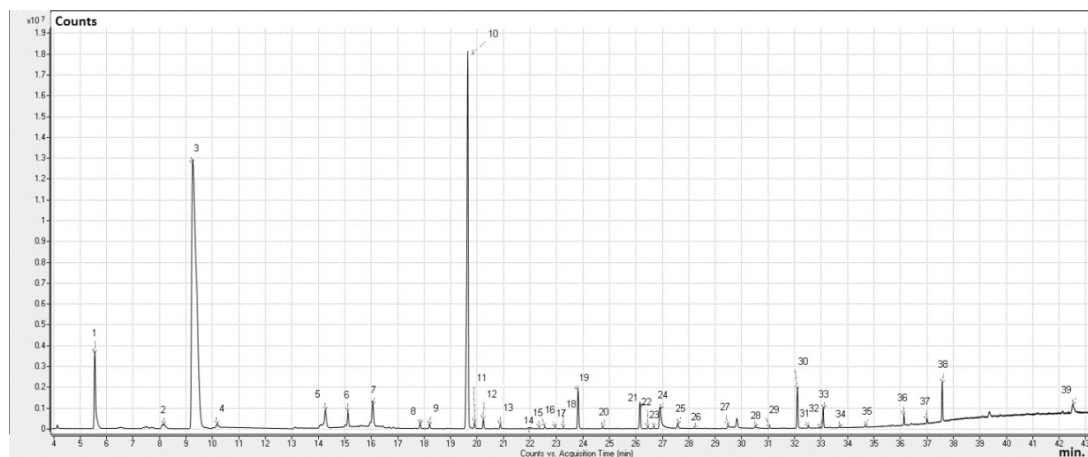
Beyond alcoholic strength, all concentrations obtained by GC-MS analysis are expressed in mg dm⁻³±SD (standard deviation); ^a sum of both 2-methyl-1-butanol and 3-methyl-1-butanol isomers, and quantified in terms of 3-methyl-1-butanol equivalent concentration; ^b sum of both isomers; ^c commercial *Filuferru* sample, here considered only for comparison purposes; ^d average values calculated before rounding made for harmonizing the number of significant digits of each analytical data according the relevant uncertainty.

Classes/Compounds	Sample																						RI
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22 ^a	
<u>Aldehydes</u>																							
Acetaldehyde	x	x	-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	710
3-methylbutanal	x	x	-	x	-	x	x	x	x	x	x	x	-	-	x	x	-	-	x	x	-	x	930
Nonanal	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1409
Furfural	x	x	x	-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	1496
Benzaldehyde	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	-	x	-	x	x	x	1567
<u>Ketones and hydroxyketones</u>																							
Acetoin	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	-	1333
1-hydroxy-2-propanone	x	-	x	x	x	x	x	-	x	x	x	-	-	x	x	x	x	-	x	x	x	x	1343
2-hydroxy-2-cyclopenten-1-one	x	-	-	-	x	-	x	-	x	-	-	-	-	-	-	-	-	-	-	-	x	x	1808
Pyranone	x	-	-	-	x	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	2339
<u>Acetals</u>																							
Acetaldehyde, ethyl methyl acetal	x	x	-	x	x	-	x	x	x	x	x	x	-	-	x	x	-	x	x	-	x	x	854
Acetaldehyde, diethyl acetal	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	907
Isovaleraldehyde, diethyl acetal	x	x	-	x	x	x	x	x	x	x	x	x	x	-	x	x	-	x	x	x	-	x	1085
Acetaldehyde, ethyl amyl acetal	-	-	-	x	x	-	-	-	-	-	x	-	-	-	-	-	-	-	-	x	-	-	1102
Hexanal, diethyl acetal	x	x	-	x	x	x	x	x	x	x	x	x	x	-	x	x	-	-	x	x	-	-	1220
1,1-diethoxy-2-propanone	-	-	-	x	x	-	-	-	x	-	-	-	x	-	-	-	-	x	x	-	-	-	1298
<u>Esters</u>																							
Ethyl acetate	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	901
Ethyl butanoate	-	x	-	-	-	-	-	-	-	-	-	-	-	x	x	x	-	x	-	-	-	x	1055
3-methyl-1-butanol acetate	-	-	-	x	x	x	x	-	x	x	x	x	-	x	x	x	x	-	-	x	-	x	1131
Ethyl hexanoate	-	-	-	-	x	-	x	-	-	-	-	-	x	-	-	-	-	x	x	x	-	x	1238
Ethyl lactate	-	x	x	x	-	x	x	x	x	x	-	x	-	x	-	x	x	-	-	x	x	-	1370
Ethyl octanoate	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	-	1438
Ethyl decanoate	x	x	-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x	1649
Diethyl butanedioate	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x	x	-	1693
<u>Alcohols, enols and polyols</u>																							
Methanol ^b	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
2-butanol												x						x			x		1042
1-propanol	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	1062
2-methyl-1-propanol	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	1117
1-butanol	x	x	-	x	x	x	x	-	-	x	x	-	x	x	x	x	-	x	x	x	-	x	1174
Pent-1-en-3-ol	-	x	-	x	x	x	x	x	-	-	x	x	x	-	x	-	-	x	x	x	-	x	1184
2-methyl-1-butanol + 3-methyl – 1-butanol	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	1234
1-pentanol	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x	x	x	-	1272
3-methylbut-3-en-1-ol	-	x	-	-	-	-	-	-	-	-	x	-	-	x	x	x	-	-	-	-	-	-	1277
2-ethyl-1-butanol	-	-	-	-	-	-	x	-	-	-	x	-	-	-	-	-	-	-	x	-	-	-	1323
(Z)-pent-2-en-1-ol	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x	-	x	x	x	x	-	1339
1-hexanol	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x	1366
(Z)-hex-3-en-1-ol	-	-	-	-	-	x	x	-	-	x	x	-	-	-	-	-	-	-	-	-	-	-	1379
3-ethoxy-1-propanol	x	-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	1401

