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Analytical Methods

Discrimination of varietal wines according to their volatiles

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ABSTRACT

A method is being proposed in order to discriminate bottled wines of different varieties when no other information is known. The advantages of the method consist in the fact that anyone who wants to certify the variety, which is written on the label or the area of origin, can use such a technique to achieve the conformity. Additionally, the method can be easily applied by laboratories equipped with a GC. The differentiation has been achieved by using only seven of the total extracted volatiles, mainly higher alcohols and higher alcohol esters, namely 3-methyl-1-butanol, 2,3-butanediol, ethyl lactate, 3-methyl-1-butyl acetate, 2-phenylethanol, phenyl ethyl acetate and p-hydroxy phenyl ethanol. These key compounds are not relevant to a single variety. The proposed method does not take into account variables such as the year of vintage and fermentation procedures (agitation, temperature).

1. Introduction

Traceability and origin identification become increasingly important when referring to foodstuffs. As far as wine is concerned, vine variety and origin are, among other factors, criteria that determine quality and commercial added value.

Various methods have been proposed in order to identify the origin of the various wines. Discrimination of Spanish wines according to their geographical origin has been achieved using Stepwise Discriminant Analysis (Huerta-Diaz-Reganon, Salinas, & Masoud, 1997). In a similar way, French red wines have been classified using multivariate analysis based on chemical data (acids, alcohols, esters, total phenols, pH, and colour) (Sivertsen, Hollen, Nicolaysen, & Risvik, 1999). Wines of Ribeira Sacra Certified Brand of Origin (CBO) have been differentiated from wines of two others CBOs in Galicia, using multivariate chemometric techniques and trace elements analysis data (Latorre, García-Jares, Mèdina, & Herrero, 1994). Other authors investigated the composition and concentration of volatiles (at the germplasm level) in 42 grape cultivars belonging to seven genotypic groups using headspace solid-phase microextraction with GC–MS in order to improve the fruit quality by understanding effects of fruit aroma (Yang et al., 2009). Determination of volatile compounds from wines made with seven clones of Monastrell grapes was performed using ultrasound extraction of the compounds and Linear Discriminant Analysis (Gómez-Plaza, Gil-Muñoz, Carreño-Espín, Fernández-López, & Martínez-Cutillas, 1999).

In wines, production of higher alcohols is influenced by the amino acid composition of the grapes and the yeast strain. As claimed by Rapp and Versini (1995), there is a strong correlation between the amino acid spectrum in must and the absolute and relative levels of higher alcohols in wine. The variation of amino acid profiles in must depend on variety, fertilisation, composition of soil and other factors related to ecological and environmental conditions (Rapp & Versini, 1995). Previous attempts to discriminate wines based only on amino acids (glutamic, aspartic, proline, leucine, alanine and serine) were not successful (Rapp & Versini, 1995).

According to Riberéau-Gaynon, Dubourdieu, Donèche, and Lonvaud (1998), 10% of higher alcohols come from corresponding amino acids through transamination, decarboxylation and hydrogenation. Another 25% are derived from the sugar skeleton and the remaining 65% from other amino acids. Based on this, it has been suggested that the composition of higher alcohols is close

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related to the amino acid pattern of must or grapes. The higher alcohols used for discrimination of wines, come mainly from three amino acids; leucine is the main precursor of 3-methyl-1-butanol and its acetate, phenylalanine of 2-phenylethanol and tyrosine of p-hydroxy phenyl ethanol (=tyrosol) (Dall'Asta et al., 2011; Dickinson, 2008; Yang et al., 2009).

Regarding the relationships of higher alcohols to amino acids, linearity was assessed for 3-methyl-1-butanol and total free amino acids in must and found to be significant (>95%) (Rapp & Versini, 1995). Rapp and Versini (1995) observed the same significance positive linear correlation between 3-methyl-1-butyl acetate and total free amino acids. However, this correlation is observed only within the same vintage year. In the case of 2-phenylethanol, the increase in free amino acids concentration in must leads to a decrease in 2-phenylethanol with the correlation significant at more than 95% (Rapp & Versini, 1995).

