DISCRIMINATION AND RISK ASSESSMENT DUE TO THE VOLATILE COMPOUNDS AND THE INORGANIC ELEMENTS PRESENT IN THE GREEK MARC DISTILLATES TSIPOURO AND TSIKOUDIA

DISCRIMINATION ET ESTIMATION DES RISQUES ÉVENTUELS SUR LA SANTÉ DUS AUX COMPOSÉS VOLATILS ET AUX ÉLÉMENTS INORGANIQUES PRÉSENTS DANS LES DISTILLATS DE MARC GRECS TSIPOURO ET TSIKOUDIA

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Abstract: Tsipouro and Tsikoudia are two denominations with geographic indications of the traditional Greek marc distillate, which is produced in continental Greece and in the island of Crete respectively. It is produced by the Greek wine-growers as well as by the professional distillers. To provide data on the safety of domestic Tsipouro and Tsikoudia distillates for human consumption and to draw conclusions from any essential results, which might have an impact on the quality of this product, 23 samples were analysed for: (a) Volatile compounds by the use of gas chromatography, (b) Inorganic elements, using atomic absorption spectrometry and (c) the pH values through-out using standard methods. Data revealed differences between these two denominations, which have been confirmed by the application of a statistic analysis and a PCA. Thus, Tsikoudia was found to contain statistically higher amounts of acetalde-hyde. However, the levels that have been observed did not exceed the official limits. Most of the Tsipouro and Tsikoudia samples also contained low concentrations of estragol, an anise compound, lead and copper, which do not represent a risk to consumer health due to their toxicity. On the other hand, the total concentration of higher alcohols was higher than the official minimum limit (140 $g.hl^{-1}$ Absolute Alcohol-AA), while the amylic alcohols rarely exceeded 300 g.hl-1 AA. The high concentrations of ethyl acetate (>300 $g.hl^{-1}$ AA) and ethyl lactate in a few samples showed the necessity of limiting unwanted fermentations in the grape pomace. The analytical study showed that the quality of the marc distillate is, generally, satisfactory. However, it revealed great differences between Tsipouro and Tsikoudia even among the samples of each denomination and, con-sequently, these domestic distillates need standardization and a more systematic production.

Résumé: « Tsipouro » et « Tsikoudia » sont deux dénominations du distillat grec de marc, avec des indications géographiques, produit en Grèce continentale et à l'île de Crète respectivement. Il est produit par les vignerons grecs ainsi que par les distillateurs professionnels. Afin de fournir des données concernant l'influence (la sûreté) de Tsipouro et de Tsikoudia produits par les vignerons sur la santé des consommateurs et de tirer des conclusions de certains résultats essentiels, qui pourraient avoir un impact sur la qualité de ce produit, 23 échantillons ont été analysés en dosant: (a) les composés volatils à l'aide de la chromatographie en phase gazeuse, (b) les éléments inorganiques, en utilisant la spectrométrie par absorption atomique et (c) les valeurs du pH, en utilisant des méthodes standards.

Les données ont indiqué des différences significatives entre ces deux dénominations, qui ont été confirmées par la statistique et l'application de l'ACP. Les échantillons de Tsikoudia se sont avérés avoir des concentrations statistiquement plus élevées en acétaldéhyde. Cependant, les niveaux qui sont observés n'ont pas dépassé les limites officielles. La plupart des échantillons de Tsipouro et de Tsikoudia présentent également des faibles concentrations en estragol, plomb et cuivre, qui ne présentent pas, à cause de leur toxicité, un risque pour la santé du consommateur. D'autre part, la concentration totale des alcools supérieurs est plus élevée que la limite minimale officielle exigée (140 $g.hl^{-1}$ d'Alcool Absolu-AA), alors que les alcools amyliques excédaient rarement les 300 $g.hl^{-1}$ AA. Les concentrations élevées de l'acétate d'éthyle (>300 $g.hl^{-1}$ AA) et du lactate d'éthyle dans quelques échantillons montrent la nécessité de limiter les fermentations non désirées du marc de raisins. L'étude analytique a prouvé que la qualité du distillat de marc est généralement satisfaisante. Cependant, elle indique de grandes différences entre le Tsipouro et la Tsikoudia par conséquent ces distillats domestiques nécessitent une standardisation et une production plus systématique.

Keywords: grape pomace, Tsipouro, Tsikoudia, volatile compounds, inorganic elements, trace elements, atomic absorption.

Mots-clés : marc, Tsipouro, Tsikoudia, composés volatils, éléments inorganiques.

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INTRODUCTION

Tsipouro and Tsikoudia are the two denominations of the Greek distillate from grape pomace, which are in-cluded in the European Community's legislation 1576/89. Tsipouro is produced in continental Greece from several grape varieties along with seeds of aromatic plants (*Pimpinella anisum*, *Foeniculum vulgare*, etc), while Tsikoudia is produced in the island of Crete (R. 1576/89) without aromatic plants. According to European and national legislation, these two marc distillates, when produced by professional distillers, can be qualified by geographic indications. Thus, there is Tsipouro of Macedonia, Thessaly and Tyrnavos, when it is produced in these homonymous regions, and Tsikoudia of Crete.

Tsipouro and Tsikoudia distillates are the result of the long Greek tradition in distillation process, which started in ancient times according to Issiodos (7th century), Hippocratis and Aristotle (5th and 4th century respectively) and in Egypt from the Hellenistic (Alexandrian) years; further it was developed during Byzantine years from the Greek distillers of Mount Athos, Constantinople and Smyrna.

Marc distillates, similar to Tsipouro and Tsikoudia, are produced in several countries: the well known grappa from Italy, the baggaceiras from Portugal, the aguardiente from Spain, the marc or eau-de-vie de marc de raisin from France etc (SOUFLEROS et BERTRAND, 1987; SILVA *et al.*, 1996; SILVA and MALCATA, 1998 and 1999; ROGERSON and FREITAS, 2001). Other traditional distillates from different fermented raw materials are the Greek mouro (from mulberry tree fruits) (SOUFLEROS *et al.*, 2004) and koumaro (from *Arbutus* unedo fruits), the Brazilian cachaças (from sugar cane) (NASCIMENTO *et al.*, 1999), the Japanese Shochu (from rice), the Yugoslavian Slivovica (from plum), the French pear, the international Kirsch, etc.

The first Greek legislation, concerning alcohol and distilled products of viticulture origin such as Tsipouro, was created as National Law 971/1917. According to this law, only viticulturists and alambic owners granted a permission to produce a marc distillate. The recent national law 2989/2001, which has lead to the harmonisation of the Greek legislation with the one of the European Community (R. 1576/89), gave also the right to the professional distillers to produce this spirit.

The aims of this research were a) to provide data on the safety of domestic Tsipouro and Tsikoudia distillates for the consumers without any repercussion to their health from methanol, estragol or heavy metal (Cu and Pb) levels and b) to form conclusions from any essential results, which might affect the quality of this product. Furthermore, the purpose of the present paper is to provide knowledge on the fermentation and distillation procedures followed for distilled product from grape pomace. This knowledge should lead to a better standardization process and a more uniform quality of this spirit in order to avoid some health risks.

MATERIALS AND METHODS

I - PROCESSING AND FERMENTATION OF GRAPES

The grape pomace allocated for domestic spirit production originates from various white and red grape varieties vinificated without any pressing process, containing an amount of 30 to 40 percent wine. The fermentation process may last up to two or four weeks depending on the vinification type and takes place in wooden barrels with a capacity of 500 to 1 000 litres.

II - DISTILLATION

The distillation of the fermented grape pomace, applied by the winegrowers, takes place usually from the beginning of October until the end of December. The marc distillates are produced either from a single or double distillation. Depending on the region, the distillation may occur with the use of aromatic plants and seeds (*Pimpinella anisum*, *Foeniculum vulgare*, *Pistacia lentiscus Chia* or *Latifolia* sp. etc.) or without any such additions. In the second case, the distillate is allowed to maintain the primary aroma of the grape pomace and sometimes the characteristics of the specific cultivars used can be detected.