Classes/Compounds	Sample																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22 ^a	RI
<u>Alcohols, enols and polyols</u>																							
(Z)-hex-2-en-1-ol	-	-	-	-	-	-	-	-	-	-	x	-	x	-	-	x	-	-	-	x	-	-	1419
Oct-1-en-3-ol	-	x	x	-	x	-	x	x	x	-	x	x	x	-	x	-	-	x	x	x	-	-	1455
1-heptanol	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	-	-	1463
Hept-4-en-1-ol	-	x	x	x	x	-	x	-	x	-	-	x	-	-	x	-	-	x	x	-	-	-	1516
2,3-butanediol isomer I	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	-	1559
2,3-butanediol isomer II	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	1591
Propylene glycol	-	-	x	-	-	-	x	x	x	x	-	x	-	-	-	-	-	-	x	-	x	-	1610
1-nonanol	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x	x	x	x	x	-	-	1670
2-furanmethanol	x	-	x	x	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1682
α-terpineol	x	-	-	-	-	x	x	-	-	x	x	-	-	-	-	x	x	-	x	-	-	-	1723
5-methyl-2-furanmethanol	x	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1743
1-decanol	x	-	-	x	-	x	x	-	x	x	x	x	x	-	x	x	x	x	x	x	-	-	1771
Citronellol	-	-	-	-	-	x	-	-	-	x	x	-	-	x	-	x	x	-	-	-	-	-	1777
Phenylmethanol	x	x	x	x	-	-	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	-	1914
2-phenylethanol	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	1954
<u>Carboxylic acids</u>																							
Acetic acid	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	1473
2-methylpropanoic acid	-	x	x	x	-	x	-	x	x	x	x	-	-	x	x	x	-	-	-	-	-	-	1588
3-methylbutanoic acid	-	x	x	-	-	x	-	-	-	x	x	x	-	x	x	x	-	x	x	-	x	x	1690
Hexanoic acid	x	-	x	x	x	x	-	x	x	x	x	-	-	x	x	-	x	-	x	-	-	x	1865
Octanoic acid	x	-	-	x	x	-	x	-	-	-	x	-	-	-	-	-	x	-	-	-	x	x	2075
Decanoic acid	x	-	-	-	x	-	x	-	-	-	-	-	-	-	-	-	x	x	x	x	-	x	2287
<u>Hydrocarbons</u>																							
2,4-dimethyl-1-heptene	-	x	-	-	-	-	x	-	-	x	x	-	-	-	-	x	-	x	-	-	x		883

Compounds (x = qualitatively present, - = qualitatively not detectable) are listed for classes according to the increasing retention time in VF-WAX column. RI: retention index; a sample 22 is a commercial Filuferru sample, here considered only for comparison purposes; b the qualitative presence of methanol has been ascertained by means of a GC-FID method.

Figure 3. Typical GC-MS chromatogram of a *Filuferru* distillate by grape marc from Sardinia, Italy (sample 19, from Orotelli).



Peak attribution: 1, Acetaldehyde; 2, Acetaldehyde, ethyl methyl acetal; 3, Ethyl acetate and acetaldehyde, diethyl acetal (not resolved); 4, 3-methyl-butanal; 5, 1-propanol; 6, Isovaleraldehyde, diethyl acetal; 7, 2-methyl-1-propanol; 8,

1-butanol; **9**, Pent-1-en-3-ol; **10**, Hexanal, diethyl acetal; **11**, 2-methyl-1-butanol and 3-methyl-1-butanol (not resolved); **12**, Ethyl hexanoate; **13**, 1-pentanol; **14**, 1,1-diethoxy-2-propanone; **15**, 2-ethyl-1-butanol; **16**, acetoin; **17**, 2-(Z)-pent-2-en-1-ol; **18**, 1-hydroxy-2-propanone; **19**, 1-hexanol; **20**, 3-ethoxy-1-propanol; **21**, Ethyl octanoate; **22**, Oct-1-en-3-ol; **23**, 1-heptanol; **24**, Acetic acid; **25**, Furfural; **26**, Hept-4-en-1-ol; **27**, 2,3-butanediol (isomer I); **28**, 2,3-butanediol (isomer II); **29**, Propylene glycol; **30**, Ethyl decanoate; **31**, 1-nonanol; **32**, 3-methylbutanoic acid; **33**, Diethyl butanedioate; **34**, α -terpineol; **35**, 1-decanol; **36**, hexanoic acid; **37**, Phenylmethanol; **38**, 2-phenylethanol; **39**, n-decanoic acid.