Alcohols and polyols of 93 red wines produced from the grape varieties Cabernet Sauvignon, Tempranillo, Monastrell and Bobal were analysed by Discriminant Analysis (Aleixandre, Lizama, Álvarez, & García, 2000). These authors showed that isoamyl alcohol (Cabernet Sauvignon), cis-3-hexenol and isobutyl alcohol (Tempranillo), methanol and cis-3-hexenol (Monastrell), and 2,3-butanediol (Bobal) were the most important components in differentiation of the varieties.

Concerning statistical analysis of the results Principal Component Analysis (PCA) has been also used to study other foodstuffs besides wines, discriminating Robusta and Arabica coffees (Casal, Oliveira, Alves, & Ferreira, 2000) as well as different blends (Bicchi, Panero, Pellegrino, & Vanni, 1997). Also, PCA and discrimination analysis (DA) were used to identify olive oil adulteration with various other seed oils, discrimination of animal fats from vegetable oils, and animal fat adulteration with seed oils as well as olive oils on the basis of their geographical origin (Dourtoglou, Dourtoglou, Diamadopoulou, & Lalas, 2013).

In this work a method is being proposed to allow discrimination of bottled wines made from different grape varieties when no other information is known, based on the volatiles present in the wine. The driving idea was to use extracted volatiles instead of amino acids (which are influenced by many factors) in must or in wine. The method was applied to two varietal wines, Agiorgitiko and Moschofilero (one red and one white), which are cultivated mainly in the Peloponnese (southern Greece) in delimited area. The volatiles used were higher alcohols and associated esters. The dataset from GC analysis was subjected to PCA and DA. Additional samples from other individual wine varieties (Xinomavro, Cabernet Sauvignon, Chardonnay), a mixed wine (made of Roditis, Savattiano, Cabernet Sauvignon, Merlot, Pinot Noir, Sauvignon Blanc, Robola and Vilana varieties), and ferment model solutions were used for comparison in order to test the discrimination potential of the proposed method.

2. Materials and methods

2.1. Wine samples

Commercial bottled wines were purchased from a local shop, which was able to confirm variety and geographical origin. A1–A15 were different wines of the Agiorgitiko variety (Group 1 – Agiorgitiko) while M1–M11 were various wines of the Moschofilero variety (Group 2 – Moschofilero). The brands and the vintages of all wines used are presented in Table 1.

Additionally, an experimental wine (MIX) was prepared in order to test the discrimination ability of the method. It was made of 57% of Roditis (white Greek variety), 38% of Savattiano (white Greek variety) and 5% of Cabernet Sauvignon, Merlot, Pinot Noir, Sauvignon Blanc, Robola (Greek variety) and Vilana (Greek variety). In MIX no extraction of compounds from the grape skins has taken place (produced as white wine). For the production of MIX, after pressing the grapes, the must was transferred to a stainless steel tank. All fermentation procedures were carried out under controlled temperature ranging from 16 °C to 17 °C. The must was fermented by its native strains (no addition of commercial *Saccharomyces cerevisiae* strains). The MIX, Xinomavro (XIN) (a red variety originated from the northern part of Greece) and Cabernet Sauvignon (S1) made up a third group (Group 3 – Other). A fourth group (Group 4 – Chardonnay) was made up of Chardonnay wines (CT, CA, CW, CJ, CP).

Table 1
Brand names of wines.

Code	Name	Vintage
M1	Mantinia Spyropoulos	1997
M2	Mantinia Megapanos	1997
M3	Mantinia Megapanos	1998
M4	Mantinia Cavino	1997
M5	Mantinia Vinifera	1998
M6	Mega Oinos Skouras	1998
M7	Paraskevopoulos	1999
M8	Mantinia Antonopoulos	1997
M9	Mantinia Spyropoulos	1998
M10	Mantinia Tselepos	1997
M11	Boutaris	1998
A1	Paraskevopoulos 14%vol	1999
A2	Paraskevopoulos 12.8%vol	1999
A3	Paraskevopoulos rose	1999
A4	Paraskevopoulos native strain	1999
A5	Skouras	1997
A6	Epilogi	1996
A7	Leontios Oinos	1995
A8	Nemea Reserve Cambas	1994
A9	Kouros	1996
A10	Kourtakis	1998
A11	Chateau Gaia	1997
A12	Chateau Gaia	1998
A13	Chateau Gaia	1999
A14	Paraskevopoulos	2000
A15	Paraskevopoulos	2000
XIN	Naousa Boutari	1998
S1	Cabernet Sauvignon	1997
CT	Tselepos Chardonnay	1999
CA	Antonopoulos Chardonnay	1999
CW	Wente Chardonnay California	1999
CJ	Jacob's Creek Chardonnay Australia	1999
CP	Papantoniou Chardonnay	1999