During the first distillation, the fermented marc, the wine lees, an amount of 25 to 30 % (v/v) of wine or water (when the marc is dry) and the « tails » from previous distillations are all transferred to traditional copper-made alambics of 130 l capacity, approximately like one of charentais (Cognac) type. The alambics are filled up to 3/4 of their capacity, closed properly with dough or cinder pulp in order to prevent any vapor leakage and then put on the fire. When the temperature reaches at 80-90 °C, the liquid spirit starts to run in glass bottles of 30 to 100 l known as damitzanes. The first 0,5 to 1 l of the distilled product corresponds to the beginning of the distillation procedure and is removed as «head». Then, and globally for about 2,5 hours, a pure spirit is distilled at lower than 86 % vol (34 Descartes grade approximately). In continental Greece, for the Tsipouro production, the distillate fraction named « heart » is obtained until 18 grada (45,5 % vol), while the « tail » is obtained until 15 grada (32 % vol), when the distillation is definitely stopped. In Crete Island, in order to produce the Tsikoudia distillate, the two aforementioned cuts are usually made at lower levels that are 15 and 12 (11 % vol) grada correspondingly. The limited official available time to the winegrowers and the relatively high cost of distillation tend to perpetuate

Table I - Volatile and inorganic components analysis of Tsipouro and Tsikoudia distillate beverages' samples. Concentration in g.hl^{-1}, except if there is a different indication

Analyse des composés volatiles et des éléments inorganiques des distillats Tsipouro et Tsikoudia Concentration en g.hl^{-1}, sauf autre indication

		Tsipourodia				Tsikoudia				t-test
	Codes	Min	Max	Mean	STDEVP	Min	Max	Mean	STDEVP	P
pH	ph	3,76	8,02	4,96	1,33	3,08	4,81	3,98	0,50	1*
Alcohols										
Ethanol % vol	eoh	31,84	71,55	45,79	9,70	26,22	48,27	35,54	4,98	1*
Methanol	moh	90,57	414	180,10	84,78	30,31	482,78	207,76	119,63	
1-butanol	but	1,06	23,58	6,92	5,97	4,00	44,75	16,52	12,70	1*
2-methyl-1-propanol (isobutyl alcohol)	m2p	22,52	87,47	51,89	16,66	10,26	70,37	30,74	18,00	1*
v 2-methyl-1-butanol (isopentyl alcohol)	m2b	20,52	138,28	60,76	26,97	10,26	68,21	31,06	15,85	1*
3-methyl-1-butanol (isoamyl alcohol)	m3b	86,51	264,44	187,10	51,25	81,94	387,54	142,24	79,13	
1-hexanol	hex	0,38	2,34	1,12	0,48	0,17	2,95	1,44	0,85	
Trans-3-hexen-ol-1	t3h	n.d.	0,04	0,01	0,01	n.d.	0,09	0,02	0,03	
Cis-3-hexen-ol-1	c3h	n.d.	0,19	0,04	0,06	n.d.	0,38	0,05	0,11	
Trans-2-hexen-ol-1	t2h	n.d.	0,03	0,01	0,01	n.d.	0,08	0,01	0,02	
2-phenylethanol	poh	0,63	19,26	4,95	4,69	n.d.	27,40	13,55	7,30	1*
Total higher alcohols (*)		178,21	474,84	312,80	106,10	127,76	566,67	235,63	133,99	1*
Acetaldehyde	ace	14,82	93,40	47,11	26,76	14,53	245,31	84,01	64,90	
Esters										
Ethyl hexanoate (ethyl caproate)	eh	0,06	0,49	0,23	0,12	n.d.	0,90	0,17	0,28	
Ethyl octanoate (ethyl caprylate)	eoc	0,18	1,22	0,68	0,35	n.d.	0,15	0,08	0,05	2*
Ethyl decanoate (ethyl caprate)	ede	0,22	2,27	0,65	0,52	n.d.	0,10	0,05	0,04	2*
Ethyl myristate (ethyl decatetranoate)	emy	n.d.	0,03	0,01	0,01	n.d.	n.d.	n.d.	n.d.	1*
Isoamyl acetate (3-methyl-butyl acetate)	ia	0.05	0,58	0,31	0,19	n.d.	0,67	0,23	0,19	
Hexyl acetate (1,3-dimethyl butyle acetate)	ha	n.d.	0,08	0,02	0,03	n.d.	n.d.	n.d.	n.d.	1*
Phenylethyl acetate	pa	n.d.	0,14	0,05	0,04	n.d.	0,08	0,02	0,03	1*
Ethyl acetate	ea	41,43	1374,87	422,69	355,74	16,37	608,97	165,22	185,43	1*
Ethyl lactate (ethyl 2-hydroxypropanoate)	el	0,50	55,51	14,09	16,56	3,00	62,58	28,60	19,66	1*
Diethyl succinate (diethyl butanedioic acid)	sde	0,17	5,95	1,09	1,51	0,24	3,90	1,08	0,97	
Acids										
Butanoic acid	ba	n.d.	0,73	0,38	0,25	n.d.	28.41	7.28	9.59	1*
Isobutanoic acid (2-methyl propanoic acid)	iba	n.d.	2,98	0,62	0,83	n.d.	17.00	1.67	4.70	
Isovaleric acid (methyl butyric acid)	iva	1,16	5,52	3,01	1,25	0,06	0,37	0,14	0,08	2*
Hexanoic acid (caproic acid)	hea	n.d.	0,82	0,23	0,22	0,03	5,02	1,16	1,50	1*
Octanoic acid (caprylic acid)	oca	0,07	0,94	0,45	0,22	0,30	1,50	0,94	0,34	2*
Decanoic acid (capric acid)	dea	0,06	0,94	0,45	0,24	0,20	1,34	0,74	0,33	1*
Dodecanoic acid (lauric acid)	doa	n.d.	0,44	0,11	0,11	0,07	0,36	0,18	0,10	
Anise compounds										
Anethole (1-methyoxy-4-propenylbenzene)		0,02	98,28	40,63	27,14	0,10	1,26	0,74	0,38	
Anisaldehyde		n.d.	11,62	4,72	3,02	n.d.	0,10	0,02	0,04	
Estragol (1-allyl-4-methoxybenzene)		0,02	0,87	0,48	0,22	n.d.	0,05	0,01	0,02	
Eugenol (2-methoxy-4-(2-propenyl) phenol)		0,05	0,41	0,17	0,10	n.d.	0,03	0,01	0,01	
Metals										
Fe (mg.l-1)	fe	n.d.	0,22	0,06	0,08	n.d.	9,10	1,05	2,47	
Ca (mg.l-1)	ca	3,00	40,50	13,57	10,94	3,80	1050,0	97,13	287,39	
Cu (mg.l-1)	cu	0,23	13,70	4,45	3,53	1,78	21,80	8,52	5,51	1*
Pb (µg.l-1)	pb	1,50	2520,00	512,08	695,74	17,60	190,20	57,63	43,90	1*

(*) In the Total higher alcohols are not included methanol and ethanol. n.d.: not detected
1*: Statistically significant difference at the 5% level (P<0,05) between the averages. 2*: Statistically significant difference at the 1% level (P<0,01) between the averages.

disadvantages. The « heart » of spirit resulting from the first distillation usually comes up to 10 or 20 % of the grape pomace used, depending on its alcoholic content and the distillation technique.

The « heart » of the first distillation is led back to the alambic for a second distillation or « metavrasma » along with an amount of 5-6 % (w/v) of *Pimpinella anisum* or *Foeniculum vulgare* seeds, in order to produce a smoother and more aromatic spirit. In the cases where no second distillation occurs, the flavoring of the Tsipouro is given in the first distillation. During the second distillation, the « head » and « tail » are eliminated while the « heart » of the spirit, which has an alcohol content between 60 and 70 % vol, is collected.

The « tails », commonly known as « aporaka », « ahamko » or « hamko » are used in a subsequent single distillation process with the new lot of the fermented grape pomace.

The various lots of the second distillation spirit are blended and sometimes - when they are produced without any aromatic plants - are placed, before the dilution with water, into oak barrels and left to age for one year before consumption.

III - SAMPLING

All the analysed samples were produced by Greek winegrowers-distillers, following the traditional procedure. Eleven Tsipouro samples were obtained from Macedonia, Epirus, Thessaly and Peloponnese. Most of these samples were distilled with aromatic plants and some of them were taken as clear fractions before dilution with water. All 12 Tsikoudia samples, acquired from several areas of Crete Island, were distilled without the use of any aromatic plants or seeds. All distilled samples were placed into glass bottles of 0,5 l capacity and stored in the dark until they were analysed.

IV - ANALYTICAL METHODS

The analysis of the distillate samples was realised by classical, chromatographic and atomic absorption spectrometry methods. Values of pH, 32 volatile compounds and 4 inorganic elements were determined.