Whereas the presence of acetals is quite common also in grappa spirit, the presence of isovaleraldehyde diethyl acetal is not. This compound, recently observed in such matrix for the first time by Masino and coworkers [14], seems to be not so uncommon in the *Filuferru* composition. More rare is the identification of the acetaldehyde ethyl amyl acetal and of the 1,1-diethoxy-2-propanone, both present in not more than one third of the samples. Among the different classes of volatile compounds, the esters are one of the most representative compounds in concurring to the definition of the aroma of the grape marc [15]. Among the lighter esters (up to five atoms of carbon), the ethyl acetate is always present, often as a principal constituent, in all *Filuferru* samples, whereas the ethyl octanoate, the ethyl decanoate and the diethyl butanedioate are the esters at medium molecular weight (up to twelve atoms of carbon) identified in the almost totality of the samples. Also the ethyl lactate (present in the grape marc due the malolactic fermentation) and the 3-methyl-1-butanol acetate have been frequently identified in more than the half of the samples, whereas more rare is the qualitative recognition of the ethyl butanoate and the ethyl hexanoate, present only in not more than one third of the samples. It is not possible to ascertain in any sample of *Filuferru* the qualitative presence of esters of fatty acid, well-known to provide an unpleasant aroma to distilled spirits [15], whereas the presence of esters of low- and medium-molecular weight is responsible to attribute to *Filuferru* a fruity smell (light and undefined for ethyl acetate, more pronounced and resembling the aroma of fruits like apple, pear, banana or pineapple for the medium molecular weight esters). The presence of ethyl esters (from ethyl acetate to ethyl decanoate) depends on fatty acid synthesis mediated by yeasts during the alcoholic fermentation [16].

As expected, alcohols are the class of compounds most represented in the qualitative analysis of volatile compounds in *Filuferru*. Obviously beyond ethanol, practically all the categories of alcohols from three to ten carbon atoms are represented in its volatile profile. Primary and secondary alcohols, with linear or branched carbon chain containing (or not) insaturations, are largely present in the composition of the beverage. Among others, 1-propanol, 2-methyl-1-propanol, the 2-phenylethanol and the couple of isomers 2-methyl-1-butanol + 3-methyl-1-butanol (not completely resolved in the chromatographic separation) are always present in each sample examined. In addition, primary alcohols from C4 to C10, with the sole exception of 1-heptanol, are almost always present in more than the 90% of the volatile profile of *Filuferru*. Also enols like pent-1-en-3-ol, (Z)-pent-2-en-1-ol and oct-1-en-3-ol have been identified in more than 50% of samples. Also present, albeit with a minor frequency, the unsubstituted and the 5-methyl substituted 2-furanmethanol, monoterpenoids from the grape like citronellol and α -terpineol, and polyols as propylene glycol. Quite surprisingly, the 2-butanol has been retrieved only in very few *Filuferru* samples (i.e. the samples 11, 17 20 and the commercial sample 22). This is unexpected, because it is well known in the literature that this alcohol concurs, together with the 1-propanol, the 3-methyl-1-butanol and the 2-methyl-1-butanol (all present in meaningful amounts in the *Filuferru* spirit) to confer favorable key notes to distilled spirits [15]. In addition, 2-butanol is described in literature as a discriminant compound among wine and grape marc distillates, being basically absent in wine distillates, and constantly present in grape marc distillates [17]. Finally, acetic acid is obviously the most represented compound belonging to the group of the carboxylic acids. The recognition of the 3-methylbutanoic acid has been frequent (in more than the half of the samples) whereas the qualitative detection of the 2-methylpropanoic acid, of the octanoic acid and of the decanoic acid (all present between the 35 and the 50% of the samples analyzed) is less frequent. The GC-MS method previously described was unable to detect the presence of methanol hence, in order to overcome this drawback, a semiquantitative GC-FID method has allowed to confirm the presence of the methanol in all the samples considered.

3.3.2. Quantitative analysis

Quantification has been accomplished on the most abundant and on the most frequently retrieved species identified in the previous qualitative step. Hence, table 4 reports the concentrations of acetaldehyde, of diethyl acetal, of ethyl acetate, of acetic acid and of seven alcohols (i.e. 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 2-phenylethanol and two isomers of the 2,3-butandiol). Very high concentrations of the lightest volatile compounds like acetaldehyde, diethyl acetal and ethyl acetate (average concentration of 1875, 873 and 1398 mg dm⁻³) are present in *Filuferru* spirits, whereas the total average alcoholic concentration (beyond ethanol) is 1907 mg dm⁻³. Finally, the average concentration of acetic acid is 1020 mg dm⁻³. For each analyte, the range spans over two (i.e. acetaldehyde, 1-hexanol, ethyl acetate and acetic acid) but also three (i.e. diethyl acetate) orders of magnitude of concentration, reaching also amounts higher than 9 g dm⁻³, as measured for ethyl acetate in sample 20. In the absence of any previous quantitative characterization of the volatile profile of the *Filuferru*, we tried a comparison between the data obtained in this study and the literature data from the same characterization made for the Italian Grappa, i.e. the distilled spirit that – for many aspects – is more close to *Filuferru*. Data reported in literature for the volatile composition of organic compounds in grappa are very often expressed in mass units on a volume of pure ethanol. Hence, for the sake of clarity and in order to facilitate the comparison of these results with the previous data, Table 6 reports the data contained in Table 4 converted in mg dm⁻³ of pure ethanol.