Three *S. cerevisiae* (SC) strains from Mantinia and Nemea Region (production areas of Moschofilero and Agiorgitiko wines, respectively) were isolated, purified and cultivated using standard procedures. These strains were used to ferment model solutions (sugar solutions) containing 20 g/L sucrose, 1% meat peptone and 1% yeast extract. Each SC strain fermented two identical solutions, and the volatiles produced were analysed by GC.

All procedures were conducted at the experimental winery of the Technological Educational Institution of Athens (Greece), at the Faculty of Food Science, Department of Oenology and Beverage Technology.

2.2. Extraction method

For the extraction of volatiles from wines or SC-fermented sugar solutions the following procedure was used: 20 g of NaCl were added into 100 g of wine, which was then extracted twice, using a mixture of 100 mL of pentane and 100 mL of diethyl ether. Where an emulsion was created during the extraction, 10 mL of saturated solution of NaCl was added. The organic layer was dried with Na_2SO_4, filtered through paper and, finally, the solvent was removed by heating in a water bath at 50–60 °C using a pear-form flask equipped with a Vigreux column 20–25 cm long.

In all cases, the residue was 0.2 g after the evaporation of the solvents. Provided the extraction procedure is the same for all samples, the loss of volatiles should be the same, maintaining the relative ratio. The extraction method applied in this work was not typical (Ferreira, Rapp, Cacho, Hastrich, & Yavas, 1993; Guth, 1997; Schneider, Baumes, Bayonove, & Razungles, 1998; Schultz et al., 1977). However, as denoted by Schneider et al. (1998), the recovery of components from model hydroalcoholic and real wine systems was 96–100%. Quantities of the extracted volatile compounds were calculated based on recovery of 3-octanol.

2.3. Analysis of volatiles

The initial identification of compounds was performed by GC/MS analysis using a Hewlett-Packard 6890 gas chromatograph connected to a Hewlett-Packard 5973 series mass selective detector operating in EI (Electron Impact) mode. A Hewlett-Packard HP-1 fused silica capillary column (30 m length, 0.32 mm i.d., 0.25 μm film thickness) (Hewlett-Packard, UK) was used for the analysis of all wine samples. The sample volume was 0.1 μL and the split ratio 1:100. The injector temperature was set at 180 °C. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The analysis was conducted according to the following program: hold at 50 °C for 3 min, raise from 50 °C to 80 °C at 2 °C/min and from 80 °C to 240 °C at 3.5 °C/min, and, finally, hold at 240 °C for 5 min. Mass spectra were interpreted by spectral matching, using either the mass spectrometer data system library (NIST 2000), or other collections of reference spectra. Linear retention indices (LRI values) of compounds were obtained by reference to a series of standards, run under the same conditions. Compound identifications were confirmed, by matching, using either the mass spectrometer data system library or other reference collections. Linear retention indices (LRI values) for compounds were obtained by reference to a series of standards run under the same conditions. Compound identification was confirmed by comparing experimental LRI values with those of authenticated compounds.

The GC/FID analysis was carried out using the same column for all analyses. The sample volume was 0.1 μL and the split ratio 1:57. The injector temperature was set at 220 °C and the FID temperature at 280 °C. The analysis was conducted as described above. Nitrogen was used as the carrier gas at a flow rate of 2.5 mL/min.

2.4. Statistical analysis

For all samples, two bottles were analysed twice and the results are presented as average of the four analyses (Maximum Standard Deviation 10%). The data set, which was composed of values obtained from GC analysis, was used for PCA and DA. The statistical program SPSS v10.0.7 for Windows (SPSS Inc., USA) was used to calculate and plot data from PCA and DA.

