DEVELOPMENT FOR A PROCEDURE FOR THE DETERMINATION OF 2-PHENYLETHANOL IN HELLENIC WINE DISTILLATES (*VITIS VINIFERA* L.) AND THEIR CHANGES DURING DISTILLATION

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Abstract

Aims: The aim of this work was to study the concentration, during distillation, of 2-phenylethanol, an aromatic compound, in different fractions of wine spirits and in a traditional copper alambic. Its contribution as an odorant is important due to its quality and olfactory character. This aromatic alcohol is valuable for the distilleries in order to collect the fractions with the higher participation of this compound and to specify the grape variety which gives the best results. Wine distillates or brandies are important spirits in the commercial world and are produced in many countries. The differences in the concentration of 2-phenylethanol in combination with the other congeners makes a great difference with respect to the quality of produced brandy.

Methods and results: Twenty nine red and white monovarietal wine distillates (*Vitis vinifera* L.) 15 red and 14 white grapes (*Vitis vinifera* L.), have been analysed after double distillation of monovarietal wine samples, a liquid–liquid continuous extraction with dichloromethane and an analysis by gas chromatography(FID). Pentan-3-ol was used as an internal standard. The content of 2-phenylethanol which introduces a pleasant aroma to wine distillates, resembling to rose, was determined. The concentration of this alcohol in different fractions during distillation in traditional alambic has important technological consequences.

Conclusion: The commercial fining factors in the present research affected the knowledge of the attitude of 2-phenylethanol in monovarietal spirits from selected grapes (*Vitis vinifera* L).This compound with this unique character of roses, plays an important organoleptioc role in commercial wine spirits, especially for the consumers which enjoy the mystagogue of brandies in order to improve the distillation technique and knowledge in wine spirits.

Significant and impact study: The knowledge of the impact of the commercial fining agent in 2-phenylethanol content during the distillation in different fractions is important for monovarietal wine spirits in order to produce qualified wine brandies. This study is also particular important for brandies production in commercial distilleries in order to separate at the right alcoholic strenght and to take fractions with the higher concentration of this alcohol which in combination of course with the minor and major other congeners gives an qualified organoleptic character.

Kʃey words : volatile determination, wine distillates, aroma, alcoholic beverages, fractions, gas chromatography

Résumé

Objectifs : Le but de ce travail est d'étudier, pendant la distillation, la concentration du 2-phényléthanol, un composé aromatique, dans différentes fractions d'eaux-de-vie de vins et dans un alambic de cuivre traditionnel. Sa contribution à l'arôme est importante en raison de sa qualité et de son odeur. Cet alcool aromatique est important pour les distilleries. Il convient donc de rassembler les fractions possédant les teneurs les plus élevées de ce composé et de préciser la variété de raisin qui donne les meilleurs résultats. Les distillats ou les brandies sont importants pour le commerce et sont produits dans beaucoup de pays. Les différences de concentration du 2-phényléthanol en association avec les autres produits volatils contribuent de manière importante à la qualité de l'eau-de-vie.

Méthodes et résultats : Vingt-neuf distillats de vins monovariétaux (*Vitis vinifera* L.), 15 provenant de raisins rouges et 14 de raisins blancs, ont été analysés après double distillation des échantillons, extraction en continu liquide-liquide avec du dichlorométhane des distillats et analyse par chromatographie en phase gazeuse (FID) de l'extrait. Le pentan-3-ol a été utilisé comme norme interne. La teneur en 2-phényléthanol, qui donne aux distillats un arôme agréable rappelant la rose, a été déterminée. La concentration de cet alcool dans différentes fractions pendant la distillation dans un alambic traditionnel a des conséquences technologiques importantes.

Conclusion : Dans cette étude, il a été observé que les auxiliaires technologiques utilisés pour la clarification ont une conséquence sur la teneur en 2-phényléthanol dans des spiritueux de vins monovariétaux issus des raisins sélectionnés (*Vitis vinifera* L.). Ce composé, avec ce caractère unique d'odeur de roses, joue un rôle organoleptique important dans les eaux-de-vie de vins, en particulier pour les consommateurs qui apprécient l'arôme des brandies dans son aspect mystérieux. Sa connaissance permet d'améliorer la technique et la connaissance de distillation dans des spiritueux à base de vin.