By the comparison with literature data for Grappa [14,18-22] it is evident that the craft *Filuferru* contains higher amounts of all compounds quantified in this study. Extending the comparison between the volatile composition of *Filuferru* and Grappa to similar European distillates from grape marc like the Portuguese *Bagaceira* and the Spanish *Orujo* [18], the average amount of acetaldehyde found in craft *Filuferru* is really impressive (3640 mg dm⁻³ of absolute ethanol), exceeding several times the maximum amount allowed in these Countries for the relevant spirits (i.e. 1000 mg dm⁻³ of absolute ethanol). Also the average concentration of ethyl acetate found in *Filuferru* (2767 mg dm⁻³ of absolute ethanol) exceeded the limit of 2500 mg dm⁻³ of absolute ethanol posed for this analyte. Before to prematurely draw any conclusion on the quality of the product, it is of the utmost importance to keep in mind that these values have been almost entirely measured on craft productions, accomplished in a traditional way and in the absence of any regulation of production. As a matter of fact, the amount of acetaldehyde and ethyl acetate in the commercial sample 22 (i.e. 570 mg dm⁻³ of absolute ethanol for acetaldehyde and 140 mg dm⁻³ of absolute ethanol for ethyl acetate) is well below not only the values measured for the craft productions (with the exception of sample 3 for both analytes, and sample 6 for the acetaldehyde), but also the limits shared by Italy, Spain and Portugal for these compounds.

However, the high difference measured for both analytes among all craft *Filuferru* samples (two orders of magnitude) suggests a key influence of the technology of production (e.g., a wrong cut off of the heads in the distillation process as well as an incorrect fermentation process of the grape marc before distillation) on the concentration of acetaldehyde and ethyl acetate in the distillate. Furthermore, whereas high amounts of ethyl acetate in *Filuferru* can affect only sensorial properties, conferring to the beverage an intense and unpleasant herbaceous smell [15], a recent study [23] envisages potential health risk related to the consumption of alcoholic beverages containing high concentrations of acetaldehyde. Finally, the results from GC-FID semiquantitative determination of methanol of all samples of *Filuferru* show that this analyte is present only as a minor constituent of this distillate, being its concentration range between 30 and 150 mg dm⁻³ (the upper limit for Italian, Portuguese and Spanish distillates is fixed in 10000 mg dm⁻³ of absolute alcohol).

3.4. Elemental composition

Table 7 reports the total concentration of 18 elements (i.e. Al, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sb, Sn, Sr, W and Zn) in 22 samples of *Filuferru* distilled spirit. Six elements of ascertained toxicity for humans, six oligoelements and six elements generally recognized as not toxic have been chosen for this characterization. Data reported give account for a wide variability of concentration measured for almost all analytes. Whereas the Cr concentration has always below the LoD for all samples analyzed, quantification for B has been possible – at levels of few parts for billion – only for samples 20 and 21, i.e. two *Filuferru* samples produced by the same distiller in the same vintage by grape marc from two different vineyards.

Table 6. Amounts of the principal volatile compounds in *Filuferru* samples (data expressed as mg dm⁻³ of absolute ethanol)

Sample	Acetaldehyde	Acetaldehyde diethyl acetal	Ethyl acetate	1-propanol	2-methyl-1-propanol	3-methyl-1-butanol ^a	1-hexanol	2-phenylethanol	2,3-butanediol ^b	Acetic acid
1	2200	610	1000	670	400	2300	79	76	190	1200
2	3400	1780	1800	220	660	3080	76	116	130	1100
3	89	9	20	95	380	700	36	34	60	190
4	7800	3300	2600	380	1130	2600	81	58	85	1000
5	15000	7000	7400	700	800	3600	130	90	200	4800
6	300	100	300	85	740	690	21	21	51	26
7	5500	2700	4900	700	420	2500	220	52	300	2900
8	3500	1500	2400	350	735	3400	60	150	240	1500
9	4700	1410	1900	730	945	3650	158	140	400	2100
10	3100	1300	1110	360	860	1700	140	59	100	800
11	4400	1900	2900	670	600	2800	76	60	100	2200
12	4000	1300	1400	330	490	1300	47	50	160	800
13	10100	1010	2200	340	350	1900	67	46	350	900
14	2000	1000	2100	380	800	3300	51	80	110	1000
15	2300	730	3300	300	775	3600	120	120	190	2600
16	1050	390	600	310	900	4500	58	150	130	390
17	1120	270	2600	470	400	2000	110	82	250	2600
18	3340	3200	2900	310	700	2550	85	111	168	1500
19	2700	1450	850	380	310	1200	98	50	150	700
20	2500	6100	18200	800	630	2050	14	71	160	5100
21	410	90	260	400	350	1300	1300	170	540	11000
22 ^c	570	130	140	600	550	960	136	40	600	740
Average ^d	3640	1694	2767	435	633	2349	144	83	212	2052
Range	89 - 15000	9 - 7000	20 - 18200	85 - 800	310 - 1130	690 - 4500	14 - 1300	21 - 170	51 - 600	26 -

^a sum of both 2-methyl-1-butanol and 3-methyl-1-butanol isomers, and quantified in terms of 3-methyl-1-butanol equivalent concentration; ^b sum of both isomers; ^c commercial *Filuferru* sample, here considered only for comparison purposes; ^d average values calculated before rounding made for harmonizing the number of significant digits of each analytical data according the relevant uncertainty.