Seven compounds from wines (3-methyl-1-butanol, 2,3-butanediol, ethyl lactate, 3-methyl-1-butyl acetate, 2-phenylethanol, phenyl ethyl acetate and p-hydroxy phenyl ethanol) were selected by Discrimination Analysis and were used as key compounds. The analytical data of volatiles were subjected to Principal Component Analysis (PCA). The compounds were selected in order to evaluate the predictive accuracy of the discrimination analysis and a classification matrix was calculated. The results were cross validated, meaning that each case was classified by the functions derived from all cases other than that case. That was because the original results may provide overly optimistic estimates and cross validation attempts to remedy this problem (SPSS, 1999). The classification results were compared with wines that could be classified correctly by chance, according to the maximum chance criterion, taking into account their group sizes. Maximum chance criterion is a measure of predictive accuracy in the classification matrix that compares the percentages correctly classified with the percentage of respondents in the largest group (Hair, Anderson, Tatham, & Black, 1995).

Table 2
Concentrations of volatile compounds in Agiorgitiko wines (mg/100 g of wine).

Code	Compound	Wines									
	Name	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
C1	3-Methyl-1-butanol	12.564	7.551	11.872	10.743	12.648	10.270	6.376	8.229	12.152	4.312
C2	Acetyl methyl carbinol	1.639	1.948	1.948	1.948	1.948	1.948	1.061	3.285	1.948	1.808
C3	Ethyl isobutyrate	0.118	0.764	0.103	0.050	0.252	0.252	0.122	0.545	0.252	0.060
C4	Butanediol	4.725	2.596	2.490	6.135	3.468	3.420	3.149	3.626	4.639	3.663
C5	2-Methylpropanoic acid	1.745	0.884	0.729	1.838	1.629	1.012	1.350	1.319	1.829	1.367
C6	Ethyl lactate	4.094	3.267	7.418	4.047	4.179	7.841	5.185	3.466	4.759	3.407
C7	3-Methyl-1-butyl acetate	0.630	0.887	0.931	1.009	0.897	1.289	0.224	0.510	0.675	0.309
C8	2.3-Butanediol	1.483	6.509	1.938	1.704	2.583	2.965	3.096	1.835	3.005	2.946
C9	3-Methyl thiopropanol	0.199	0.254	0.324	0.221	0.307	0.300	0.245	0.125	0.239	0.180
	3-Octanol (IS)	11.450	10.700	11.500	10.700	11.300	11.950	12.300	12.000	12.000	12.300
C10	2 Hydroxy pentanoic acid	0.657	0.228	0.234	0.291	0.354	0.267	0.216	0.157	0.301	0.301
C11	Iso amyl lactate	1.289	2.056	3.034	0.849	0.896	1.488	0.526	0.314	1.306	1.306
C12	2-Phenylethanol	6.389	6.551	5.823	4.098	9.466	5.012	5.724	3.978	5.445	5.210
C13	Diethyl butanedioate	2.589	3.411	1.981	1.736	2.239	2.199	1.191	0.701	1.350	0.609
C14	Monoethyl butanedioate	4.991	4.192	4.351	6.536	16.036	12.376	11.741	9.626	13.383	9.679
C15	Phenyl ethyl acetate	0.576	0.566	0.363	0.188	0.350	0.329	0.188	0.109	0.334	0.334
C16	Ethyl-p-hydroxy phenyl propionate	0.274	0.255	0.319	0.238	0.365	0.507	0.335	0.205	0.272	0.308
C17	Di iso amyl butanedioate	2.393	2.025	2.370	1.345	3.604	1.732	2.060	1.293	1.862	1.796
C18	p-Hydroxy phenyl ethanol	1.200	1.760	1.112	0.449	0.708	0.528	0.355	0.205	0.733	0.281
C19	Phenyl ethyl lactate	0.185	0.295	0.384	0.093	0.291	0.650	0.294	0.176	0.296	0.296
C20	Monoamyl butanedioate	0.295	0.379	0.694	0.190	0.580	0.364	0.224	0.389	0.389	0.389
C21	*n*-Acetyl tyramine	0.459	0.668	0.283	0.210	0.211	0.307	0.208	0.157	0.313	0.313
C22	Hexyl butanedioate	0.181	0.172	0.228	0.116	0.233	0.220	0.181	0.181	0.181	0.116
C23	Indole-3-ethanol	0.288	0.359	0.417	0.136	0.365	0.208	0.196	0.240	0.240	0.188
C24	*n*-Amino acetyl tyramine	0.189	0.189	0.189	0.248	0.189	0.189	0.143	0.187	0.189	0.180
C25	Phenyl ethyl butanedioate	0.288	0.253	0.222	0.442	0.270	0.237	0.220	0.217	0.338	0.180
C26	Ethyl-p-hydroxy cinnamate	0.255	0.284	0.115	0.221	0.169	0.200	0.122	0.152	0.305	0.180
C27	p-Hydroxy cinnamic acid	0.154	0.154	0.156	0.151	0.154	0.154	0.154	0.154	0.154	0.154

Table 3
Concentrations of volatile compounds in Moschofilero wines (mg/100 g of wine).