1) Alcoholic title

The real alcoholometric title was determined after distillation, according to the official method of OIV (1994) and the results are expressed as a % vol.

2) pH

pH measurement was performed with a microprocessor pH/ion meter pmx2000 wtw.

3) Volatile compounds

The volatile compounds were determined with gas chromatography. Acetaldehyde, ethyl acetate, methanol and higher alcohols (table I) were analysed by the official method of OIV (1994) with direct injection of the diluted distillate on a Carbowax 400 + Hallcomid M. 1801 (7,5 m x 2,3 mm) classic column, as described previously (SOUFLEROS *et al.*, 2004).

Higher esters, fatty acids, anise compounds and some other higher alcohols (table I) were analysed by the official method of OIV (1994), slightly modified, after their extraction from the samples by a mixture of solvents (SILVA *et al*., 1996; SILVA and MALCATA, 1998 and 1999; FERREIRA *et al.*, 1999) as in previous publication (SOUFLEROS *et al.*, 2004). The extract is injected in a capillary column CP Wax 57 CB (50 m x 0,22 mm x 0,2 μm) (Chrompack).

4) Inorganic element profile

The inorganic elements Fe, Ca and Cu were determined by flame atomic absorption spectrometry (NASCIMENTO *et al.*, 1999; MUNTEAN *et al.*, 1998; CAMEAN *et al.*, 1998) and Pb by graphite furnace atomic absorption spectromentry (NASCIMENTO *et al.*, 1999; MUNTEAN *et al.*, 1998; CAMEAN *et al.*, 1998; AKRIDA-DEMERTZI, 1999; CHEN, 1999; RODUSHKIN, 1999) as in a previous work (SOUFLEROS *et al.*, 2004).

5) Statistical analysis

a) An independent two-sample t-test on the analytical data of Tsipouro and Tsikoudia samples was performed in order to detect potential differences between these two spirits. b) A Principal Component Analysis (PCA) was applied to the whole data set, except the anise compounds. Statistical data processing was performed using SPSS, v.10 statistical package (SPSS Inc., 2000) (Headquarters, 233 S. Wacker Drive, Chicago, Illinois 60606, USA).

RESULTS AND DISCUSSION

The results are given in table I and further discussed in relation to distillates from wine, grape pomace, pomace of other fruits, etc (table II).

I - VOLATILE COMPOUNDS

There was an attempt to compare the Greek distillates to other distillates of grape pomace or several fermented raw materials as well as to well known international spirits (SOUFLEROS et BERTRAND, 1987; ORRIOLS and BERTRAND, 1990; SILVA *et al.*, 1996; BAUER-CHRISTOPH *et al.* 1997; SILVA and MALCATA, 1998 and 1999; LEHTONEN *et al.*, 1999; Fitzerald *et al.*, 2002; ROGERSON and FREITAS, 2001; CORTES *et al.* (2002) and others (CORDONNIER, 1971; AMERINE *et al.*, 1972; Bertrand, 1975; LAFON *et al.*, 1973; VERSINI and MONETTI, 1991; CASTAGNER et CAPPELLERI, 1991). For easier comparison, all the results were expressed as $g.hl^{-1}$ Absolute Alcohol (AA), making the necessary changes according to the alcoholic title of each distilled alcoholic beverage.

II - ETHANOL

The alcoholic title of nearly all -except for one- Tsipouro samples (table I) was found to be higher than the official European limit of 37,5 % vol (Reg. 1576/89). On the other hand, Tsikoudia samples present a rather low alcoholic title (P<0,01) (table I), often lower than 37,5 % vol but close to the national limit of 35 % vol allowed for the wine-grower's grape pomace distillate (Greek law 2989/2001). The variation of the alcoholic title, observed in table I, suggests that Tsipouro and Tsikoudia distillates need standardization and more systematic production in order to ensure a more homogenised product.

III - METHANOL

As it originates from crushed grapes, its concentration in the final distillate depends on the grape variety and the extraction time of the pomace (SILVA *et al.*, 1996; SILVA and MALCATA, 1998 and 1999).

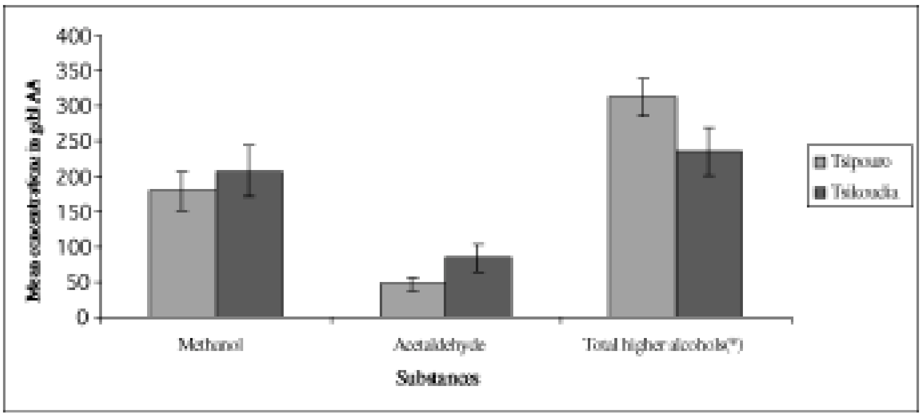
Because of its toxicity, methanol content in the alcoholic beverages from grape pomace must be lower than 1 000 $g.hl^{-1}$ AA (EEC 1576/89). Since all of the samples (table I ; figure 1) have concentrations much lower than this limit, it is unlikely that they may pose any health risks, even if they are consumed on a daily base; for this reason they are qualified for safe consumption. Since the spirits were found to be low in methanol we conclude that a second distillation or at least the early cut of the « tails » may have taken place, because methanol is a « tail» product (SOUFLEROS et BERTRAND, 1990). On the other hand, methanol levels lower than its perception threshold (table III) have a positive impact on this spirit quality.

The international literature (table II) demonstrated lower values of methanol for Tsipouro (SOUFLEROS et BERTRAND, 1987) and much higher mean values for Spain aguardiente (ORRIOLS and BERTRAND, 1990; CORTES *et al.*, 2002) and Portuguese bagaceiras (SILVA *et al.*, 1996; SILVA and MALCATA, 1998 and 1999), depending on the conservation state of grape pomace, the distillate fraction, the extraction time and grape variety. High concentrations of methanol in grape pomace distillates are, also, given by other authors (BAUER-CHRISTOPH *et al.*, 1997; CORDONNIER, 1971; AMERINE *et al.*, 1972; BERTRAND, 1975) (table II).

Mean values of methanol, higher than ours, are given for fruit distillates (apple, cherry, pear, plum and marc) (BAUER-CHRISTOPH *et al.*, 1997) and much lower, similar to ours, for mouro (SOUFLEROS *et al.*, 2004) (table II).

IV - HIGHER ALCOHOLS

They are produced during alcoholic fermentation and constitute the group with the highest concentration, which provides a pleasant aroma to the distillate (SILVA and MALCATA, 1999 ; FERREIRA *et al.*, 1999). For this reason, the minimum requirement of the European legislation (Reg. 1576/89) for these aromatic substances is higher than 140 $g.hl^{-1}$ AA. However, some alcohols (e.g. the amylic) in very high concentrations (table III) are responsible for certain toxicity. These compounds are concentrated in the first fractions of the distillates (SILVA and



(*) In the total higher alcohols are not included methanol and ethanol

Fig. 1 - Averages of volatile compounds in Tsipouro and Tsikoudia

Valeurs moyennes de composants volatils de Tsipouro et Tsikoudia

Table II - Concentrations of volatile and inorganic components of various distillate beverages, according to the international literature
(Concentration in g.hl^{-1} AA, except if there is a different indication)

Concentrations des composés volatils et des éléments inorganiques de diverses boissons alcoolisées, suivant la littérature internationale. Concentration en g.hl^{-1}, sauf autre indication

