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Differentiation of Young Red Wines Based on Chemometrics of Minor Polyphenolic Constituents

STAMATINA KALLITHRAKA,[†] ANDREAS MAMALOS,[‡] AND DIMITRIS P. MAKRIS*,[§]

Vine & Wine Institute, National Agricultural Research Foundation (N.AG.RE.F.) 1, S. Venizelou Street, 14123, Lycovrysi, Athens, Department of Chemistry, University of Athens, Panepistimiopolis, 15771, Zografou, Athens, and Department of Food Quality & Chemistry of Natural Products, Mediterranean Agronomic Institute of Chania (M.A.I.Ch.), P.O. Box 85, 73100, Chania, Greece

For reasons of examining any possible discrimination of young red wines based on selected, minor polyphenols, a survey was carried out including 35 samples originating from three Hellenic native and three international *Vitis vinifera* cultivars from various regions of Greece. All samples were experimental wines vinified and stored under identical conditions, in an effort to minimize the effect of different winemaking technologies as well as aging. The polyphenols analyzed belonged to two categories: benzoic acid derivatives, including gallic acid, protocatechuic acid, vanillic acid, and syringic acid, and stilbenes, including astringin, piceid, and resveratrol (all *trans* isomers). Data handling employing discriminant analysis (DA) yielded very satisfactory categorization of samples in terms of both cultivar and geographical region of origin. This outcome was discussed with regard to the value of certain minor polyphenols that could serve as characteristic indices for discrimination of varietal red wines, after appropriate implementation of chemometrics.

KEYWORDS: Benzoic acids; chemometrics; differentiation; discriminant analysis; polyphenols; red wines; stilbenes

INTRODUCTION

For the wine industry and market sector, it is particularly essential that the intended value traits created via genetics (variety), origin of production (typicité), and unique inputs or processing methods (vinification technology) are preserved. In other words, it must be ensured that a product's label is accurate and not misleading, since consumers distinguish peculiar commodities from a mass of other similar ones, on the belief that they bear a superior quality. However, wine is a product that can be easily adulterated, and for this reason, wine authenticity is guaranteed by strict guidelines laid down by responsible national authorities and includes sensory evaluation, chemical analyses, and examination of the records kept by wine producers.

The chemical composition of wines is of fundamental importance to quality control and authenticity, and in recognition of this fact, a large body of data regarding various classes of chemical constituents has been built. Nevertheless, meaningful interpretation of vast amounts of such information, to allow for a credible assessment of quality and authenticity, becomes impractical or even problematic when conventional statistical approaches are employed. Nowadays, the implementation of suitable multivariate statistical analyses, so-called "chemometrics", has been proven a versatile and valuable tool for the assurance of wine authenticity and quality (*1*). Several inorganic elements (*2*−*5*) and organic wine constituents (*6*, *7*), as well as data from sensory studies (*8*), have been the basis for differentiation of wines according to vinification technology or classification according to region and variety.

The polyphenolic profile of a given grape cultivar and, consequently, the wines produced from it are subject to tight genetic (varietal) control, but environmental parameters, including the type of soil, rainfalls, sun exposure, etc., may be of equal importance in this regard. Therefore, the polyphenolic composition can be the platform for reliable wine discrimination via chemometrics, and this option has been very well-illustrated by recent studies (*9*). The study presented herein had as an objective to examine the possibility of using several minor polyphenols as critical parameters for categorizing young, nonaged, varietal wines according to region of origin and cultivar, thus eliminating the impact of oak wood, as well as the reactions that might take place during aging in an oak barrel. Furthermore, vinification and storage of all samples were carried out under identical conditions, to ascertain the minimal influence deriving from implementation of techniques or conditions that could substantially alter the polyphenolic composition.

* To whom correspondence should be addressed. Tel: +30 28210 35056. Fax: +30 28210 35001. E-mail: dimitris@maich.gr.
[†] National Agricultural Research Foundation.
[‡] University of Athens.
[§] Mediterranean Agronomic Institute of Chania.

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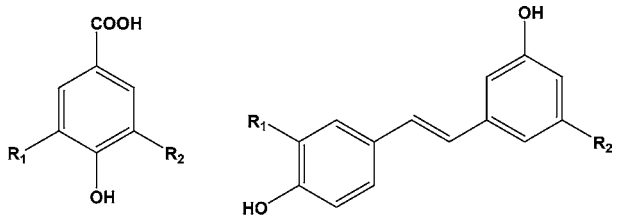

R_1, H; R_2, OH: Protocatechuic acid
R_1, H; R_2, OCH_3: Vanillic acid
R_1, OCH_3; R_2, OCH_3: Syringic acid
R_1, OH, R_2, OH: Gallic acid

R_1, H; R_2, OH: Resveratrol
R_1, H; R_2, OGl: Piceid
R_1, OH; R_2, OGl: Astringin

Figure 1. Structures of benzoate and stilbenic phytochemicals considered in this study.