Signification et impact de l'étude : La connaissance de l'effet de l'auxilaire technologique utilisé lors de la clarification sur la teneur en 2-phényléthanol pendant la distillation dans différentes fractions est importante pour des spiritueux de vins monovariétaux pour produire des eaux-de-vie de vins de qualité. Cette étude est aussi particulièrement importante pour la production d'eaux-de-vie dans les distilleries afin réaliser le coupage au bon degré alcoolique et pour prendre des fractions contenant la concentration la plus élevée de cet alcool, qui en combinaison avec les substances volatiles mineures et majeures donne un caractère organoleptique de qualité.

Mots clés: détermination volatile des subtances volatiles, distillats de vin, arôme, boissons alcoolisées, fractions, chromatographie en phase gazeuse

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INTRODUCTION

Wine spirit, according to EC regulation (EC 1576/89), is a spirit drink, obtained exclusively by distilling wines. This valuable alcoholic beverage is important in local and international trade because of its aromatic characteristics and its unique taste.

The most famous French wine distillates with geographical designations are Cognac and Armagnac but wine distillates are produced in many geographical areas all over the world with peculiar aroma characteristics deriving also from specific native grape varieties.

At present time, distilleries show a tendency to produce distillates demanded by consumer interest, with high quality and good flavour.

Wine distillates are widely consumed and are especially estimated for their unique aromatic profile. They were studied by different scientists such as BERTRAND *et al.* (1988, 1989), BOIDRON *et al.* (1998), CANTAGREL *et al.* (1991), SÉGUR *et al.* (1992), GEROGIANNAKI *et al.* (2002, 2003, 2004, 2005, 2007), SILVA *et al.* (1996, 1998, 1999), SOUFLEROS *et al.* (1987, 2001).

Wine distillates are produced either by discontinuous or continuous distillation of varietal or commercial wines. Cognac and Armagnac (Appellation origins) are famous because of their high quality and their unique aromatic profile. Cognac is obtained by discontinuous distillation whereas Armagnac in general results from a continuous distillation in the specific Armagnac still. Commercial brandies are generally manufactured in continuous industrial stills.

Various factors influence the aromatic profile of wine distillates such as, vine environment, soil and climate, grape variety, degree of grape ripeness, enological procedure used for must production, fermentation conditions, distillation process and aging of the distillates (FERREIRA *et al.*,1993 ; ORRIOLS-FERNANDEZ, 1994).

Beside ethyl alcohol and water as the major components exists a substantial number of volatile congeners in a wide concentration range. Some of them are present at high concentrations (hundreds of mg/L), but most of them are found in the low concentration range, from traces to 10 mg/L (GEROGIANNAKI *et al.*, 2003, 2004, 2005, 2007; SILVA *et al.,* 1996, 1998, 1999; SOUFLEROS *et al.*, 1987, 2001). It was found that several of these aroma compounds have a substantial effect on the aromatic flavor of the final product (HARDY, 1969; RAPP *et al.*, 1976; SALINAS *et al.*,1994).

2-phenylethanol is together with some aromatic esters one of the most pleasant aroma compounds, resembling to rose. It derives from L-phenylalanine through metabolic reaction of *Saccharomyces cerevisiae* during carbonic anaerobiosis (LAMBRECHTS et PRETORIUS *et al.* (2000), MOIO *et al.* (1995), VERNIN *et al.* (1987). 2-phenylethanol remains the less representative volatile in wines -distillates with the range of 0-2.5 mg/L besides its important role in wine, wine-distillates and alcoholic beverage degustation (GEROGIANNAKI *et al.* 2003, 2005, 2007; SOUFLEROS *et al.*, 1987, 2001; VERNIN *et al.*, 1987; GARCIA *et al.*, 1995).

Until now for the most minor volatiles(like esters, ethyl esters of fatty acids and acetates of some alcohols), there is no specific reference for their presence in different fractions during wine distillation. It is known from distillation theory that the concentrations of different volatile compounds are continuously changing during distillation depending on their volatility (ATKINS, 1986). Accordingly, it would be of interest to create a method to measure its concentrations and at the same time to follow the changes of these volatiles during distillation.