Table 7 – Selected elemental abundances in *Filuferru* samples from Sardinia, Italy

Sample	Al	B	Ca	Cd	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Pb	Sb	Sn	Sr	W	Zn
1	<13 ^c	<0.20 ^d	<30 ^d	<0.32 ^c	<2.0 ^d	1483±1	<6.7 ^d	<0.11 ^d	<1.0 ^d	8.2±0.3	771±3	37.5±0.7	18.5±0.7	3.1±0.1	172±2	13±3	68±6	148±1
2	<4.0 ^d	<0.20 ^d	<30 ^d	<0.32 ^c	<2.0 ^d	1096±4	<6.7 ^d	108±6	<1.0 ^d	<2.0 ^c	298±4	6.1±0.1	11.2±0.2	1.4±0.2	14.5±0.7	6.2±0.2	49.5±0.7	92.9±0.1
3	94.5±0.5	<0.20 ^d	251±1	<0.32 ^c	<2.0 ^d	204±6	299±1	578±4	80±1	<2.0 ^c	390±20	3.5±0.7	1313±6	<0.27 ^d	<2.8 ^d	10.5±0.7	<0.38 ^d	43±3
4	19±1	<0.20 ^d	118±2	<0.32 ^c	<2.0 ^d	3996±6	<6.7 ^d	760±20	<1.0 ^d	3.4±0.5	1720±15	4.5±0.4	183±1	<0.27 ^d	12±6	6.05±0.07	23±3	410±1
5	61±1	<0.20 ^d	<30 ^d	<0.32 ^c	<2.0 ^d	77±4	<6.7 ^d	<0.11 ^d	<1.0 ^d	2.2±0.3	580±30	<0.98 ^d	978±2	<0.90 ^c	25±10	4.5±0.7	17.5±0.7	<0.94 ^d
6	<13 ^c	<0.20 ^d	125.5±0.7	<0.32 ^c	<2.0 ^d	1450±100	<6.7 ^d	2050±50	<1.0 ^d	3.05±0.07	1850±150	<3.2 ^c	24±2	<0.27 ^d	<9.2 ^c	4.6±0.6	29±1	25±6
7	98.3±0.9	<0.20 ^d	770±50	0.4±0.1	<2.0 ^d	113±1	<6.7 ^d	<0.11 ^d	145.5±0.5	50.5±0.7	710±10	<0.98 ^d	171±8	<0.27 ^d	16.5±0.7	5.1±0.1	13±1	<0.94 ^d
8	<13 ^c	<0.20 ^d	<30 ^d	<0.32 ^c	<2.0 ^d	168.5±0.7	<6.7 ^d	<0.11 ^d	<1.0 ^d	2.2±0.2	1140±40	<0.98 ^d	34±2	<0.27 ^d	<2.8 ^d	4.4±0.5	7.2±0.3	<0.94 ^d
9	45±1	<0.20 ^d	204±1	<0.32 ^c	<2.0 ^d	678±7	<6.7 ^d	3000±60	<1.0 ^d	6.1±0.1	940±50	5.4±0.6	8.5±0.7	<0.27 ^d	<9.2 ^c	2.8±0.3	5.5±0.7	688±7
10	<13 ^c	<0.20 ^d	<30 ^d	<0.32 ^c	<2.0 ^d	4000±50	<6.7 ^d	<0.11 ^d	<1.0 ^d	<0.6 ^d	164±1	<3.2 ^c	19±4	<0.27 ^d	<9.2 ^c	2.5±0.7	<0.38 ^d	56±4
11	24.05±0.07	<0.20 ^d	1510±30	<0.32 ^c	<2.0 ^d	780±40	<6.7 ^d	630±10	214.6±0.7	<2.0 ^c	1700±600	4.6±0.9	128.5±0.7	<0.27 ^d	<2.8 ^d	4.5±0.7	<0.38 ^d	144±8
12	15.2±0.3	<0.20 ^d	<98 ^c	4.2±0.2	<2.0 ^d	259±35	<6.7 ^d	<0.11 ^d	<1.0 ^d	2.4±0.2	860±200	<0.98 ^d	15±1	<0.27 ^d	<2.8 ^d	5.5±0.8	<0.38 ^d	48±1
13	<13 ^c	<0.20 ^d	707±3	<0.32 ^c	<2.0 ^d	180±1	84±1	250±20	386±4	<2.0 ^c	2420±60	4±1	54±3	<0.27 ^d	<9.2 ^c	4.2±0.2	<0.38 ^d	112±4
14	<13 ^c	<0.20 ^d	<30 ^d	0.7±0.1	<2.0 ^d	1730±25	<6.7 ^d	21.5±0.7	<1.0 ^d	<2.0 ^c	650±50	45.3±0.4	241±2	<0.90 ^c	<2.8 ^d	2.4±0.6	<0.38 ^d	930±10
15	<4.0 ^d	<0.20 ^d	<30 ^d	1.1±0.2	<2.0 ^d	14700±10	<6.7 ^d	160±4	<1.0 ^d	3.5±0.7	420±7	4.4±0.6	18.5±0.7	<0.27 ^d	<2.8 ^d	2.2±0.4	8.6±0.6	<0.94 ^d
16	<13 ^c	<0.20 ^d	<30 ^d	<0.32 ^c	<2.0 ^d	86±4	<6.7 ^d	6330±20	<1.0 ^d	<2.0 ^c	1596±6	<0.98 ^d	1.2±0.2	<0.90 ^c	<2.8 ^d	1.95±0.07	11±3	<0.94 ^d
17	13.5±0.2	<0.20 ^d	<30 ^d	<0.32 ^c	<2.0 ^d	170±7	<6.7 ^d	6550±10	<1.0 ^d	10.5±0.9	804.6±0.5	<0.98 ^d	6.9±0.1	<0.27 ^d	<9.2 ^c	1.8±0.2	3.8±0.2	<0.94 ^d
18	24.9±0.1	<0.20 ^d	215.4±0.1	0.5±0.3	<2.0 ^d	210±10	<6.7 ^d	1389±8	<1.0 ^d	<2.0 ^c	970±30	<3.2 ^c	54±2	2.2±0.3	14±1	3.8±0.4	<0.38 ^d	37±1
19	50±2	<0.20 ^d	102±1	<0.32 ^c	<2.0 ^d	860±20	<6.7 ^d	387±4	<1.0 ^d	2.1±0.2	750±10	<3.2 ^c	191.5±0.7	<0.27 ^d	<2.8 ^d	2.7±0.4	<0.38 ^d	<0.94 ^d
20	44±4	1.94±0.08	<30 ^d	<0.32 ^c	<2.0 ^d	3480±20	<6.7 ^d	40900±200	2800±200	6.8±0.4	2325±1	<3.2 ^c	16.5±0.6	18.9±0.2	10.5±0.3	1.5±0.7	1.3±0.3	<0.94 ^d
21	20±8	13.6±0.6	<30 ^d	<0.32 ^c	<2.0 ^d	470±70	<6.7 ^d	3100±50	<1.0 ^d	<2.0 ^c	432±2	<3.2 ^c	7.5±0.3	<0.90 ^c	<2.8 ^d	3.8±0.3	<1.25 ^c	<0.94 ^d
22 ^a	20±2	<0.20 ^d	<30 ^d	<0.32 ^c	<2.0 ^d	<8.0 ^c	<6.7 ^d	244±4	<1.0 ^d	<0.6 ^d	8600±40	<0.98 ^d	<0.41 ^c	<0.90 ^c	<9.2 ^c	3.35±0.05	19.5±0.7	<0.94 ^d
Average ^b	<24	<0.89	<203	<0.43	<2.0	<1751	<24	<3022	<166	<5.4	1368	<8.1	<159	<1.53	<15	4.4	<12	<125
Range	<4.0+98.3	<0.20+13.6	<30+1510	<0.10+4.2	<2.0	<8+14700	<6.7+299	<0.11+40900	<1.0+2800	<2.0+50.5	164+8600	<0.98+45.3	<0.41+1313	<0.27+18.9	<2.8+172	1.5+13	<0.38+68	<0.94+930