The two categories of phenolics chosen for this study are, at least directly, biosynthetically irrelevant with all of the other groups used in a previously published work (*10*). This means that they can be influenced by different factors, with proportional results to their usefulness in differentiating wines. One simple example is *trans*-resveratrol. This stilbenic metabolite, unlike all other phenolics, is synthesized in response to infection or other stresses of the grape berry; thus, its presence in wine could be significantly affected by such events (*11*). Thus, it could potentially reflect environmental stimuli (onto which discrimination can be based with regard to the area of origin) that do not affect, at least in a direct manner, compounds such as flavanols, proanthocyanidins, or anthocyanins. On the other hand, there are no reports for microbiological alteration of either stilbenes or benzoates in red wines. On the basis of the previously mentioned concept, the phytochemicals selected may provide a reliable basis for a chemometric analysis. If this is so, then the whole analysis could be greatly facilitated, since the determination of seven indices could be carried out, instead of 19 used in our published study (*16*). Other combinations including different polyphenol groups could also be a matter of investigation, to choose the most appropriate indices for a rapid and reliable differentiation.

MATERIALS AND METHODS

Chemicals. Water for high-performance liquid chromatography (HPLC) analyses was nanopure. Acetonitrile and trifluoroacetic acid were of HPLC grade (Mallinckodt Baker, Deventer, Holland). Gallic acid, protocatechuic acid, vanillic acid, syringic acid, astringin, piceid, and resveratrol (**Figure 1**) were from Sigma Chemical Co. (St. Louis, MO).

Wine Samples. All samples examined were young (nonaged) wines produced and stored under identical conditions. Briefly, grapes were harvested at technological maturity, based on indices of sugar content and acidity established by the Vine & Wine Institute, destemmed, and crushed. Musts were inoculated with selected yeast strain (*Saccharomyces cerevisiae* var. *cerevisiae*) and fermented under controlled temperature (26−30 °C) to dryness (reducing sugar content, <4 g L^{-1}), with a pomace contact period of 7 days. Following this, wines were racked, SO_2 was added (30 mg L^{-1}), and they were bottled and stored at 15 ± 2°C in the dark for no longer than 3 months.

HPLC Analysis. Equipment consisting of a HP 1050 chromatography apparatus coupled to a HP 1100 diode array detector was employed. Analyses were performed on a Lichrospher, 5 μm, 250 mm × 4 mm, at a flow rate of 1 mL min^{-1}, using a 20 μL injection volume. Detection was accomplished at 280 and 310 nm, for benzoates and stilbenes, respectively. Details concerning eluents and elution program were analytically described elsewhere (*12*). Identification was based on comparing retention times of the peaks detected with those of original compounds and on UV−vis on-line spectral data. Quantification was carried out using external standard, and results were expressed as mg L^{-1}.

Table 1. Origin of Grapes Used for the Production of Experimental Wines[a]

cultivar	code	sample no.	area	location
Merlot	M003	1	Drama	Eastern Macedonia
	M007	2	Imathia	Western Macedonia
	M013	3	Kavala	Eastern Macedonia
	M021	4	Arkadia	Peloponnese
Cabernet Sauvignon	M004	1	Phthiotida	Sterea Ellada
	M034	2	Messinia	Peloponnese
	M009	3	Larisa	Thessaly
	M051	4	Arkadia	Peloponnese
Syrah	MAR029	1	Karditsa	Thessaly
	MAR005	2	Attica	Sterea Ellada
	OIN022	3	Lakonia	Peloponnese
	MAR024	4	Karditsa	Thessaly
	OIN031	5	Florina	Western Macedonia
	OIN032	6	Pieria	Western Macedonia
	OIN033	7	Kilkis	Central Macedonia
	MAR038	8	Drama	Eastern Macedonia
Agiorgitiko	ASP040	1	Attica	Sterea Ellada
	ET-AG	2	Attica	Sterea Ellada
	EYX	3	Attica	Sterea Ellada
	TEI	4	Korinthia	Peloponnese
	M041	5	Argolida	Peloponnese
	LAK	6	Lakonia	Peloponnese
Xinomavro	OIN052	1	Florina	Western Macedonia
	M030	2	Magnesia	Thessaly
	OIN041	3	Imathia	Western Macedonia
	OIN047	4	Imathia	Western Macedonia
	OIN051	5	Imathia	Western Macedonia
	M059	6	Kilkis	Central Macedonia
	M048	7	Pieria	Western Macedonia
	MAR046	8	Imathia	Western Macedonia
Mandilaria	M028	1	Paros	Aegean Isles
	M018	2	Heraklio	Crete
	M019	3	Heraklio	Crete
	M027	4	Rhodes	Aegean Isles
	M057	5	Rhodes	Aegean Isles

[a] Letters N, C, and S denote Northern, Central, and Southern Greece, respectively.