Gas chromatographic techniques coupled to extraction-concentration procedures have been used for the analysis of many volatiles and especially 2-phenylethanol. These techniques include liquid-liquid extraction (SILVA *et al.*, 1998, 1999; SOUFLEROS *et al.,* 2001) liquid-liquid extraction with ultrasound (BOIDRON *et al.*, 1998) simultaneous distillation-extraction (FERREIRA *et al.* (1993) and other techniques (SILVA *et al.*, 1996, 1998, 1999; MOIO *et al.*,1995).

The above techniques are generally labor-intensive and are characterized by relatively low reproducibility. Sample preparation is mainly concerned to obtain more concentrated samples, but the elimination of interfering substances is also important. The specific advantages and disadvantages of these methods are always considered when selecting the most adequate technique for a given condition.

The aim of this work was to concentrate and analyse the content of the aromatic 2-phenylethanol obtained from monovarietal distilled wines from white and red grape varieties (*Vitis vinifera* L.) This knowledge is essential for the understanding of its attitude during the distillation. Its concentration is important for commercial distilleries in order to separate the fractions at the right alcoholic strenght and to take fractions with the higher concentration of this important aromatic alcohol in Hellenic monovarietal wine-distillates (brandies). Previously, we could not find any literature citation related to the results presented here.

In our research, the concentration of 2-phenylethanol was identified according the methods described by

PRISER *et al.* (1997). After the double distillation of wines in traditional distillation apparatus which are used by commercial and local distillers, the distillate was separated into five equal volume fractions.

A gas chromatograph (GC) equipped with flame ionization detector was used for the ana1ysis of 2-phenylethanol of the produced samples.

The aim of the present study was:

- To study factors affecting 2-phenylethanol aggregation such as ethanol percentage in different fractions during single and double distillation.

- To determine the 2-phenylethanol concentrations in equal different fractions of monovarietal wine distillates.

- To evaluate this concentration in wine-distillates from white and red grape varieties (*Vitis vinifera* L.).

MATERIALS AND METHODS

1. Samples

The collected samples (FAO/WHO,1986) consisted of a total of 29 monovarietal wines corresponding to 29 varieties of grape cultivated in private vineyards and made under the same conditions of vinification.

2. Chemical and physicochemical analysis of wine and must samples

Grape musts from the grape samples collected from the various field plots of commercial and private vineyard, as is reported above, were measured for their °Brix and pH values and for total acidity according to the relevant EEC Directive (2984/98). °Brix was measured with a portable Brix meter. pH was measured with a digital pH meter (Orion, model 520 A, Boston, MA). Acidity was measured by volumetric titration with 0.1 N NaOH using phenolphthalein as indicator. Acidity was expressed as tartaric acid EEC Directive (2984/98). Table 1 indicates the physicochemical parameters of wine-must of the wines used for distillation. These data were measured in order to evaluate the quality and the stage of maturity of collected grapes.

3. Distillation

Monovarietal wine samples were double distilled in a traditional copper distillation apparatus. Distillation products were collected as five equal volume fractions. 2-phenylethanol was extracted and concentrated with combination and modification of the methods described by MOIO *et al.* (1995) and PRISER *et al.* (1997). Wine distillate (200 mL) which produced after double-distillation (about two-hours each distillation), dichloromethane (5 mL) and sodium chloride (50 g) were added in a spherical flask. The spherical flask was cooled in melting ice and purged with nitrogen for 1 min in order to remove air. The mixture was stirred at 500 rpm for 2 h. The emulsion, formed during the stirring, was broken down by passing through a special filter (GHP Acrodisc Syringe Filters, GF 0.45) with the help of a gas-tight syringe in order to have the process in the absence of air. The organic extract was dried over anhydrous sodium sulfate and then filtered once more.

With the combination and modification of the methods described by MOIO *et al.* (1995) and PRISER *et al.* (1997), we have qualified and quantified characterization in the wine distilled fractions according to Raoult's law.

Filtrates were transferred into small glass-vials and stored at -5 °C, until analysis. The alcoholic strength of the collected fractions was measured by the use of refractometer as described by GEROGIANNAKI *et al.* (2002).

4. Chemical analysis

A standard (chromatographic grade) of 2-phenylethanol was purchased from Merck, Darmstadt.