All elemental concentrations are expressed in $\mu\text{g dm}^{-3}$; ^a commercial *Filuferru* sample, distilled through a stainless steel apparatus rather than a copper apparatus, and here considered only for comparison purposes; ^b values preceded by a “<” symbol are obtained considering in the calculation of the average value (calculated before rounding made for harmonizing the number of significant digits of each analytical data according the relevant uncertainty) at least one sample showing concentration below LoQ; ^cLoQ value; ^d LoD value.

On the other hand, quantification of Na, Sr, Cu and Pb in craft *Filuferru* has been always possible, whereas the quantification of Na and Sr has been possible also for the commercial *Filuferru* sample, distilled in a stainless steel apparatus rather than in a copper apparatus, and this – in our opinion – is the reason explaining the substantial absence of Cu by sample 22. The concomitant absence of Pb in sample 22 likely depends by the absence, in a stainless steel apparatus, of any solders made using a Sn/Pb filler material, typically that used for make solderings between parts in copper. Whereas the concentration of Na is between 164 and 8600 $\mu\text{g dm}^{-3}$, the amount of Sr is shifted downwards by two magnitude orders (i.e. between 1.5 and 15 $\mu\text{g dm}^{-3}$), while the range of Cu concentration is the widest among all elements considered (i.e. between the LoD, 8 $\mu\text{g dm}^{-3}$, and 14700 $\mu\text{g dm}^{-3}$). However, for all samples analyzed the Cu amount is largely below the limit (1000 mg dm^{-3} of absolute ethanol) reported by Silva et al. [18]. The variability of the Cu and Pb concentrations in *Filuferru* is wide also within samples produced by the same distiller (i.e. *Filuferru* couples 18-19, produced in Orotelli in two consecutive vintage years, and 20-21, produced in Oliena in the same year by grape marc from two different vineyards). This variability should be tentatively ascribed by a number of different causes like: a different geochemical threshold for Cu, the intensity of possible Cu-based agrochemical treatments, the different bioaccumulation level of the elements on the grape marc as a function of the genotype or the pollution level and – not least – by the deterioration level of the copper pot still. In particular, the last one is the principal cause the contamination of spirits distilled in discontinuous copper apparatuses.

It is well known that the solubilization, by means of acidic alcoholic vapors, of compounds of Cu(II) like $\text{CuCO}_3\cdot\text{Cu}(\text{OH})_2$ (the so-called “verdigris”) [24,25] formed on the inner copper surfaces of the copper pot, can contaminate the distillate. Even still EC and Italian legislations do not establish a maximum copper limit for distilled spirits, in Brazil the highest Cu concentration in alcoholic distillates is fixed in 5 mg dm^{-3} [26].