Sample Collection and Experimental Design. The grapes used for the production of wine samples were from the six most cultivated red varieties (*Vitis vinifera* sp.) and originated from major viticultural areas of Greece that cover the entire Hellenic vineyard (**Table 1**), with defined soil composition, cultural practices, and climatic conditions. Samples were grouped geographically according to the vicinity of the region of origin with emphasis to peculiar microclimatic characteristics, which could potentially have a prominent impact of the polyphenolic composition (**Figure 2**). These specific areas were assigned as A1 (prefectures of Imathia, Pieria, Kilkis, Thessaloniki, Florina, Magnesia, Larisa, and Karditsa), A2 (prefectures of Attica, Phthiotida, and Evia), A3 (prefectures of Kavala and Drama), A4 (prefectures of Arkadia, Messinia, Lakonia, Argolida, and Korinthos), and A5 (the isles of Rhodes, Paros, and Crete).

Statistics and Data Processing. HPLC analyses were performed in duplicate, and data are given as average values ± standard deviation (SD). The average values of the obtained data set were subjected to discriminant analysis (DA). Samples were distributed to a predetermined number of groups, resulting in a number of discriminant functions (DFs) equal to the number of groups minus one. The maximization of the ratio variance between groups-to-variance of samples within the same group is a principle selection criterion during the calculation of the functions. SPSS 10 was used to calculate the DFs and plot the data for the DA.

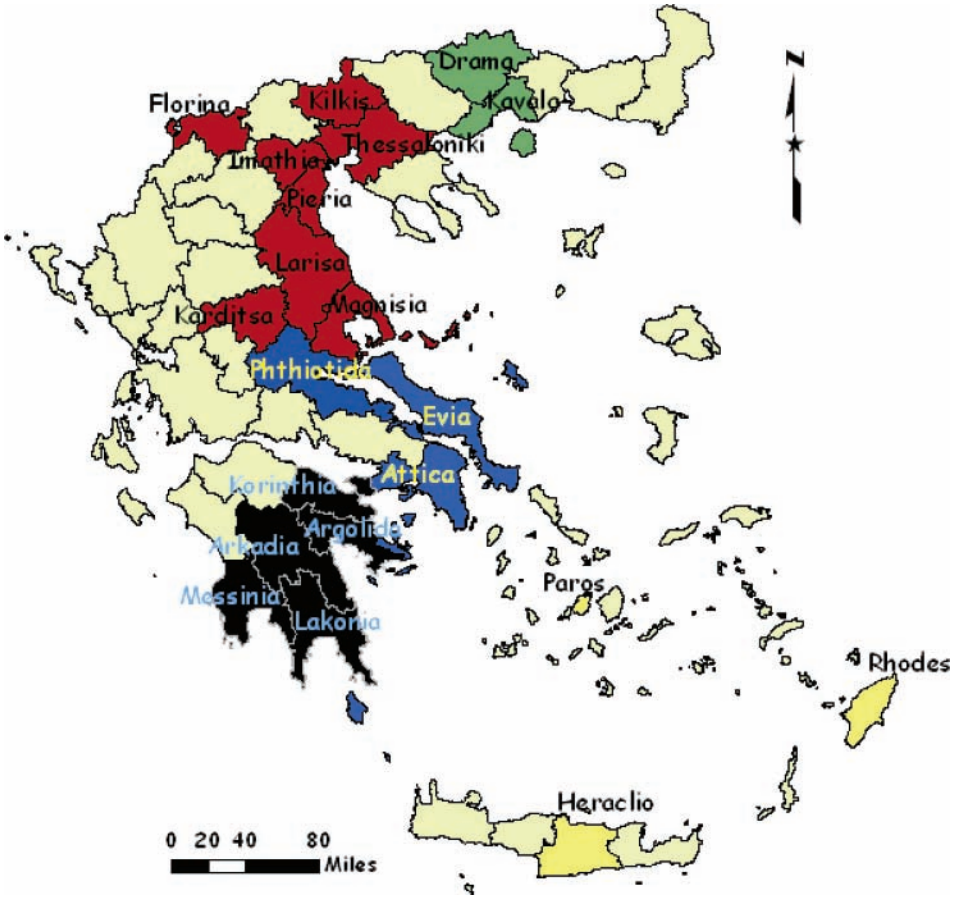

Figure 2. Map illustrating the viticultural areas used for geographical origin-based differentiation. Reproduced with permission from ref *10*. Copyright 2006 Elsevier. The varietal composition of each viticultural area may be seen in **Table 1**.