Code	Compound	Wines						
	Name	M1	M2	M3	M4	M5	M6	M7
C1	3-Methyl-1-butanol	4.772	6.743	4.487	5.665	6.622	14.038	9.159
C2	Acetyl methyl carbinol	0.645	0.859	1.540	1.140	1.922	1.140	0.732
C5	2-Methylpropanoic acid (=iso butyric acid)	1.039	1.480	1.024	0.709	0.847	1.676	0.497
C6	Ethyl lactate	4.664	3.894	0.282	2.056	0.502	0.997	2.931
C7	3-Methyl-1-butyl acetate	0.328	0.222	0.139	0.098	0.102	0.520	0.572
C8	2.3-Butanediol	0.329	0.263	0.158	0.225	0.211	0.391	1.465
	3-Octanol (IS)	9.828	11.200	10.600	15.200	12.350	11.600	12.800
C11	Iso amyl lactate	1.320	0.196	0.824	0.395	0.824	0.144	2.064
C12	2-Phenylethanol	2.443	3.176	2.078	2.905	1.567	4.347	3.888
C13	Diethyl butanedioate	2.614	5.901	0.318	4.489	0.553	0.921	0.546
C14	Monoethyl butanedioate	4.994	9.889	3.922	3.240	4.612	5.591	2.604
C28	Ethyl octanoate	0.233	0.355	0.771	0.375	0.375	0.375	0.140
C15	Phenyl ethyl acetate	0.307	0.263	0.092	0.166	0.194	0.194	0.140
C29	Diethyl hydroxy butanedioate	0.160	0.255	0.074	0.166	0.109	0.162	0.154
C16	Ethyl-p-hydroxy phenyl propionate	0.251	0.882	0.252	0.671	0.129	0.241	0.117
C17	Di iso amyl butanedioate	0.816	1.803	1.423	1.181	0.672	1.888	1.419
C18	p-Hydroxy phenyl ethanol (=tyrosol)	0.282	0.666	0.666	1.682	0.400	0.394	0.569
C21	*n*-Acetyl tyramine	0.196	0.146	0.073	0.115	0.126	0.126	0.100
C23	Endole-3-ethanol	0.255	0.255	0.230	0.212	0.255	0.255	0.323
C26	Ethyl-p-hydroxy cinnamate	0.245	0.547	0.098	0.174	0.245	0.245	0.162

3. Results and discussion

The proposed method was designed to discriminate bottled wines of different varieties when no other information is known. The advantages of the method consist in the fact that anyone who wants to certify the variety, which is written on the label or the area of origin, can use such a technique to achieve the conformity. Additionally, laboratories equipped with a GC and common basic equipment can easily apply this technique. This method does not take into account variables such as the year of vintage or the fermentation procedures (agitation, temperature). Finally, the variables used are independent of typical characteristics of varieties such as terpenic or other compounds present in the wine. During the proposed method differentiation was achieved using only seven of all the extracted volatiles. These were mainly higher alcohols and higher alcohols esters, namely 3-methyl-1-butanol

Table 4
Key volatile compounds that have been identified through GC in all wine samples. Concentrations are expressed in mg/100 g of wine.