A 5-mL sample of each concentrated extract was mixed with 50 µL of the internal standard solution (50 g of pentan-3-ol per L of ethanol). A gas chromatograph (Hewlett Packard 5890 series II) equipped with FID has been used for the ana1ysis of 2-phenylethanol. Samples of 0.2 µL were injected into the gas chromatograph. The injector of the gas chromatograph was maintained at 200 °C and operated under split mode. Elution was achieved in a 30 m x 0.25 mm i.d. x 0.2 µm capillary INNOWAX cross-linked polyethylene glycol column.

The oven temperature program was as follows: 400 °C for 5 minutes, a linear ramp from 40 to 200 °C at 30 °C /min, and 200 °C for 20 min. Detection was by FID at a temperature of 200 °C. Helium was used as the carrier gas at a split ratio of 1:60. The flow rate of the carrier gas was 2 mL/min. Air pressure was adjusted to 119 kPa (air). Chromatographic runs were carried out in triplicate, and their average was used as a single data point in the result section. The average coefficient of variation for the triplicate assays was 1.1-1.6 %.

5. Linearity

Calibration curves for 2-phenylethanol were built by plotting relative area versus concentration of standard solutions in order to check the linearity of the method.

6. Limit of detection

Diluted solutions of standard solution C (from 5 to 100 g l^{-1}) were submitted to the sampling method in order

to determine the limit of detection (LOD). The LOD for each derivative was determined until recording a signal to noise ratio of less than 3.

7. Repeatability

Repeatability was obtained from ten replicated experiments performed on the solution A (1 mg l^{-1}) and it was expressed as the relative standard deviation (RSD, %). The R^2 was 0.9985.

8. Standard additions

Standard additions experiments were realised for wine distillates. Additions of 0.5 ml of 0, 1, 1.5 and 2 mg l^{-1} of standard solution to 25 ml of sample and were performed.

RESULTS AND DISCUSSIONS

Results obtained, concerning the concentration of 2-phenylethanol in wine-distillates from the studied white and red grape varieties (*Vitis vinifera* L.) are presented in tables 1 and 2 and figures 1 and 2.

2-Phenylethanol introduces a pleasant aroma to distillates, resembling to rose (LAMBRECHTS et PRETORIUS, 2000) and derives from L-phenylalanine through metabolic reaction of *Saccharomyces cerevisiae* during carbonic anaerobiosis (LAMBRECHTS et PRETORIUS, 2000).

2-phenylethanol concentration for distilled grape pomace has 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 grape pomace distillates, reported mean values from 0.54 to 4.08 g/hl AA directly related to grape variety and extraction time.

Table 1 - Ethyl alcohol distillation profile of wine distillates from monovarietal white and red grapes (*Vitis vinifera* L.) (% vol.).

	5th fraction	4th fraction	3rd fraction	2nd fraction	1st fraction	Mean values
Red grapes (*Vitis vinifera* L.)						
Kalambaki	32	27	22,2	17	12	22,04
Muscat of Hamburg	28	22	18	14	9	18,2
Xinomavro	22	18	15	11	7	14,6
Agiorgitiko	26	21,5	19	13	8	17,5
Mantilaria	22	18	14	10	6	14
Mavrodafni	19	15	12	9	4,6	11,9
Fokiano	24	20	17	13	10	16,8
Negoska	29	26	21	15	9	20
Moschofilero	22,8	19	15	12	10	15,76
Vertzami	31	27	20	15	11	20,8
Liatiko	21	18	14	11	8	14,4
Romeiko	25	21	17	11	5,91	15,9
Vaftra	27	23	17	12	9	17,6
Kotsifali	32	26	21	18	12	21,8
Mavro Mesenikola	21	17,2	13	10,3	8	13,9
White grapes (*Vitis vinifera* L.)						
Muscat of Alexandria	35	29	21	18	15	23,6
Moschofilero	29	25	12	9	6	16,2
Savatiano	27	24	16	13	10	18
Vilana	25	21	18	13	11	17,6
Muscat of Patras	36	31	27	22	16	26,4
Tachtas	26	22	17	12	10	17,4
Vilana	23	19	14	11	8	15
Malagouzia	24	20	16	12	9	16,2
Mpatiki	31	28	21	17	13	22
Dempina	28	25	22	17	14	21,2
Robolla	30	26	21	17	14	21,6
Asyrtico	32	25	21	19	16	22,6
Aidani	34	28	24	20	17	24,6
Athiri	27	24	22	15	11	19,8

FITZERALD *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 et BERTRAND (1987) for the Greek grape distillate and varying from 2.8 to 23.4 g/hl AA.