RESULTS

Cultivar-Based Differentiation. The sets of data given in **Tables 2** and **3** composed the data matrix. Application of DA on the sample groups using all seven variables (phenolics) resulted in five DFs. The first, assigned as DF1, accounted for 68.1% of total variability, while DF2 and DF3 accounted for 20.7 and 9.6%, respectively, of the variability, the sum of which covered 98.4% of the total variance explained (**Table 4**). All variables were significant at a 95% significance level. The scores for the first three DFs were plotted as a three-dimensional (3D) scatter diagram, yielding a sufficient separation of samples into different clusters (**Figure 3**). The main discriminating axis in **Figure 3** was DF1, which was mainly correlated with the benzoic derivatives gallic acid, vanillic acid, and syringic acid, as shown in **Table 5**. Mandilaria, Xinomavro, and Syrah samples formed well-defined clusters according to DF1. On the other hand, Agiorgitiko, Cabernet Sauvignon, and Merlot differentiated according to DF3, which was strongly correlated with syringic acid, gallic acid, resveratrol, and astringin.

Geographical Area-Based Differentiation. In the same context described for cultivar-based classification, DA with regard to geographical origin resulted in four DFs. DF1 accounted for 53.6% of total variability, while DF2 and DF3 accounted for 37 and 5.4%, respectively (96% of total variance explained; **Table 6**). All variables were significant at a 95% level. The scores for the first three DFs composed the 3D scatter diagram (**Figure 4**). The main axis of differentiation (DF1) was strongly affected by gallic acid, syringic acid, and protocatechuic acid (**Table 7**). Specific areas A1, A2, and A4, which were correlated with DF1, formed separate clusters with no overlapping, whereas DF2, which was influenced by syringic acid and gallic acid (**Table 7**), was responsible for the discrimination of areas A2 and A5. DF3 was responsible for separation of areas A3 and A4 and was correlated with the stilbenic derivatives, protocatechuic and vanillic acids, as illustrated in **Table 7**.

DISCUSSION

As mentioned earlier in the text, polyphenol biosynthesis is strictly controlled by the genes of the corresponding enzymes involved in the relevant biosynthetic pathways. Thus, the polyphenolic profile of a given cultivar reflects to a great extent its genetic potential. In a similar fashion, environmental stimuli, that is, sun exposure, play critical roles in regulating activities of enzymes implicated in polyphenol biosynthesis, and this effect could as well be reflected on the polyphenolic profile. It was thus hypothesized that the differences arising from these two key parameters might be revealed after appropriate statistical analysis, which would take into consideration specific polyphenolic indices.

Two groups of polyphenols were considered as follows: benzoic acid derivatives and stilbenes, which represent minor constituents of wines, as their concentration does not usually exceed a few milligrams per liter. This choice was based on the consideration that these components are in general chemically and microbiologically stable; thus. they could be viewed as indices for a reliable differentiation. Moreover, other compounds such as ferulic acid or flavonols (aglycones), which also usually occur at low levels, were excluded, because they are not directly relevant with regard to biosynthesis to either benzoates or stilbenes; thus, their concentrations may not be

Table 2. Composition of Benzoic Acid Derivatives in the Experimental Wines Tested. Values are Expressed as mg L^{-1} and Represent Means of Duplicate Determination ± SD.