Compounds	Tsipouro (Soufleros and Bertrand, 1987 35*)	Bagaceiras (Silva *et al.*, 1996 34)	Bagaceiras (Silva and Malcata, 1998 32)	Bagaceiras (Silva and Malcata, 1999 31)	Aguardiente (Rogerson and Freitas, 2001 30)	Aguardiente good storage (Oriols and Bertrand, 1990 25)	Aguardiente bad storage (Oriols and Bertrand, 1990 25)	Aguardiente (Cortes *et al.*, 2002 11)	Grappa (Versini *et al.*, 1991 41)	Marc (Bauer-Christoph *et al.*, 1997 3)	Marc (Cordonnier *et al.*, 1971 10)	Marc (Amerine *et al.*, 1967 2)	Marc (Bertrand, 1975 4)	Marc (Lafon *et al.*, 1973 18)	Grape distillate (Castagner and Cappelleri, 1991 7)	Mouro (Soufleros *et al.*, 1994 38)
Methanol	50,4-84,0	755,0	1021-1031	346,77-3828	-	410	1545	1343	0,51	537	530-1590	39-2860	205-1157	-	0,2	145,74
1-butanol	-	5,1	0,56-2,27	0-6,16	-	-	-	2,7	2,55	2,5	-			-	1,0	7,41
2-methyl-1-propanol	29,2-65,2	80,0	77,1-85,1	28-131,0	-	109,2	105,2	76,2	70,0	66	-			-	63	24,85
2-methyl-1-butanol	31,6	62,2	39,2-50,3	11,1-56,2	-	60,7	35,3	36,2	48,0	-	-			-	41	33,67
3-methyl-1-butanol	116,25	204,4	86,8-103,6	35,2-201,6	-	216,0	122,7	125,2	181,5	-	-			-	195	145,18
1-hexanol	1,6-4,3	13,3	11,4-21,89	6,36-31,55	-	5,1	26,0	3,0	9,0	15,4	-			-	6,74	0,56
Trans-3-hexen-1-ol	-	0,22	0,21-0,27	0-0,13	-	-	-	-	0,18	-	-			-	0,15	0,01
Cis-3-hexen-1-ol	0,04-0,2	0,44	0,16-0,42	0,02-0,85	133-788,5	0,4	1,1	-	0,38	-	-			-	0,15	0,02
Trans-2-hexen-1-ol	-	0,04	0,038-0,044	0-0,23	-	-	-	-	0,19	-	-			-	0,07	0,01
2-phenylethanol	2,8-23,4	2,22	1,1-1,31	0,54-4,08	0,55-1,8	3,9	2,3	1,1	2,3	-	-			-	0,66	6,39
Acetaldehyde	4,7-40,8	133,0	69,9-86,3	3,9-135	-	-	-	7,6	115,0	-	-			80-400	3	44,84
Ethyl hexanoate	0,4-1,2	0,89	018-0,24	0,22-2,25	101,5-326	1,6	2,0	n.d.	1,85	-	-			-	0,65	0,39
Ethyl octanoate	0,8-4,0	2,44	0,10-0,45	0,25-1,84	0,69-1,57	6,3	0,5	n.d.	7,1	-	-			-	3,53	1,29
Ethyl decanoate	0,3-4,1	2,44	0,16-0,4	0,08-1,04	0,6-2,4	8,6	0,7	75,9	12,0	-	-			-	7,8	1,40
Ethyl myristate	-	-	-	-	-	-	-	n.d.	0,3	-	-			-	0,75	0,03
Isoamyl acetate	<10,0	1,33	0,4-0,96	0-0,91	183,5-398	1,2	1,6	3,3	2,54	-	-			-	0,8	0,32
Hexyl acetate	<0,5	0-0,07	0,09-0,21	0,06-1,35	-	0,06	0,6	-	0,2	-	-			-	0,03	0,00
2-Phenylethyl acetate	<0,5	0-0,07	0,004-0,05	0-0,92	10-49	0,12	0,25	n.d.	0,18	-	-			-	0,1	0,15
Ethyl acetate	58,0	44,4	314,7-494	45,1-853,8	-	215	436	118,4	163,0	-	230-330			100-280	26	185,62
Ethyl lactate	28-408,0	42,2	10,5-47,9	16,58-221,2	8-33,5	215	291	138,2	32,2	-	-			-	13,3	22,02
Diethyl succinate	1,6-8,6	1,11	0,41-0,56	0,25-12,2	0,56-1,46	2,2	0,9	n.d.	3,0	-	-			-	1,82	3,19
Butyric acid	-	-	-	-	-	tr	0,38	4196		-	-			-		0,07
Isobutyric acid	0-0,5	0,44	0,26-0,41	0-1,4	0-379,5	1,4	0,67	2,25		-	-			-		0,58
Isovaleric acid	0,1-0,6	0,67	0,19-0,20	0,034-1,94	0-556	1,3	0,85	2,7		-	-			-		0,30
Hexanoic acid	-	0,44	0,21-0,36	0,12-30	0-518	0,7	0,93	14,7		-	-			-		0,98
Octanoic acid	0,3-1,2	0,22	0,08-0,14	0,09-1,06	45,5-1662,5	1,9	0,1	21,2		-	-			-		0,41
Decanoic acid	0,2-1,4	0,89	0,17-0,36	0,04-1,6	-	2,2	0,1	24,7		-	-			-		0,66
Dodecanoic acid	0,1-0,4	0,89	0,16-0,36	0,02-0,37	0-116,5	0,5	0,1	2,7		-	-			-		0,83
Anethole	43,6-195,7	-	-	-	-					-	-			-		
Anisaldehyde	4,3-24,9	-	-	-	-					-	-			-		
Estragol	1,1-6,8	-	-	-	-					-	-			-		
Eugenol	0,1-1,8	-	-	-	1,7-2,45					-	-			-		

Parameter	Soufleros et Bertrand, 1987 35	Lehtonen *et al.*, 1999 19			Nascimento *et al.*, 1998 22			Muntean *et al.*, 1998 21		Camean *et al.*, 2001 5	Rodushkin *et al.*, 1999 29		Rizzon *et al.*, 1998 28	Vijayakumar *et al.*, 2001 42
	Tsipouro	Brandy	Whiskey	Rum	Cachaças	International beverages	Cognac	Distilled spirits	Industrial spirits	Brandy	Whiskey	Flavoured strong aperitifs	Cognac	Country liquor
pH	4,15-7,0	3,5	3,95	3,89	-	-	-	-	-	-	-	-	-	-
Fe (mg l^{-1})		-	-	-	0,009-2,24	0,013-1,28	-	-	-	0-2,3	0,068	0,0048	-	-
Ca (mg l^{-1})	-	0-14,8	-	-	1,36-44,6	9,6-27,2	-	-	-	-	-	-	11,04	-
Cu (mg l^{-1})	-	-	-	-	0-14,3	0-4,6	1,58	5	<1	0,30-5,31	0,23	<0,23	-	0,103-0,214
Pb (µg l^{-1})	-	-	-	-	0-421	0-600	420	-	-	8-224	0,0034	0,0011	-	295-486

* The numbers correspond to the reference order

MALCATA, 1998; SOUFLEROS et BERTRAND, 1990) and their levels are affected by grape variety, alcoholic title, fermentation conditions and distillation techniques.

The results (table I) show that the most important higher alcohols are 1-butanol, 2-methyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol. Concerning the two first alcohols, Tsipouro samples contain lower amounts of 1-butanol ($P<0,05$) and higher of 2-methyl-1-propanol ($P<0,01$) in relation to Tsikoudia.

The literature (table II) reported, for bagaceiras, aguardiente, grappa and grape distillate concentrations of 1-bu-tanol similar (SILVA *et al*., 1996) or lower (SILVA and MALCATA, 1998 and 1999; BAUER-CHRISTOPH *et al*., 1997; CORTES *et al*., 2002; VERSINI and MONETTI, 1991; CASTAGNER et CAPPELLERI, 1991) to ours, and the values of 2-methyl-1-propanol (SILVA *et al*., 1996; SILVA and MALCATA, 1998 and 1999; ORRIOLS and BERTRAND, 1990; CORTES *et al.*, 2002; VERSINI and MONETTI, 1991; CASTAGNER et CAPPELLERI, 1991) higher than ours; these contents depend on grape variety and extraction time. In previous works for Tsipouro (SOUFLEROS et BERTRAND, 1987) and marc (BAUER-CHRISTOPH *et al*., 1997) distillates, it was identified that the 2-methyl-1-propanol contents were similar to ours. Concerning the fruit distillates, it is reported that mouro distillate (SOUFLEROS *et al*., 2004) has presented mean concentrations of 1-butanol similar to Tsipouro and lower to Tsikoudia and for 2-methyl-1-propanol lower than ours (table II).