Chemical and physicochemical parameters of the wine-must from the 29 white and red grape varieties studied are presented on table 1. In the samples examined, °Brix values varied between 17 and 18.6, pH values varied between 3.3 and 4.1 and acidity between 5.4 and 7.4 g/L, expressed as tartaric acid. The physicochemical parameters of the grape must are important in order to prove the mature stage of monovarietal grapes for the vinification because the 2-phenylethanol produced from the from L-phenylalanine through metabolic reaction of *Saccharomyces cerevisiae* during alcoholic fermentation.

Results obtained concerning the concentrations of 2-phenylethanol are presented in figures 1 and 2. Ethyl alcohol percentage concentration are listed in table 1 distinguished between white and red grape varieties (*Vitis vinifera* L.). It has to be mentioned that during distillation the first collected fraction was the n° 5 (25.5 % vol) and the last one, the n° 1 (8.6 % vol). From a comparison of figure 1 with figure 2, it can be seen that the distillation profile of 2-phenylethanol is quite different in distinctive monovarietal wine-distillates. It exists in all fractions but has higher concentration in the first fraction of distillation with the higher alcoholic volume going lower in 4th and 3rd fraction and higher in the last fraction with 12 % vol. White grape varieties have higher (2.4 mg/L) mean values than the red (1.74 mg/l) grape varieties.

The variety with the highest concentration is Muscat of Hamburg for the red grape variety, with 3,7 mg/L and Muscat of Patras for the white grape variety, with 6,08 mg/L.

All wine distillates have water and ethyl alcohol as major components and a substantial number of volatile congeners as minor or very minor components. 2-phenylethanol is a compound which exists in a small concentration. The explanation for the differences in the distillation profile is the following.

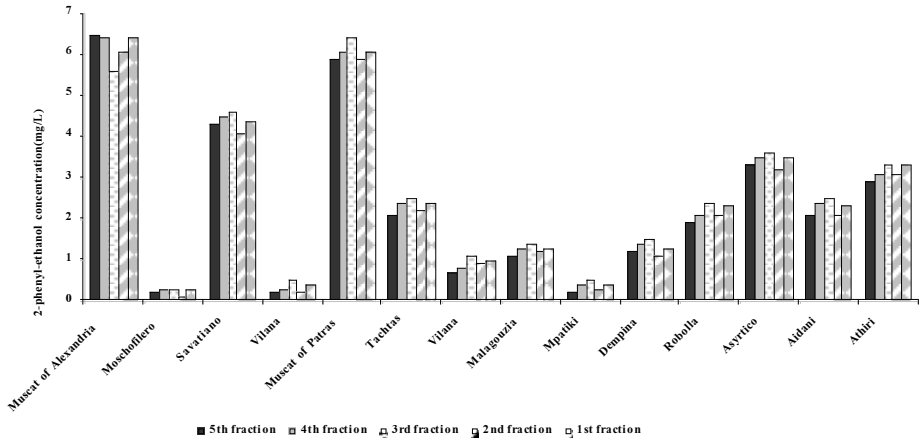

Figure 1 - Histograms of 2-phenyl- ethanol concentration of wine - distillates from monovarietal white grapes (*Vitis vinifera* L.).