sample	gallic acid	protocatechiuc acid	vanillic acid	syringic acid	total
		Merlot			
1	10.8 ± 1.3	2.9 ± 0.8	2.5 ± 0.1	0	16.1
2	8.9 ± 0.4	1.5 ± 0.5	2.8 ± 0.2	3.2 ± 0.6	16.4
3	12.7 ± 1.0	2.2 ± 0.0	4.3 ± 0.3	3.8 ± 0.5	23.0
4	11.0 ± 0.8	1.1 ± 0.2	0	0	12.1
average	10.9	1.9	2.4	1.8	16.9
		Cabernet Sauvignon			
1	17.8 ± 0.3	0.9 ± 0.0	0.9 ± 0.0	8.9 ± 0.0	28.5
2	18.6 ± 0.6	0.6 ± 0.0	1.9 ± 0.6	0	21.1
3	12.7 ± 3.1	0.7 ± 0.0	0.5 ± 0.0	3.9 ± 0.5	17.8
4	3.8 ± 0.4	7.9 ± 1.5	0	6.6 ± 0.9	18.3
average	13.2	2.5	0.8	4.9	21.4
		Syrah			
1	19.4 ± 2.1	1.7 ± 0.1	7.9 ± 0.5	8.2 ± 0.3	37.1
2	19.0 ± 2.5	0.9 ± 0.1	2.7 ± 0.8	11.5 ± 0.7	34.1
3	12.7 ± 0.7	0.8 ± 0.1	2.6 ± 0.6	0	16.1
4	44.9 ± 0.6	1.8 ± 0.2	5.5 ± 0.9	7.4 ± 0.5	59.6
5	11.7 ± 0.6	0.5 ± 0.2	0	4.5 ± 0.4	16.7
6	14.1 ± 0.8	1.2 ± 0.0	2.9 ± 0.4	7.9 ± 1.2	26.1
7	33.4 ± 0.6	2.4 ± 0.4	7.4 ± 0.2	10.7 ± 1.7	54.0
8	10.1 ± 0.2	0.7 ± 0.1	1.7 ± 0.4	1.7 ± 0.4	14.2
average	20.7	1.3	3.8	6.5	32.2
		Agiorgitiko			
1	34.5 ± 0.8	1.3 ± 0.1	1.4 ± 0.1	10.6 ± 0.8	47.8
2	44.3 ± 6.2	1.9 ± 0.1	3.1 ± 0.8	9.8 ± 0.1	59.0
3	48.0 ± 3.9	1.3 ± 0.3	2.2 ± 0.6	10.4 ± 1.6	61.7
4	6.0 ± 0.5	18.8 ± 4.6	0	6.7 ± 2.6	31.5
5	10.7 ± 0.1	4.1 ± 0.8	0	3.0 ± 0.4	17.8
6	7.5 ± 1.1	9.2 ± 1.7	0.9 ± 0.1	2.9 ± 0.6	20.4
average	25.2	6.1	1.3	7.2	39.8
		Xinomavro			
1	32.2 ± 3.9	1.7 ± 0.7	5.7 ± 0.6	4.8 ± 0.1	45.0
2	23.3 ± 1.0	1.3 ± 0.1	0	0	24.6
3	34.2 ± 0.5	1.0 ± 0.3	0	0	35.2
4	18.1 ± 3.5	0.8 ± 0.1	0	0	18.9
5	16.7 ± 3.6	0.8 ± 0.2	0	0	17.5
6	32.2 ± 1.7	0.9 ± 0.0	0	0	33.1
7	39.1 ± 1.4	1.1 ± 0.4	0	0	40.2
8	23.4 ± 0.2	1.2 ± 0.0	0	4.5 ± 0.4	29.1
average	27.4	1.1	0.7	1.2	30.4
		Mandilaria			
1	51.2 ± 5.5	0.7 ± 0.2	0	0	51.9
2	56.8 ± 1.1	0.5 ± 0.0	0	0	57.4
3	44.5 ± 0.9	1.1 ± 0.1	0	0	45.6
4	37.3 ± 0.1	0.9 ± 0.1	0	0	38.3
5	41.7 ± 9.2	0.9 ± 0.2	0	0	42.6
average	46.3	0.8	0	0	47.1

Table 3. Composition of Stilbenic Derivatives (*trans* Isomers) in the Experimental Wines Tested[a]

sample	astringin	piceid	resveratrol	total
		Merlot		
1	0	0	0.24 ± 0.01	0.24
2	0.07 ± 0.01	2.74 ± 0.20	3.20 ± 0.34	6.01
3	0	7.92 ± 0.35	0	7.92
4	0	2.24 ± 0.52	0	2.24
average	0.02	3.23	0.86	4.10
		Cabernet Sauvignon		
1	0	0	0	0
2	0.70 ± 0.16	0.06 ± 0.01	0.28 ± 0.02	1.04
3	0.26 ± 0.01	0	0.22 ± 0.03	0.48
4	0.34 ± 0.03	0	0.34 ± 0.08	0.69
average	0.33	0.02	0.21	0.55
		Syrah		
1	0	5.80 ± 0.26	2.38 ± 0.03	8.18
2	1.57 ± 0.08	0	2.83 ± 0.44	4.40
3	0	0	1.17 ± 0.05	1.17
4	0.50 ± 0.06	0	0.27 ± 0.06	0.77
5	0	0	4.01 ± 0.77	4.01
6	0.89 ± 0.04	0	3.26 ± 0.19	4.15
7	1.48 ± 0.38	0	3.65 ± 0.03	5.13
8	4.66 ± 0.64	0	2.82 ± 0.30	7.48
average	1.14	0.73	2.55	4.41
		Agiorgitiko		
1	0	0	0	0
2	0.30 ± 0.04	0	0	0.30
3	0	0	0.98 ± 0.07	0.98
4	1.16 ± 0.13	0	1.13 ± 0.06	2.28
5	0.35 ± 0.03	0	0	0.35
6	0.26 ± 0.02	0	0.27 ± 0.02	0.53
average	0.35	0	0.40	0.74
		Xinomavro		
1	0	5.09 ± 0.03	0	5.09
2	0	0.99 ± 0.06	0	0.99
3	0	0	0.30 ± 0.01	0.30
4	0	0.26 ± 0.01	0	0.26
5	0	1.02 ± 0.02	1.53 ± 0.13	2.55
6	0.65 ± 0.07	0	1.02 ± 0.19	1.67
7	0	0	1.23 ± 0.11	1.23
8	0	1.86 ± 0.31	0.73 ± 0.07	2.59
average	0.08	1.15	0.60	1.84
		Mandilaria		
1	0	2.53 ± 0.37	1.20 ± 0.21	3.72
2	0.59 ± 0.08	0	1.40 ± 0.36	2.00
3	0.80 ± 0.09	0.80 ± 0.21	1.48 ± 0.12	3.08
4	1.41 ± 0.11	2.57 ± 0.28	1.79 ± 0.18	5.77
5	0	2.78 ± 0.45	1.58 ± 0.11	4.36
average	0.56	1.74	1.49	3.79

[a] Values are expressed as mg L^{-1} and represent means of duplicate determination ± SD.