The highest concentrations of Cu and Pb measured in the *Filuferru* samples are 14700 $\mu\text{g dm}^{-3}$ for Cu (sample 15, produced in Fonni) and 1313 $\mu\text{g dm}^{-3}$ for Pb, (sample 3, produced in Orgosolo). Sn is the second element potentially correlated with the presence of solderings in a copper pot still. Unlike Cu and Pb, Sn has been quantified only in less than one third of the samples, with concentrations ranging between 10.5 and 172 $\mu\text{g dm}^{-3}$. The concentration of another toxic element like Cd is rarely over the relevant LoQ: the element is quantified only on five samples, reaching the highest concentration in sample 12 (4.2 $\mu\text{g dm}^{-3}$, from Orotelli). Oligoelements like Mn, W, Zn and Ni have been quantified in more than the half of the samples (Mn, W and Zn), and in less than one third of them (Ni). The highest concentrations measured are on levels of few tens of $\mu\text{g dm}^{-3}$ (for Mn, W and Ni) and almost a mg dm^{-3} for Zn. Potassium is the element showing the widest range (between LoD, 0.11 $\mu\text{g dm}^{-3}$, and 40900 $\mu\text{g dm}^{-3}$). This is likely due also to the potential use of potassium-containing fertilizers in vine cultivation and/or the use of potassium metabisulfite as a source of sulfur dioxide in homemade winemaking processes. Metals like Fe and Al show a very different fate in *Filuferru* samples. Whereas Al is quite frequently quantified in them, reaching concentrations also close to 100 $\mu\text{g dm}^{-3}$, Fe has been found only two times over the relevant LoD, and this happens to sample 3 (299 $\mu\text{g dm}^{-3}$, from Orgosolo) and sample 13 (84 $\mu\text{g dm}^{-3}$, from Villagrande Strisaili). Although Ca is not commonly quantified in *Filuferru* (this happens only for eight samples), the frequency of quantification for Mg is also lower (only for five samples), and the concentrations measured for both analytes reached amounts of few thousands of $\mu\text{g dm}^{-3}$. Finally, Sb has been found over the relevant LoQ only in four samples, reaching its maximum amount on sample 20 (18.9 $\mu\text{g dm}^{-3}$, from Oliena).

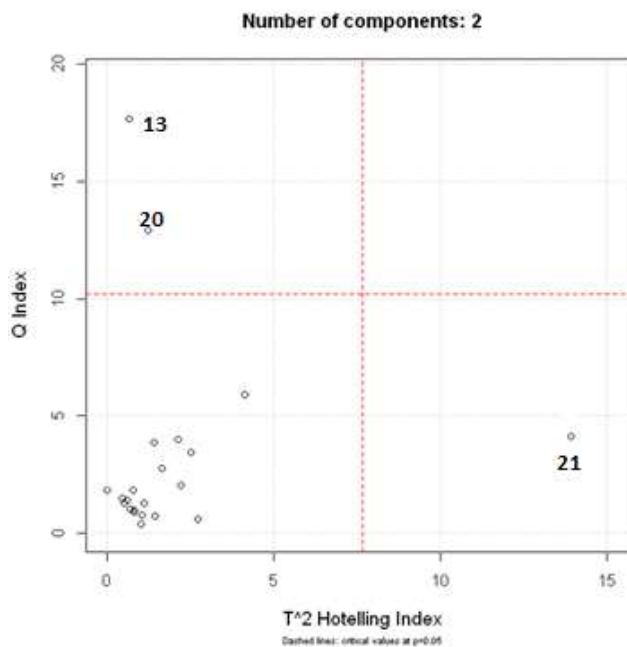
At the best of our knowledge, literature reports only very few contributions relating the determination of the trace elements in grape marc distillates, all aimed only on the evaluation of the Cu amount in similar distilled spirits from grape marc. Again, Grappa is chosen as the principal term of paragon for data obtained in this study. The amounts of Cu previously measured in this matrix ranged between 1.4 and 4.4 mg dm^{-3} (craft Grappa [24]) and from 0.4 to 0.7 mg dm^{-3} [27]. A wider comparison of our data, that keeps into account also some of other elements here measured, might be made with other traditional distilled spirits produced in other Countries. For example, very high Cu amounts (up to 105 mg dm^{-3}) have been found in wine distillates from Crete, Greece [28], as well as in a spirituous beverage from Venezuela (Cocuy, that contains amounts of Cu

between 4.5 and 86 mg dm⁻³) [29], whereas the amounts of Cu found in Zivania, a traditional Cypriot Spirit from distillation of grape marc [30] are comparable with data here reported for this element. Following the comparison between the trace amounts of elements in *Filuferru* and Zivania, it is possible to observe that the former prevails for the average concentrations of Pb and Na, whereas the latter evidences higher amounts of Ca, Cr, Mg, Mn and Sb. Beyond Cu, both distillates have comparable amounts of elements like Fe, Al and Zn. On the other hand, the amounts of Al, Cd, Mn, Fe, Cr, Ni, Pb and Zn found in the wine distillates from Crete appear to be significantly higher than those found in *Filuferru*, as well as observed for the amounts of Fe and Zn measured in Cocuy distillate from agave.