Code	Compounds						
	Isoamyl alcohol	Ethyl lactate	Iso amyl acetate	2,3-Butanediol	2-Phenyl ethanol	Phenyl ethyl acetate	p-Hydroxy phenyl ethanol
A1	12.564	4.094	0.63	1.483	6.389	0.576	1.2
A2	7.551	3.267	0.887	6.509	6.551	0.566	1.76
A3	11.872	7.418	0.931	1.938	5.823	0.363	1.112
A4	10.743	4.047	1.009	1.704	4.098	0.188	0.449
A5	12.648	4.179	0.897	2.583	9.466	0.35	0.708
A6	10.27	7.841	1.289	2.965	5.012	0.329	0.528
A7	6.376	5.185	0.224	3.096	5.724	0.188	0.355
A8	8.229	3.466	0.51	1.835	3.978	0.109	0.205
A9	12.152	4.759	0.675	3.005	5.445	0.334	0.733
A10	4.312	3.407	0.309	2.946	5.21	0.334	0.281
A11	8.124	2.974	0.476	2.893	4.418	0.334	0.733
A12	9.143	1.307	0.358	1.645	3.752	0.334	0.733
A13	8.882	3.538	0.45	2.527	5.458	0.127	0.229
A14	9.421	3.543	0.39	2.115	5.25	0.05	0.197
A15	12.634	5.072	0.872	2.706	7.274	0.102	0.311
M1	4.772	4.664	0.328	0.329	2.443	0.307	0.282
M2	6.743	3.894	0.222	0.263	3.176	0.263	0.666
M3	4.487	0.282	0.139	0.158	2.078	0.092	0.666
M4	5.665	2.056	0.098	0.225	2.905	0.166	1.682
M5	6.622	0.502	0.102	0.211	1.567	0.194	0.4
M6	14.038	0.997	0.52	0.391	4.347	0.194	0.394
M7	9.159	2.931	0.572	1.465	3.888	0.14	0.569
M8	12.521	0.21	0.27	1.661	2.073	0.141	0.587
M9	14.055	1.202	0.423	1.43	2.743	0.095	0.656
M10	11.599	0.536	0.216	1.505	2.579	0.015	0.656
M11	13.354	0.584	0.718	1.28	4.414	0.132	0.656
XIN	11.146	3.71	0.718	1.83	6.816	0.184	0.279
MIX	16.823	3.127	0.252	3.885	7.133	0.105	0.15
S1	20.173	3.935	0.484	1.943	8.176	0.334	0.733
CT	11.795	2.982	0.334	2.02	3.816	0.051	0.122
CA	5.645	0.213	0.084	0.149	1.003	0.023	0.064
CW	10.896	5.81	0.379	1.22	2.095	0.031	0.176
CJ	3.159	1.566	0.092	0.927	1.12	0.03	0.171
CP	1.301	2.119	0.075	0.87	2.127	0.014	0.119

(=iso amyl alcohol), 2,3-butanediol, ethyl lactate, 3-methyl-1-butyl acetate (=iso amyl acetate), 2-phenylethanol, phenyl ethyl acetate and p-hydroxy phenyl ethanol. These key compounds are not relevant to only one variety like terpenes for Muscat varieties or pyrazines for Cabernet sauvignon. Additionally, the combination of all key compounds was not previously used for identification.

Agiorgitiko and Moschofilero samples were analysed for volatile content (only the first ten and seven are presented in Tables 2 and 3, respectively). A preliminary data matrix using the compounds common to both varieties was created (Table 4) and used to select those for discrimination analysis by means of PCA (Kellner, Mermet, Otto, & Widmer, 1998). Choosing eigenvalues greater than one (>1), the dimensionality was reduced to two Principal Components (PC) with 4.068 for the first and 1.425 for the second one, both explaining 78.471% of the total variance (PC1 = 58.116%, PC2 = 20.356%). Scores for the two Principal Components are plotted in Fig. 1A. This scatter plot shows the distribution of wines belonging to different varieties. The groups of Agiorgitiko (Group 1), Moschofilero (Group 2) and Chardonnay wines (Group 4) can be easily discriminated from one another. In comparison the third group is near the group of Agiorgitiko wines. This is probably because this group consists of samples from many different varieties thereby creating an anomaly in the group's properties.