Table 4. Eigenvalues, % Variance, and Cumulative % Variance of Cultivar-Based Differentiation[a]

function	Eigenvalue	% of variance	cumulative %	canonical correlation
1	8.039	68.1	68.1	0.943
2	2.441	20.7	88.8	0.842
3	1.129	9.6	98.4	0.728
4	0.164	1.4	99.8	0.375
5	0.029	0.2	100.0	0.167

[a] Analysis was carried out on the basis of the first five canonical DFs.

directly influenced by the same factors. In addition to these criteria, all samples were vinified under identical conditions to minimize technological influence, but most significantly, they were nonaged. This prerequisite was met bearing in mind that the concentration of minor phenolics, such as gallic, vanillic, and syringic acids, could dramatically be altered as they can be formed through oak wood lignin and tannin hydrolysis and, therefore, yield misleading results.

All seven phenolics exhibited notable variations even in samples made from the same cultivar, evidencing an important impact of cultural practices and climatic conditions. This was particularly obvious for samples made from Mandilaria that were characterized by complete lack of both vanillic and syringic acids (**Table 2**). The same holds true for Syrah and Agiorgitiko samples, which were found to contain no or trivial amounts of piceid. This stilbenic metabolite was the least abundant in all samples (**Table 3**), and presumably, for this reason, it played virtually no role in differentiation. Cultivar-based DA showed that the levels of gallic, vanillic, and syringic acids were the most important factors for the differentiation of Mandilaria,

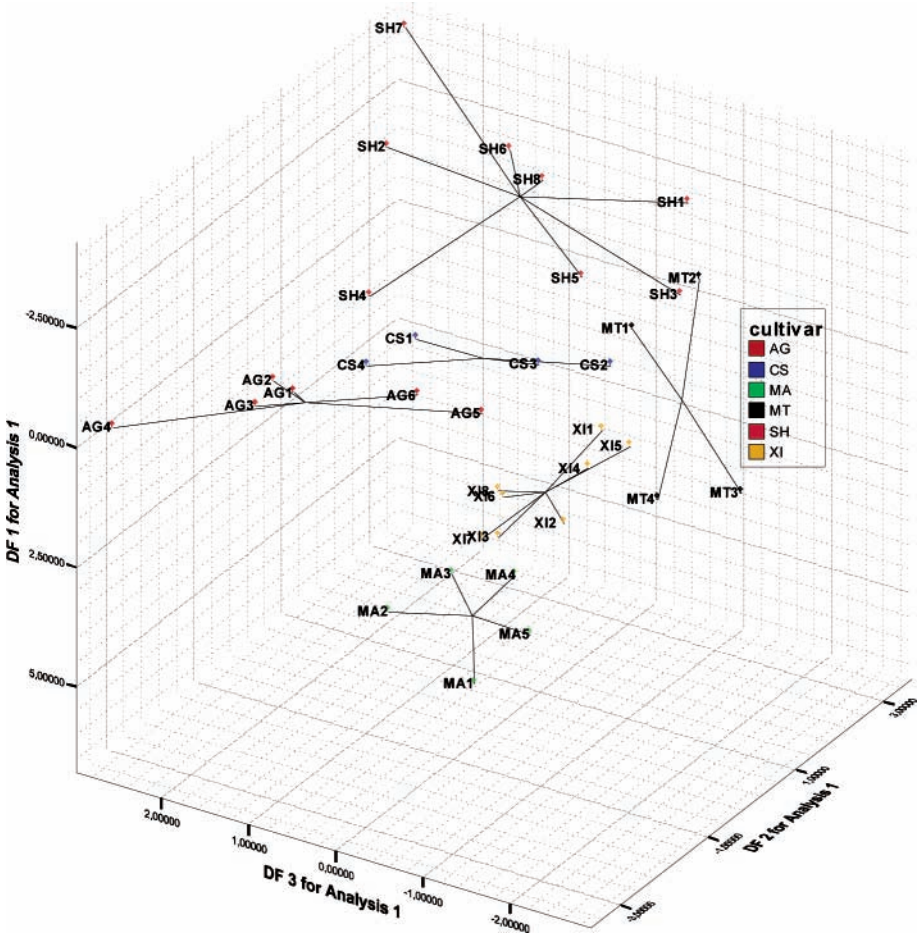

Figure 3. Three-dimensional plot showing cultivar-based differentiation of samples. Assignments: AG, Agiorgitiko; CS, Cabernet Sauvignon; MA, Mandilaria; MT, Merlot; SH, Syrah; and XI, Xinomavro.