3.5. Principal Components Analysis.

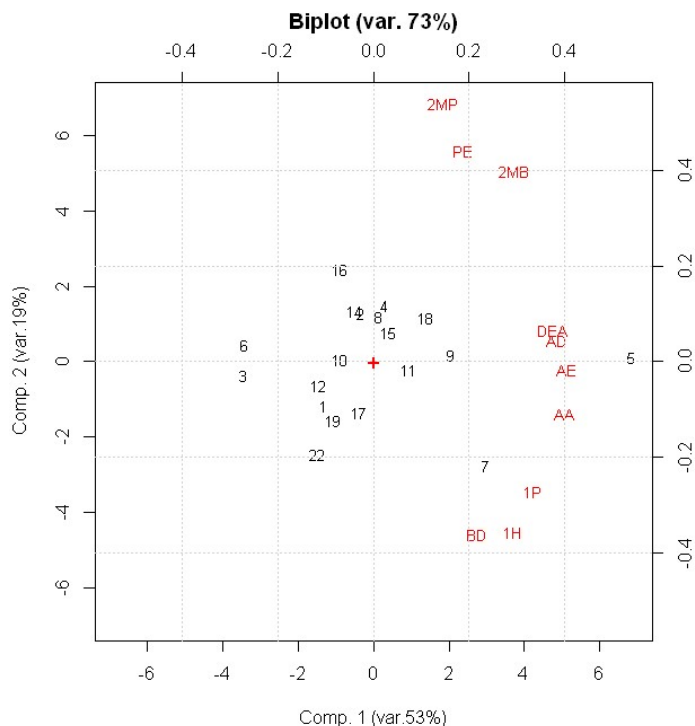
On first analysis, all 22 *Filuferru* samples, and all data from quantitative analysis of organic volatile compounds (all analytes reported in Table 6, which have been provided data higher than the relevant LoQ in every sample) and all data from elements which concentration was over the relevant LoQ in at least 50% of samples (i.e. Al, Cu, K, Mn, Na, Pb, Sr and Zn), for a total of 18 analytes, were kept into consideration for the Principal Component Analysis (PCA). Unfortunately, with this choice, the variance resulted very fragmented, as demonstrated by the amount of the total variance explained by the first two components, able to explain only less than 40% of the cumulative variance. Two PCA performed separately on the group of inorganic and volatile organic analytes, respectively, provided evidence that the highest amount of variance was explained by the last group; for this reason all inorganic analytes were not more considered in the model. In this way the cumulative variance explained by the two principal components rise up to 68% of the total variance. Hence, the T² vs Q diagnostic plot, reported in Figure 4, provide evidence that sample 21 was clearly different from others, whereas samples 20 and – mainly – sample 13 were badly described, as revealed by its very high residual variance. For this reason these data were considered outliers and removed from the dataset.

Figure 4 - T² vs Q diagnostic plot of the PCA performed on the whole *Filuferru* dataset.



Hence, PCA analysis, which generates the biplot reported in Figure 5, was referred to 19 scores and 10 objects.

Figure 5 – Biplot for PCA analysis (19 *Filuferru* samples, 10 analytical variables)



Sample origin: 1, Galtelli; 2, Atzara; 3, Orgosolo; 4, 5, 7, 10, 12, 18 and 19, Orotelli; 6, 9, Silanus; 8, 14, Sarule; 11, Arzana; 15, 17, Fonni; 22, commercial *Filuferru*. Acronyms of volatile compounds; DEA, diethyl acetal; AD, acetaldehyde; AE, ethyl acetate; AA, acetic acid; 1P, 1-propanol; 2MP, 2-methyl-1-propanol; 2MB, the sum of 2-methyl-1-butanol and 3-methyl-1-butanol; 1H, 1-hexanol; PE, 2-phenylethanol; BD, 2,3-butanediol.

The two principal components were able to explain 73% of the total variance. From the biplot it is evident that the diethyl acetal (DEA), the acetaldehyde (AD), the ethyl acetate (AE) and the acetic acid (AA) describe the PC1, whereas all the alcohols (1-propanol, 1P; 2-methyl-1-propanol, 2MP; the two isomers 2-methyl-1-butanol and 3-methyl-1-butanol, both indicated as 2MB; 1-hexanol, 1H; 2-phenylethanol, PE and the 2,3-butanediol, BD) describe the PC2. With the exception of sample 5, from Orotelli, and of samples 6 and 3 (from Silanus and Orgosolo, respectively), all the remaining samples were described roughly in the same way by PC1 and PC2. In particular, sample 5 is characterized by high concentrations of low-boiling volatiles (i.e. DEA, AD and AE) and AA, whereas samples 6 and 3 are different for a very low amounts of these compounds. PC2 differentiate samples 2, 4, 8, 14, 15, 16 and 18 by samples 1, 12, 17, 19 and 22 in terms of 2MB, 2MP and PE (higher amounts for the first group rather than in the second group).

4. Conclusions

A preliminary screening of a wide number of craft samples of traditional *Filuferru*, a typical distillate of grape marc produced from centuries in Sardinia, Italy, has allowed for the first time to define a number of chemical properties like the alcoholic strength, the qualitative profile of the organic volatile compounds, the quantitative determination of the most abundant of them, and the quantitative determination of 18 trace elements. Data obtained have allowed to highlight a number of analogies and differences between the most close Italian spirit, the Grappa. The PC analysis allowed the differentiation of the ten organic analytes quantified in two groups: the first, described by PC1, accounting for acetic acid, ethyl acetate, diethyl acetal and acetaldehyde, and the second, described by PC2, accounted for the remaining six superior alcohols. The great variation in composition substantiates, on the one hand, in an incontrovertible way the artisanal nature of these spirits. On the other hand it reveals a number of potential quality issues (i.e. the high concentration in the distillate of low-boiling point compounds and of acetic acid, and a quite high concentration of Cu) that should keep into account in a next future in order to establish a regulation for this ancient

and valuable traditional production. The key points where a careful quality control should be applied are the quality the grape marc, its fermentation and storage processes, the optimization of both the distillation phase and the care and maintenance of the apparatus of distillation.

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