The mean contents of amylic alcohols [2-methyl-1-butanol ($P<0,01$) and 3-methyl-1-butanol ($P<0,1$)] were higher for Tsipouro than for Tsikoudia (table I) and for both of these distillates exceeded largely the perception threshold (table III), contributing positively to the aroma of the distillates. At the same time, because of the removal of head, their concentrations remain within acceptable limits (European commission, 2001).

The literature (table II) demonstrates that most of the results concerning the amylic alcohols of grape or grape pomace distillates coincide with ours (table I). The similarity of our distillate composition with those given for two Greek distillates (Tsipouro and Mouro) (table II), produced from raw material absolutely different, shows the significance of the alambic type and the technique of distillation on their chemical composition.

1-hexanol is an alcohol originating exclusively from grape pomace (SOUFLEROS et BERTRAND, 1987). The mean concentrations for Tsipouro and Tsikoudia distillates (table I), being higher than 0,5 $g.hl^{-1}$ AA (table III), are con-sidered to have a positive ef-fect on their aroma and taste (SOUFLEROS *et al*., 2004 ; FERREIRA *et al*., 1999). At the same time, with an average slightly higher of 1,0 $g.hl^{-1}$ AA, they are far below the levels (>10 g. hl^{-1}) that would reveal a « grassy » note. In previous work (SOUFLEROS et BERTRAND, 1987) on Tsipouro, 1-hexanol concentrations ranged from 1,6 to 4,3 g.hl-1AA. All other values, given by the international literature (table II) for marc or grape distillate and fruit distillates, are more or less higher than ours. This alcohol is typically a « heart » product and for this reason its content could not have been affected by the distillation technique.

2-phenylethanol is an aromatic compound, which introduces to distillates a pleasant aroma, resembling the rose. Higher contents of it result from carbonic anaerobiosis (SILVA and MALCATA, 1998 and 1999 ; SOUFLEROS *et al*., 2001; STARK *et al*., 2001). 2-phenylethanol values were lower than its perception threshold (table III) for both Tsipouro and Tsikoudia samples probably owing to early cutting of the « tails », since this substance comes out at the end of distillation. The fact that Tsikoudia samples presented three times higher mean values, than Tsipouro (table I) ($P<0,01$), shows that for Tsikoudia distillates the « tails » were cut at lower alcohol levels than Tsipouro. Similar results were also demonstrated by SOUFLEROS and BERTRAND (1987) for Tsipouro dis-tillates, which with those referring to the mouro distillate (SOUFLEROS *et al*., 2004) are the biggest one (table II) and confirm once more the influence on their composition of alambic type and distillation technique, used in Greece. However, other authors reported mean values (table II) directly related to grape variety (SILVA and MALCATA, 1999), extraction time (SILVA *et al.*, 1996; SILVA and MALCATA, 1998), distillate fractions (SILVA and MALCATA, 1999) and marc state of con-servation (ORRIOLS and BERTRAND, 1990).

Generally, all the distillate samples satisfy the threshold of 140 $g.hl^{-1}$ AA given by the European legislation for higher alcohols (table I; figure 1) and have concentrations within desirable limits (CANTAGREL *et al.*, 1998) (table III). However, the lower concentrations of total higher alcohols in Tsikoudia samples ($P<0,05$) are due to the fact that the « tails » are cut later during its distillation so that Tsikoudia, in contrast to Tsipouro, is poorer in « head » products (alcohols) and richer in « tail » products (alcohols).

5) Acetaldehyde

It is a compound derived from the fermented raw materials and increased during distillation and spirit aging (SILVA and MALCATA, 1998). It is also considered to be mainly the result of spontaneous or microbial mediated oxidation (SILVA and MALCATA, 1999). According to our results (table I ; fig. 1), acetaldehyde mean concentration for Tsipouro is half in relation to that for

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Table III - Perception threshold for some compounds and their effect on the quality of the distillates.

Seuil de perception de certains composés et leur effet sur la qualité des distillats

	Perception Threshold g.hl^{-1} AA	Perception Threshold mg.l^{-1} 40 % vol	Effects	Bibliographic References
Alcohols				
Ethanol % vol				
Methanol	1000	4000	(-)	25, 40
1-butanol				
2-methyl-1-propanol				
2-methyl-1-butanol	6	24	(+) Aroma > 6 g.hl^{-1}, (-) Aroma < 6 g.hl^{-1}	35, 25
3-methyl-1-butanol	6	24	(+) Aroma > 6 g.hl^{-1}, (-) Aroma < 6 g.hl^{-1}	35, 25
1-hexanol	0.5	2	0,5 < (+) < 10 g.hl^{-1}, (-) >10 g.hl^{-1}	35, 40
Trans-3-hexen-1-ol				
Cis-3-hexen-1-ol				
Trans-2-hexen-1-ol				
2-phenylethanol	32	125	(+) Roses aroma	35, 12
Total higher alcohols			250 < (+) < 600 or 875 g.hl^{-1}	35, 6
Acetaldehyde	6 – 7	25	(-) Sharp smell/ (+) fruity character	12
Esters				
Ethyl acetate	50 - 300	200-1200	(-) Solvent or glue nuances in higher concentrations	19
Isoamyl acetate			(+) Banana-like aroma	26
Hexyl acetate			(+) Apple-like aroma	26
Phenylethyl acetate			(+) Rose-like aroma	26
Ethyl hexanoate				
Ethyl lactate			(+) Fixation of fruity and floral aroma	13
Ethyl octanoate				
Ethyl decanoate				
Ethyl myristate				
Diethyl succinate				
Acids				
Butyric acid	2,72*10-5	1,09*10 -4	(-) Rancid butter or cheese	39, 33
Isobutyric acid	3,5*10-6	1,4*10 -5	(-) Rancid butter or cheese	13
Isovaleric acid	5,0*10-6	2*10 -5	(-) Rancid butter or cheese	13
Hexanoic acid				
Octanoic acid	4	15	Attenuated contribution to aroma	35, 26
Decanoic acid	2	8	Attenuated contribution to aroma	35, 26
Dodecanoic acid				
Anise compounds				
Anethole, trans				
Anisaldehyde				
Estragol		50 µg.Kg^{-1} person-1	(-) > 50 µg.Kg^{-1} person-1	Council of Europe, 2001
Eugenol				
Inorganic elements				
Ca (mg.l^{-1})				
Fe (mg.l^{-1})				
Cu (mg.l^{-1})		1 to 3 or 5	Tolerated	21, 44
Pb (µg.l^{-1})		250-300 µg.day-1	Tolerated	21

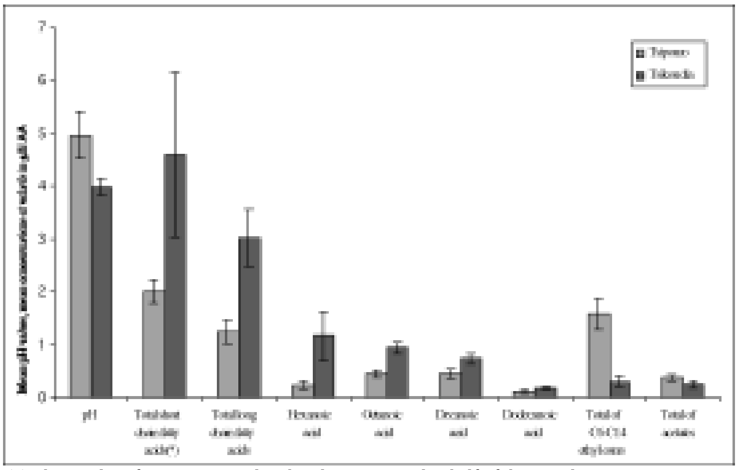
(+) Positive effect, (-) Negative effect

Tsikoudia (P=0,05) and both are superior to its perception threshold (De ROSA and CASTAGNER, 1994) (table III). These results show that most Tsipouro samples correspond to values lower than the official European limit (73-500 g.hl^{-1} AA) (Reg. 1576/89) but those of Tsikoudia are near that limit. This means that both distillates are produced under the conditions and techniques rather appropriate to limit the presence of high levels of acetaldehyde. Really, the cut of « head » during the distillation can remove the high content of this substance, because it comes out early. Comparing the acetaldehyde contents with those of ethyl acetate, it is worth noting that, in contrary, Tsipouro is richer in ethyl acetate than Tsikoudia. This observation suggests that acetaldehyde is more likely a result of glucidic fermentation (VERSINI and MONETTI, 1991) than of oxidative degradation of ethanol to acetic acid. Similar (SOUFLEROS et BERTRAND, 1987; SILVA and MALCATA, 1998 and 1999) or higher (SILVA *et al*., 1996; VERSINI and MONETTI, 1991) levels of acetaldehyde were given for marc distillates from other authors (table II).