Table 5. Pooled Within-Groups Correlations between Discriminating Variables and Standardized Canonical DFs (Cultivar-Based Analysis)[a]

	function				
	1	2	3	4	5
resveratrol	–0.055	0.620	0.394	0.093	0.241
syringic acid	0.287	–0.093	0.536	0.273	0.165
gallic acid	0.311	0.049	0.440	–0.247	–0.363
piceid	0.085	0.170	–0.365	0.727	0.241
protocatechuic acid	–0.072	–0.316	0.233	0.666	–0.050
vanillic acid	–0.206	0.314	0.101	0.469	–0.347
astringin	–0.061	0.222	0.335	–0.148	0.567

[a] Variables are ordered by absolute size of correlation within the function. The largest absolute correlation between each variable and any DF.

Table 6. Eigenvalues, % Variance, and Cumulative % Variance of Geographical Area-Based Differentiation[a]

function	Eigenvalue	% of variance	cumulative %	canonical correlation
1	5.088	53.6	53.6	0.914
2	3.514	37.0	90.6	0.882
3	0.512	5.4	96.0	0.582
4	0.380	4.0	100.0	0.525

[a] Analysis was performed on the basis of the first four canonical DFs.

Xinomavro, and Syrah samples (**Table 5**). However, differentiation of Agiorgitiko, Cabernet Sauvignon, and Merlot samples

Table 7. Pooled Within-Groups Correlations between Discriminating Variables and Standardized Canonical DFs (Geographical Area-Based Analysis)[a]

	function			
	1	2	3	4
gallic acid	0.495	0.330	–0.019	0.130
syringic acid	0.248	–0.498	0.156	–0.053
astringin	–0.073	0.049	0.522	0.262
piceid	–0.043	0.168	0.361	–0.166
protocatechuic acid	–0.215	–0.116	–0.424	0.561
vanillic acid	–0.012	–0.119	0.352	–0.486
resveratrol	0.059	0.126	0.067	–0.422

[a] Variables were ordered by absolute size of correlation within the function. Largest absolute correlation between each variable and any DF.

also depended on astringin and resveratrol concentrations. Likewise, geographical origin-based analysis was significantly affected by DFs that depended on gallic acid, although a less prominent influence was exerted by stilbenes (**Table 7**). In both analyses, grouping of samples yielded distinct differentiation without overlapping, which clearly demonstrates the validity of the procedure for credible classification. This outcome could be of practical importance and may be regarded as an additional criterion for studies pertaining to red wine quality control and authenticity, in addition to minor (*13*) and major components, including several polyphenolic classes (*9*, *14*) and/or other compositional parameters (*15*−*17*). Fur-

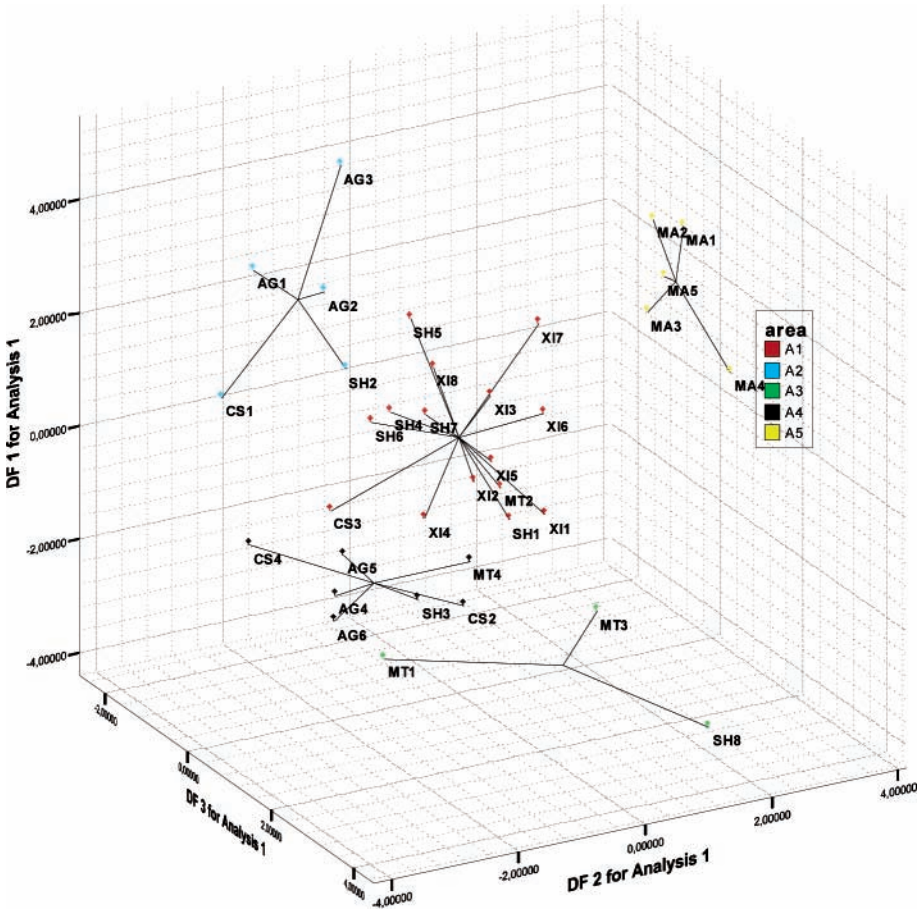

Figure 4. Three-dimensional plot showing geographical area-based differentiation of samples (see also the Materials and Methods and **Figure 2**).

thermore, as the profile of simple phenolic acids depends substantially on aging in oak barrels, their value may be of significance for differentiating samples aged in different types of oak, as well as identifying variations in the aging period.