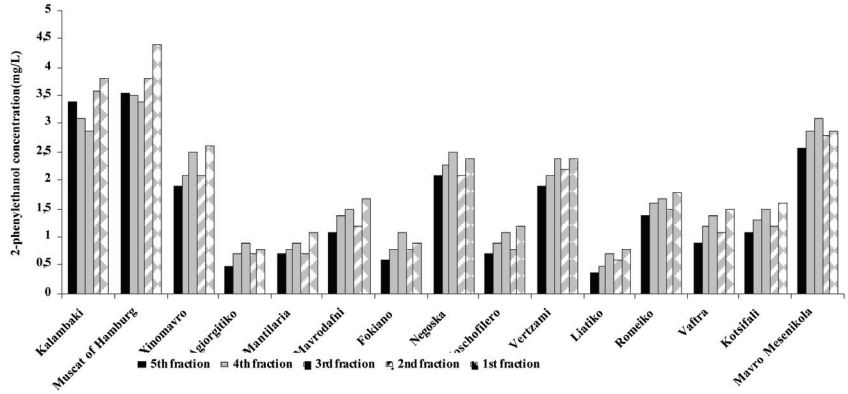

Figure 2 - Histograms of 2-phenyl- ethanol concentration of wine - distillates from monovarietal red grapes (*Vitis vinifera* L.).

Table 2 - Chemical and physicochemical parameters of grape-must from white and red grape varieties (*Vitis vinifera* L.).

Grape varieties	pH	^{0}Brix	Acidity g/L expressed in tartaric acid
Reds			
Kalambaki	3.6	17.3	5.6
Muscat of Hamburg	3.5	18.2	6.1
Xinomavro	3.5	16.1	5.7
Agiorgitiko	3.7	15.8	6.3
Mantilaria	4.3	16.3	7.2
Mavrodafni	3.9	17.4	5.5
Fokiano	4.1	16.9	4.9
Negoska	3.8	18.3	5.3
Moschofilero	3.9	16.4	5.9
Vertzami	4.2	17.1	4.6
Liatiko	3.6	15.5	4.9
Romeiko	4.2	16.2	5.2
Vaftra	3.7	17.8	5.4
Kotsifali	3.4	14.9	6.1
Mavro Mesenikola	4.1	16.5	5.5
Whites			
Muscat of Alexandria	4.2	18.7	4.9
Moschofilero	4.1	17.1	4.1
Savatiano	3.9	16.6	5.3
Vilana	3.7	18.5	5.5
Muscat of Patras	4.1	14.9	4.9
Tachtas	3.7	15.6	5.2
Vilana	3.5	16.9	6.3
Malagouzia	4.2	17.2	4.9
Mpatiki	3.8	14.9	5.1
Dempina	3.3	16.7	4.7
Robolla	3.7	16.8	5.2
Asyrtico	4.1	17.6	4.4
Aidani	4.3	17.1	4.9
Athiri	3.9	18.1	5.1

It is known from standard distillation theory [1] that (a) the concentration of different volatile compounds are continuously changing during distillation depending on their volatility and (b) each volatile begins to distil when the solution temperature is near its boiling point temperature. According to Raoult's law, the vapor pressure (Pi) of a volatile component (i) above a solution is the product of the vapor pressure (Pi0) of the pure component and of the mole fraction (Xi) of the component (i) in the solution (Pi = Pi0.Xi). From Dalton's law, it can be calculated that the Xi (gas phase) = Pi/Ptotal, where Ptotal is the sum of all partial pressures of volatile components of the alcoholic solution. Due to the very high concentrations of water and ethyl alcohol and to the very low concentration of all components presented it can be calculated that Xi (gas phase) is 10^{-4} to 10^{-5} of Xi (solution). Due to this phenomenon, volatile compounds of small or very small concentrations have very small mole fractions in the gas phase. Accordingly, during distillation, these components distill at a slow and rather uniform rate throughout the whole distillation process. This attitude has important consequences for producers of distilled alcoholic beverages. It means that it is impossible to get rid of some minor unwanted components through distillation cut and some other ways have to be applied.

Consequently, this report on the composition of 2-phenylethanol in wine distillates from selected monovarietal white and red grape varieties is a first approach on a subject that requires and is already under further investigation.

CONCLUSION

It can be seen that the attitude of 2-phenylethanol in distillates varies in depending on the alcoholic strength of the fraction. This research has been applied in order to understand the attitude of this aromatic alcohol during

distillation and to try to characterise the varieties by the means of their 2-phenylethanol content. For this reason, systematic approach and better standardization of the distillation procedures would offer an opportunity to improve the distillation process and to obtain a more aromatic and qualitatively better wine-distillates from monovarietal Hellenic grape varieties (*Vitis vinifera* L.).

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