When a simultaneous DA was applied to include all seven independent variables for the discrimination of four groups, three discriminant functions were deduced. The first canonical discriminant function (DF1) accounts for 73% of the total dispersion with a correlation of 0.868, which measures the association among the discriminant scores and the groups (SPSS, 1999). The second discriminant function (DF2) accounts for 16.7% of the total variance with a canonical correlation of 0.642. Finally, the third canonical discriminant function (DF3) accounts for 10.3% of the total dispersion with a correlation of 0.549 (total variance explained 100%). The scores of the first two canonical discriminant functions (DF1, DF2) were plotted with a cumulative of 89.7% (Fig. 1B and C).This plot suggests that DF1 is responsible for the discrimination

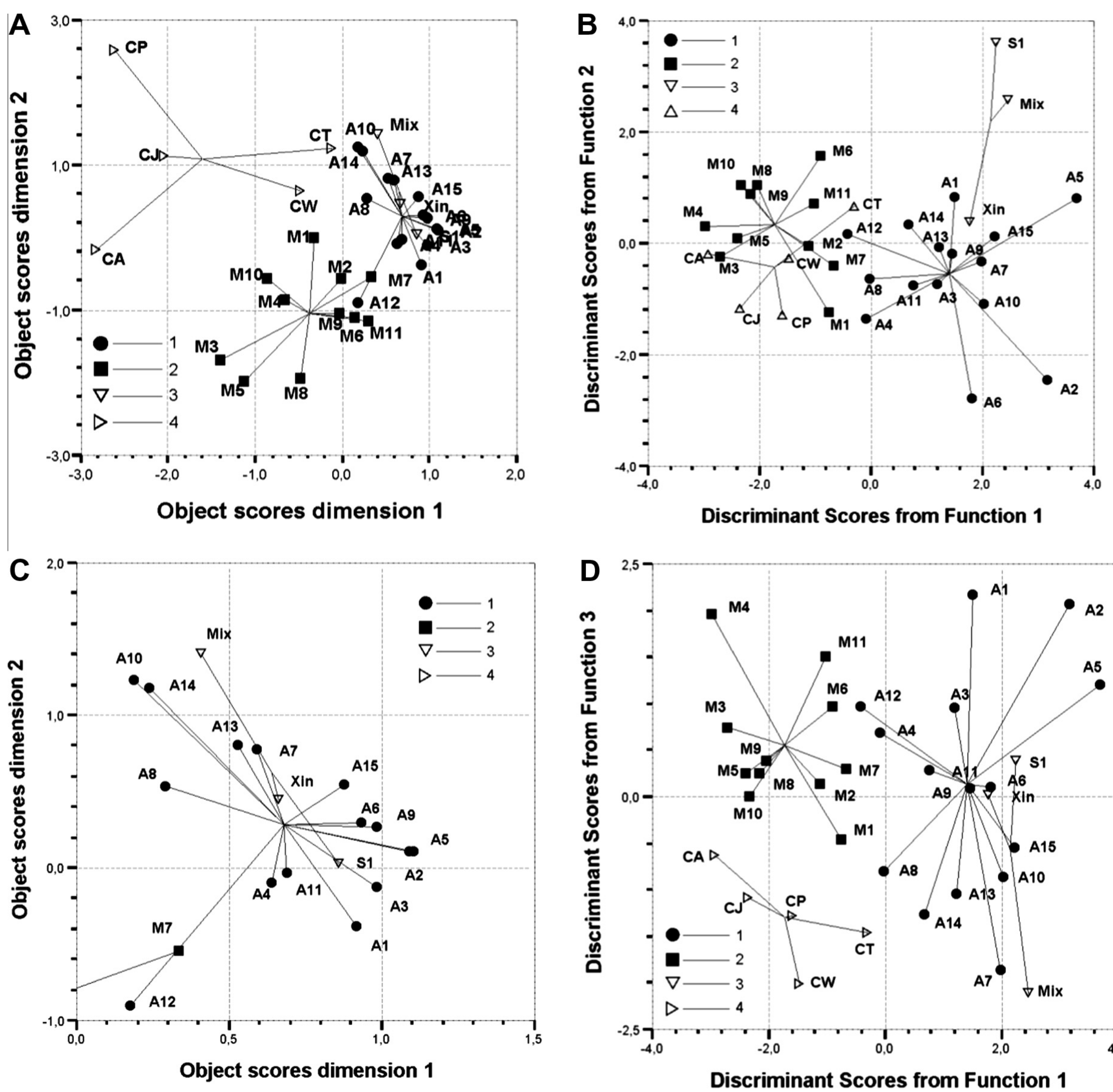

Fig. 1. (**A**) Scatter plot of objects Scores from PCA. Group 1, Agiorgitiko; Group 2, Moschofilero; Group 3, Other; Group 4, Chardonnay. PC1 = 58.116% of total variance, PC2 = 20.356% of total variance. (**B**) Scatter plot of Discriminant Scores from Functions 1 and 2, (DF1 vs. DF2). Group 1, Agiorgitiko; Group 2, Moschofilero; Group 3, Other; Group 4, Chardonnay. DF1 = 73.0% of total variance, DF2 = 16.7% of total variance. (**C**) Zoom of Objects Scores from PCA of Fig. 1B. (**D**) Scatter plot of Discriminant Scores from Functions 1 and 3, (DF1 vs. DF3). Group 1, Agiorgitiko; Group 2, Moschofilero; Group 3, Other; Group 4, Chardonnay. DF1 = 73.0% of total variance, DF3 = 10.3% of total variance.

Table 5
Classification results.[a,b]

		Group	Predicted group membership				Total
			1	2	3	4	
Original	Count	Agiorgitiko (1)	14	1	0	0	15
		Moschofilero (2)	0	10	0	1	11
		Other (3)	1	0	2	0	3
		Chardonnay (4)	0	1	0	4	5
	%	Agiorgitiko (1)	93.3	6.7	0	0	100.0
		Moschofilero (2)	0	90.9	0	9.1	100.0
		Other (3)	33.3	0	66.7	0	100.0
		Chardonnay (4)	0	20.0	0	80.0	100.0
Cross-validated	Count	Agiorgitiko (1)	12	2	1	0	15
		Moschofilero (2)	1	10	0	0	11
		Other (3)	1	0	2	0	3
		Chardonnay (4)	1	1	0	3	5
	%	Agiorgitiko (1)	80.0	13.3	6.7	0	100
		Moschofilero (2)	9.1	90.9	0	0	100
		Other (3)	33.3	0	66.7	0	100
		Chardonnay (4)	20.0	20.0	0.0	60.0	100

[a] 88.2% of original grouped cases correctly classified.
[b] 79.4% of cross-validated group cases correctly classified.

between Agiorgitiko and Moschofilero varieties, while DF2 is responsible for the discrimination of the third group (XIN, S1 and MIX). The scores of the DF1 and DF3 were plotted with cumulative of 83.3% (Fig. 1D). This suggested that Chardonnay wines can be discriminated by DF3.