6) Esters

The majority of these substances is considered to be secondary products of the alcoholic fermentation and contributes positively to the flavor of the wines and spirits and is responsible for their fruity and floral smell (SILVA and MALCATA, 1998 and 1999; FERREIRA *et al*., 1999 ; SOUFLEROS *et al*., 2001). However, some others like ethyl acetate, ethyl lactate and diethyl succinate are mainly produced by bacterial alteration of various raw material components and they prevail in acidic character, lowering the quality of the distillates. Ethyl hexanoate, ethyl octa-noate and ethyl decanoate pass through distillation to the distillates and increases during aging (SILVA and 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 bound after fermentation (CAUMEIL, 1983). These three esters have quantitatively a small part as compared to the higher alcohols, but they contribute to a fine aromatic character to the spirits (FERREIRA *et al*., 1999).

As mentioned in table I, the mean values of the ethyl esters hexanoate, octanoate (P<0,01) and decanoate (P<0,01) are slightly higher or much higher for Tsipouro than for Tsikoudia (fig. 2). Taking into consideration that these three ethyl esters are located in the « tail » fractions and that, in the case of Tsikoudia, the « tail »-cut occurred relatively late during the distillation, we waited to have higher concentrations from these three compounds. According to ORIOLS and BERTRAND (1990), the concentrations of the long chain fatty acid esters (C6, C8, C10 and C12) in distillates produced from badly conditioned grape pomace are low, in contrast to well conditioned grape mass which presents higher concentrations. In a previous work for Tsipouro, SOUFLEROS and BERTRAND (1987) reported higher values for ethyl hexanoate and much higher values for ethyl octanoate and ethyl decanoate. Similar or slightly higher results for these three ethyl esters have been given by SOUFLEROS *et al.* (2004) in regard to a Greek fruit distillate (mouro), produced with the same technique. Concerning the other marcs and grape distillates (table II), most of them (SILVA *et al*., 1996; SILVA and MALCATA, 1999; VERSINI and MONETTI, 1991; CASTAGNER et CAPPELLERI, 1991) have mean values for these three esters higher than ours, while in another case (ROGERSON and FREITAS, 2001) the values for ethyl hexanoate were extremely high. The authors attributed the results to the grape variety and extraction time (SILVA and MALCATA, 1998 and 1999) and, on the other hand, to the conservation state of the grape pomace (ORRIOLS and BERTRAND, 1990).



(*) These values for Tsipouro and Tsikoudia correspond to half of the actual content (devised by 2)

Fig. 2 - Averages of pH and volatile compounds in Tsipouro and Tsikoudia

Valeurs moyennes du pH et de composants volatils de Tsipouro et Tsikoudia

Generally, Tsipouro and Tsikoudia have low concentrations of ethyl esters (table I) because these compounds, appearing in the « tail » fractions (CORTES *et al*., 2002), were eliminated in a big part during the early « tail »-cut.

Isoamyl acetate, hexyl acetate and phenylethyl acetate constitute the acetic acid esters that are mostly responsible for the flowering and fruity aroma of distillates (SILVA and MALCATA, 1999; FERREIRA *et al*., 1999). Table I shows that isoamyl acetate has the highest mean values of 0,31 and 0,23 g.hl^{-1} AA for Tsipouro and Tsikoudia correspondingly. The other two acetates have trace levels (fig. 2), because they are located in the « tail »

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fractions and consequently eliminated during the « tail »-cut or the second distillation.

Except for a study (table II) on aguardiente (ROGERSON and FREITAS, 2001), which gives amounts considered to be extremely higher, the international literature presents, for bagaceiras (SILVA *et al*., 1996; SILVA and MALCATA, 1998 and 1999), aguardiente (ORRIOLS and BERTRAND, 1990; CORTES *et al*., 2002), marc (BAUER-CHRISTOPH *et al*., 1997; VERSINI and MONETTI, 1991) and grape (CASTAGNER et CAPPELLERI, 1991) distillates, values lightly higher. The variations observed depended mostly on the grape varieties (SILVA and MALCATA, 1999) used and the extraction time (SILVA and MALCATA, 1998 and 1999). On the other hand, SOUFLEROS and BERTRAND (1987) gave, for Tsipouro, values lower than 1,0 $g.hl^{-1}$ AA for isoamyl acetate, while SOUFLEROS *et al.* (2004) for mouro distillate, have found even lower mean concentration of 0,32 $g.hl^{-1}$ AA.

Ethyl acetate, ethyl lactate and diethyl succinate derive mainly from bacterial spoilage of the distilled marc (SOUFLEROS et BERTRAND, 1987; SILVA and MALCATA, 1998 and 1999). Ethyl acetate is the ester with the highest concentration, which above the perception threshold of 180 $g.hl^{-1}$ AA (table III) gives to the spirit an unpleasant flavor (SILVA and MALCATA, 1998) and acidic character (FERREIRA *et al*., 1999). The mean value of ethyl acetate in Tsipouro samples is considered to be relatively high and three times higher than those of Tsikoudia ($P<0,05$) (table I). Ethyl lactate presents a yeast extract, wet and bakery in character to the distillate, whereas, diethyl succinate is fusel-like (FERREIRA *et al.*, 1999). On the other hand, SOUFLEROS and BERTRAND (1987) note that ethyl lactate, at relatively low amounts, stabilises the odor and smoothens the firm character of certain substances. Ethyl acetate is found in the « head » fractions, while ethyl lactate and diethyl succinate were distilled within the « tail » products.

SOUFLEROS and BERTRAND (1987), for Tsipouro (table II), demonstrated concentrations for ethyl acetate lower than ours, very high values for ethyl lactate and for diethyl succinate lightly higher then ours.

About ethyl acetate, comparing our results (table I) with those of the literature (table II), we conclude that it was present at concentrations considered rather acceptable for Tsikoudia but relatively or very high for Tsipouro. This means that the storage of the raw material (grape pomace) for Tsipouro was done in defective conditions and that undesirable acetic fermentation took place. In contrast, ethyl lactate and diethyl succinate were found in low concentrations. These two substances, appearing in the « tail » fractions, were proportionally eliminated during the early « tail »-cut or the second distillation.

According to literature (table II), ethyl acetate, ethyl lactate and diethyl succinate concentrations increase within the time of grape pomace fermentation (SILVA and MALCATA, 1999). In contrary, pH lowering of the grape pomace (SILVA and MALCATA, 1998) leads to lower contents of these substances.

7) Volatile acids

Short chain fatty acids (butyric, isobutyric, isovaleric) are minor compounds but often with a strong smell equal to acetic acid (SILVA and MALCATA, 1999; SOUFLEROS *et al.*, 2001) that is reminiscent of rancid butter or cheese (CORTES *et al*., 2002) and consequently with an important contribution to the aroma (table III) of distillates (SILVA and MALCATA, 1999; FERREIRA *et al.*, 1999). For this reason, they are usually indicative of the low quality of the raw material that has been distilled (ORRIOLS and BERTRAND, 1990).

Regarding the Tsipouro samples (table I), the total average for these three volatile acids is 4,01 $g.hl^{-1}$ AA (fig. 2) with isovaleric acid constituting of this total. The most concentrated acid, for Tsikoudia, is butyric acid ($x=7,28$ $g.hl^{-1}$ AA) with a total mean concentration for these three acids equal to 9,09 $g.hl^{-1}$ AA (table I ; figure 2). Their concentration can be influenced by the storage conditions of the marc and by the distillation technique (« tail »-cut or second distillation), since these compounds are mainly products of « tail ».

According to table III, the presence of short chain fatty acids in these concentrations, which are far above their perception threshold, decreases the distillate quality (DESAUZIERS *et al.*, 2000).

The mean levels concerning isovaleric acid of Tsipouro samples and butyric acid of Tsikoudia (table I) were higher than all the concentrations given in table II, except for those reported by ROGERSON and FREITAS (2001). Short chain fatty acid contents increase as a consequence of extended fermentation (SILVA and MALCATA, 1999) of grape pomace, improper conditions of storage (ORRIOLS and BERTRAND, 1990; DESAUZIERS *et al.*, 2000) and origin of distillate from « tail » fraction (SILVA and MALCATA, 1999).