According to the classification results, 88.2% and 79.4% of all original grouped cases were classified correctly before and after cross-validation. These percentages were greater than 44.1% that could be classified by chance, and, according to the maximum chance criterion, the discriminant model is acceptable. Table 5 shows the predicted members for each group. After cross-validation, 80.0% of Agiorgitiko wines were classified correctly, while that for Moschofilero wines was 90.9%, for Group 3 samples was 66.7% and for Chardonnay 60.0%.

Antonelli, Castellari, Zambonelli, and Carnacini (1999) proved that the composition of higher alcohols in wines can be affected by the yeast strain. For this reason, selected yeast strains from the area producing Agiorgitiko and Moschofilero have been used to ferment sugar solutions and the same key compounds were examined to determine whether DA can differentiate wines from sugar solutions. The results revealed that the strains examined were able to produce all the key compounds. Nevertheless, concentrations significantly differed (results not presented) from those in the real wine. This is in agreement with the work of Marchetti and Guerzoni (1987), who investigating 28 wines of different varieties and showed that the influence of the must on the production of higher alcohols was greater than that of 16 different yeast strains. Grape varieties differ from each other regarding the amount of certain common amino acids.

4. Conclusions

The method was able to discriminate bottled wines made with different grape varieties when no other information is available. It uses extracted volatiles instead of amino acids in must or wine, and can be applied easily by laboratories equipped with a GC and other common equipment. Differentiation was achieved using only seven of the volatiles extracted. These were mainly higher alcohols and higher alcohols esters namely, 3-methyl-1-butanol (=iso amyl alcohol), 2,3-butanediol, ethyl lactate, 3-methyl-1-butyl acetate (=iso amyl acetate), 2-phenylethanol, phenyl ethyl acetate and p-hydroxy phenyl ethanol. These key compounds are not specific to the variety. The proposed method does not rely on variables such as the year of vintage or fermentation procedures (agitation, temperature). In addition, the variables used are independent to the typical aroma volatiles characteristics of certain varieties such as terpenes for muscat type wines or pyrazines for Sauvignon.

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