On the other hand, long chain fatty acids (hexanoic, octanoic, decanoic and dodecanoic) (figure 2) seem to have an effect on the spirit aroma more attenuated (SOUFLEROS et BERTRAND, 1987; SILVA and MALCATA, 1999; SOUFLEROS *et al*., 2001). The mean values of these compounds, in contrast to their ethyl esters, are slightly lower in Tsipouro than in Tsikoudia ($P < 0,01$)

(table I ; figure 2) and in both cases lower than their perception thresholds (table III).

Except for ROGERSON and FREITAS (2001) who, for aguardiente, reported extremely high concentrations of hexanoic, octanoic and dodecanoic acid (table II), the international literature presents, for marc (SILVA *et al.*, 1996; SILVA and MALCATA, 1998 and 1999; ORRIOLS and BERTRAND, 1990; BAUER-CHRISTOPH *et al.*, 1997; CORTES *et al.*, 2002; VERSINI and MONETTI, 1991) and grape (CASTAGNER et CAPPELLERI, 1991) distillates, values generally similar or relatively higher than ours. The variations observed in table II depend mostly on the type of distillate sample (SOUFLEROS et BERTRAND, 1987; SILVA *et al.*, 1996; ROGERSON and FREITAS, 2001; SOUFLEROS *et al.*, 2004; ORRIOLS and BERTRAND, 1990; BAUER-CHRISTOPH *et al.*, 1997; VERSINI and MONETTI, 1991; CASTAGNER et CAPPELLERI, 1991), the grape varieties used (SILVA and MALCATA, 1999), the skin contact time (SILVA and MALCATA, 1998 and 1999) and distillation technique (CORTES *et al*., 2002) (whether « tail » fractions are removed or not).

8) Anise compounds

These compounds are the flavoring substances delivered by the *Pimpinella anisum* and other aromatic plants used during distillation. Trans-anethole, anisaldehyde, estragol and eugenol are the most important. Usually the ratio of trans-anethole and anisaldehyde is 10:1 for a natural extract and it is well known that anisaldehyde occurs mostly from anethole's oxidation.

Estragol is an aromatic, strongly flavored and caustic aldehyde, which mostly originates from aromatic plants. Its concentration in essential oils is for sweet fennel 5-20 %, for anise star 5-6 % and for anise 1 % (European commission, 2001). According to the Committee of Experts on Flavoring Substances (CEFS) of the Council of Europe, estragol is considered as «…a naturally occurring genotoxic carcinogen in experimental animals after chronic exposure or after a few repeated doses…». For this reason, «…a limit of 0,05 mg.Kg^{-1} per person (detection limit) is recommended…» (Office of environmental health hazard assessment, 1999). The Scientific Committee on Food of the European Commission (2001), concludes : « Estragol has been demonstrated to be genotoxic and carcinogenic. Therefore, the existence of a threshold cannot be assumed and the Committee could not establish a safe exposure limit. Consequently, reductions in exposure and restrictions in use levels are indicated…».

Eugenol is a flavorful compound found in carnation flowers. According to FERREIRA *et al.* (1999), it is an aromatic substance adding to wines and spirits a nearly liquorice, fruity like peach, cinnamon aroma and flavor. Estragol and eugenol are also considered to be substances of Pimpinella anisum extract (LIDDLE and BOSSARD, 1984).

From our results (table I), it occurs that the Tsipouro samples -with only one exception- were aromatized with anise plants, whereas, this is not the case for Tsikoudia samples. Usually, in Tsikoudia production no aromatic plants are used.

Anise compounds in Tsipouro (table I) confirm the aforementioned ratio (10:1) between t-anethole and anisaldehyde, while estragol content seems to be higher than that of eugenol. However, the estragol content does not

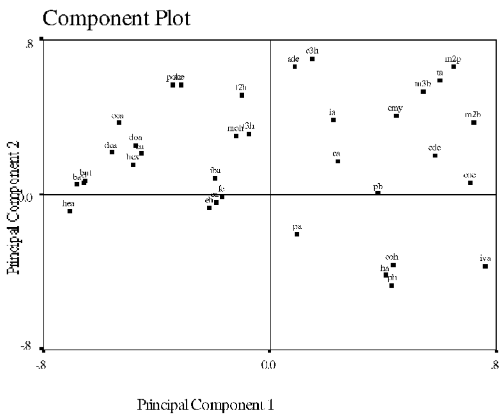

Fig. 3 - Loading plot of the compounds found in the Tsipouro and Tsikoudia samples, except anise ones.
The compound code names are given in table I.

Représentation des composés trouvés dans les échantillons de Tsipouro et de Tsikoudia, excepté pour les substances anisées.
Les codes des composés figurent dans le tableau I.

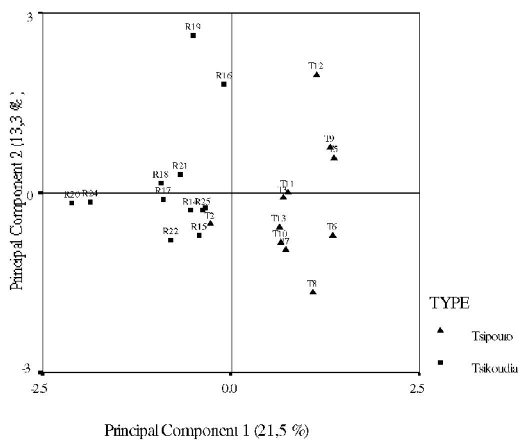

Fig. 4 - Loading plot of Tsipouro and Tsikoudia distillate samples

Représentation des échantillons de distillats Tsipouro et Tsikoudia

exceed the maximum limit for alcoholic beverages proposed at the European Council by the French Committee (20 ppm) and on the other hand by the European Union of Spirit Producers and also by French Industry of Foods (100 ppm).

For the anise components, few researchers studied the marc distillates. SOUFLEROS and BERTRAND (1987), for Tsipouro, gave for trans-anethole, anisaldehyde, estragol and eugenol (table II) values higher than ours. Even higher concentrations, for eugenol, were given by ROGERSON and FREITAS (2001) for aguardiente.

pH. The pH mean values were 4,96 for Tsipouro and 3,98 for Tsikoudia ($P<0,05$) (table I). This pH difference is in accordance with the important variations found between the concentrations of the volatile acids (fig. 2). According to many authors (SOUFLEROS et BERTRAND, 1987; LAFON *et al.*, 1973; CAUMEIL, 1983) these pH values characterise distillates of 1-2 years old. Researches for Tsipouro (SOUFLEROS et BERTRAND, 1987) and for aguardiente (ORRIOLS and BERTRAND, 1990) presented pH values similar to ours, while others (LEHTONEN *et al.*, 1999) gave for brandy the lowest pH value, followed by whiskey and rum (table II).

9) Inorganic elements

The importance of the metal analysis is due to the toxicity of Cu, Pb that mostly derives from the alambics. In general, the inorganic elements in food and drinks might originate from the soil and fertilisers, the equipment containers and utensils used for food processing, storage or cooking and the industrial activity (MUNTEAN *et al.*, 1998; KARADJOVA *et al.*, 1998). Sometimes the inorganic elements might also originate from water, which is used for dilution of the distillate.

a) Fe

Values of iron appear to be lower for Tsipouro than for Tsikoudia distillates (table I). These values, in most cases, are lower in comparison to the results (table II) reported for cachaças (NASCIMENTO *et al.*, 1999) (Brazilian sugar cane spirit) and other international spirits as well as for brandy (CAMEAN *et al.*, 1998). Even lower mean values (RODUSHKIN, 1999), which are similar or less than ours, were demonstrated for whiskey and flavored strong aperitifs.

b) Ca

Its presence in the spirit is due to the dilution of the distillate with hard water rich in salts. Table I shows that, except a sample of Tsikoudia, there are no significant difference of calcium content in comparison to Tsipouro. Calcium concentrations similar to those found in our distillate samples were reported for cognac (RIZON *et al.*, 1998), for cachaças spirit (NASCIMENTO *et al.*, 1999) and for international beverages (NASCIMENTO *et al.*, 1999) (table II), while a smaller range was issued for brandy (LEHTONEN *et al.*, 1999).

c) Cu

A traditional alambic is entirely made from copper due mainly to its specificity to bind numerous malodorous volatile substances, optimising in this manner the distillate's aroma. Therefore, the principal sources of Cu to the distillates are the alambics and the funnel of the freezer, while lower concentrations of copper come from the treatment of the grapes with $CuSO_4$. The copper mean content in Tsikoudia is almost double than in Tsipouro ($P<0,05$) (table I). These contents of copper are similar to those reported for the traditional cachaças (NASCIMENTO *et al.*, 1999) and for distilled spirits (MUNTEAN *et al.*, 1998), but higher than the content cited for other industrial alcoholic beverages (cognac, brandy, whiskey, flavored strong aperitifs, etc) (NASCIMENTO *et al.*, 1999; MUNTEAN *et al.*, 1998; CAMEAN *et al.*, 1998; RODUSHKIN, 1999) or country liquors (VILAY-KUMAR and ASHWINI, 2001) (table II).

According to the Brazilian legislation, copper concentration in distillates is limited to 5,0 $mg.l^{-1}$ (Portaria L.371, 18/09/74). The same limit is also adopted from the Latvia Republic (Reg. L101, 6/03/2001) (Republic of Latvia Cabinet, 2001). Research data show that the daily intake of copper for normal adults' diet is usually between 1 and 3 mg (MUNTEAN *et al.*, 1998), doses which correspond to the intake levels recommended by most authorities (WHO, 1973 and 1984). Based on our results, copper levels could not be harmful in any way, unless extreme consumption of the distillates is observed. However, the producers must take care of the cleanliness of the alambics and standardization of the product.

d) Pb

The lead in the distillates is coming mainly from the repaired parts of alambics. The daily intake (MUNTEAN *et al.*, 1998) of lead for an adult through his food and drinks usually comes up to 250-300 micrograms. These amounts were adopted from Latvia Republic (Reg. L. 101,6/03/2001) as a higher limit of the daily human intake.

Tsipouro distillates presented values higher than those of Tsikoudia ($P<0,05$) (table I).

The concentrations of lead (table I) are in concordance with the majority of the values given in the bibliography (NASCIMENTO *et al.*, 1999; CAMEAN *et al.*, 1998; VILAYKUMAR and ASHWINI, 2001) (table II) and those adopted from many authorities (table III). Only in a few (2-3) samples of Tsipouro lead concentrations correspond to values much higher than the allowed limit of 250-300 micrograms per day. However, it is considered that the usual daily consumption of a distilled alcoholic beverage is 50-100 ml. These Tsipouro samples can be consumed without any health hazards. Nevertheless, this analysis showed the necessity of a proper control in alambic state and safety and homogenous quality of produced distillates.

DISCRIMINATION BETWEEN TSIPOURO AND TSIKOUDIA

Principal Component Analysis (PCA) was applied to the whole data set, except for the anise compounds, and explained 34,8 % of the variability in the data in the first two dimensions. PC1 accounted for 21,5 % and PC2 for an additional 13,3 % of the variability. Anise compounds were excluded because they are met only in Tsipouro. Although the first two components explained a low percentage of the variability, the discrimination between Tsipouro and Tsikoudia samples was almost perfect. Thus, in the plot of the scores in the coordinate plane, it defined by the principal components of the first two functions (figure 4) and the samples of Tsipouro (Ti), with one exception, are positioned on the right part of the plot, while samples of Tsikoudia (Ri) are positioned on the left part. The separation of the distillates may be ascribed:

a) to isovaleric acid, higher alcohols (2-methyl-1-butanol, 2-methyl-1-propanol and 3-methyl-1-butanol) and ethyl octanoate, which had high loadings to the positive part of PC1 (fig. 3) and are mainly responsible for the positioning of Tsipouro samples at the right part of the plot. The compound code names are given in table I.

b) to hexanoic acid, butyric acid and 1-butanol, which had high loadings to the negative part of PC2 (fig. 3) and appear to be connected with Tsikoudia samples (fig. 4). As they were located further to the left, distillates R20 and R24 had the highest butyric acid and ethyl lactate content, so these compounds are related to lactic bacteria development. Generally, in figure 3, the compounds locating in the upper left quadrant mainly have either disagreeable aroma or a negative effect on wine quality, such as hexanol, methanol, 1-butanol, acetaldehyde and ethyl lactate. Additionally, in the same quadrant fatty acids are gathered (fig. 3) including copper. This suggests that the alambics used for distillation are not properly cleaned and consequently the blue mixture, consisting of fatty acids and copper, may pass to the distillate. 2-phenylethanol is probably the only compound – in this quadrant - that has a pleasant aroma. This is due to the fact that this compound is one of the « tail » products. All the other compounds that are considered positive - like higher alcohols, isoamyl acetate and most of the ethyl esters, which are associated with fruity and floral aromas - are located at the upper right quadrant. Tsipouro distillates contain higher levels of the « positive compounds » (table I).

As regards to Tsikoudia distillates, the presence of methanol, 2-phenylethanol, ethyl lactate, hexanol and other « tail » compounds in high levels suggests that these samples had undergone a single distillation without « tail » cutting or this cutting happened at very low alcohol degree. The opposite stands for Tsipouro samples, which appear to contain less « tail »-compounds.

Figure 3 shows that the horizontal axis is related to inorganic elements, as the three out of four inorganic elements are positioned along this axis. The elevated contents of iron (Fe-) and copper (Cu) found in Tsikoudia and high lead (Pb) levels found in Tsipouro greatly contributed to properly discriminating between the two distillates.

CONCLUSIONS

The composition analysis of Tsipouro and Tsikoudia revealed significant variations to their alcoholic content, emphasising the fact that the distillation process of these alcoholic beverages needs standardization. Generally, Tsipouro has a higher alcoholic title than Tsikoudia ($P<0,01$). Methanol and acetaldehyde mean contents are higher in Tsikoudia samples than in Tsipouro ones, but without significant differences; these contents, in all cases, are lower than the European limits (Reg. 1576/89).

The total concentration of higher alcohols was higher than the European threshold of 140 $g.hl^{-1}$ AA and lower (in all cases) than 600 $g.hl^{-1}$ AA (Average < 313 $g.hl^{-1}$ AA). Amylic alcohols, a major part of higher alcohols, rarely exceeded 300 $g.hl^{-1}$ AA. On the contrary, the marginal concentrations of 2-phenylethanol, isoamyl acetate and ethyl esters with C6, C8, C10 and C14, responsible for the flowering and fruity aromas, were relatively low.

The relatively high concentration of ethyl acetate (>300 $g.hl^{-1}$ AA) and ethyl lactate in some of the samples analysed shows the necessity of limiting unwanted fermentations in the grape pomace, a precaution that could improve the distillation process.

Regarding volatile acids, it is shown that short-chain acids, usually indicative of the low quality of the grape pomace, are present in higher concentrations than long-chain acids and that Tsikoudia samples contain higher amount of volatile acids, mainly those with long chain ($P<0,01$), compared to Tsipouro.

The variability, which is observed in the concentrations of volatile acids among the samples, can be attributed mainly to the conservation state of raw material, at the time of the « tail »-cut performing and in the appli-

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cation or not of the second distillation, since these components in their majority are products of « tail ».

The relative similarity in volatile composition between our results and in previous works for two Greek distillates, Tsipouro (from grape pomace) and mouro (from fruit pomace), indicate the important effect that the use of the same technique of distillation and the same type of alambic have on their chemical composition.

The anise compounds are present only in Tsipouro samples. From this group, estragol - which due to its toxicity is currently being discussed in the European Council - was found in concentrations much lower than those proposed by the European Union of Spirit Producers.

The inorganic element profile of the distillates shows that the elements causing a health risk due to their toxicity (Cu and Pb) were found, in most cases, in low concentrations. Finally, PCA applied to the whole data set has confirmed the compositional differences between Tsipouro and Tsikoudia and allowed for their discrimination.

The overall perception is that the domestic marc distillates produced by the Greek winegrowers need a continuous quality control and a more systematic approach in order to be successfully scaled-up, a procedure that will contribute to the finished product quality and help increase the producer's income. Adequate conditions during fermentation and storage of the grape pomace, redistillation and the removal of « head » and « tail » fractions would improve the marc distillate quality.

Acknowledgements: The authors wish to thank Professor Keramidas Vissarion for his support providing a flame atomic absorption spec-trometer for inorganic element analysis. They also thank all those who provided the distillate samples for our analysis.

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Manuscrit reçu le 12 novembre 2004 ; accepté pour publication, après modifications le 9 mars 2005