It is essential to stress the importance of the group of compounds considered in similar studies. In a previous work (*10*), it was demonstrated that multivariate discrimination based on major polyphenolic constituents of young red wines depends mainly on compounds with relatively high concentrations, including malvin (malvidin 3-*O*-glucoside), caftaric acid, and procyanidins B1 and B2, whereas other classes with significantly lower concentrations, such as flavonols, play a rather trivial role. The exclusive use of selected minor phytochemicals, as performed in this study, however, may accentuate the potential role of these compounds and provide further insights into the usefulness of chemometrics in wine quality control.

ABBREVIATIONS USED

DA, discriminant analysis; DF, discriminant function; SD, standard deviation.

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LITERATURE CITED

(1) Arvanitoyannis, I. S.; Katsota, M. N.; Psarra, E. P.; Soufleros, E. H.; Kallithraka, S. Application of quality control methods for assessing wine authenticity: Use of multivariate analysis (chemometrics). *Trends Food Sci. Technol.* **1999**, *10*, 321−336.

(2) Núñez, M.; Peña, R. M.; Herrero, C.; García-Martín, S. Analysis of some metals in wine by means of capillary electrophoresis. Application to the differentiation of Ribeira Sacra Spanish red wines. *Analusis* **2000**, *28*, 432−437.

(3) Frías, S.; Pérez Trujillo, J. P.; Peña, E. M.; Conde, J. E. Classification and differentiation of bottled sweet wines of Canary Islands (Spain) by their metallic content. *Eur. Food Res. Technol.* **2001**, *213*, 145−149.

(4) Kment, P.; Mihaljevič, M.; Ettler, V.; Šebek, O.; Strnad, L.; Rohlová, L. Differentiation of Czech wines using multielement composition−A comparison with vineyard soil. *Food Chem.* **2005**, *91*, 157−165.

(5) Šperková, J.; Suchánek, M. Multivariate classification of wines from different Bohemian regions (Czech Republic). *Food Chem.* **2005**, *93*, 659−663.

(6) Siversen, H. K.; Holen, B.; Nicolaysen, F.; Risvik, E. Classification of French red wines according to their geographical origin by the use of multivariate analysis. *J. Sci. Food Agric.* **1999**, *79*, 107−115.

(7) Arozarena, I.; Casp, A.; Marín, R.; Navarro, M. Multivariate differentiation of Spanish red wines according to region and variety. *J. Sci. Food Agric.* **2000**, *80*, 1909−1917.

(8) Koussissi, E.; Paterson, A.; Piggott, J. R. Sensory flavour discrimination of Greek dry red wines. *J. Sci. Food Agric.* **2003**, *83*, 797−808.

(9) De Villiers, A.; Majek, P.; Lynen, F.; Crouch, A.; Lauer, H.; Sandra, P. Classification of South African red and white wines according to grape variety based on the non-coloured phenolic content. *Eur. Food Res. Technol.* **2005**, *221*, 520−528.

(10) Makris, D. P.; Kallithraka, S.; Mamalos, A. Differentiation of young red wines based on cultivar and geographical origin with application of chemometrics of principal polyphenolic constituents. *Talanta* **2006**, *70*, 1143−1152.

(11) Jeandet, P.; Doullet-Breuil, A.-C.; Bessis, R.; Debord, S.; Sbaghi, M.; Adrian, M. Phytoalexins from Vitaceae: Biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. *J. Agric. Food Chem.* **2002**, *50*, 2731−2741.

(12) Arnous, A.; Makris, D. P.; Kefalas, P. Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines. *J. Agric. Food Chem.* **2001**, *49*, 5736−5742.

(13) Kallithraka, S.; Arvanitoyannis, I.; El-Zajouli A.; Kefalas, P. The application of an improved method for trans-resveratrol to determine the origin of Greek red wines. *Food Chem.* **2001**, *75*, 355−363.

(14) Tinttunen, S.; Lehtonen, P. Distinguishing organic wines from normal wines on the basis of concentrations of phenolic compounds and spectral data. *Eur. Food Res. Technol.* **2001**, *212*, 390−394.

(15) Kallithraka, S.; Arvanitoyannis, I.; Kefalas, P.; El-Zajouli A.; Soufleros, E.; Psarra, E. Instrumental and sensory analysis of Greek wines; implementation of principal component analysis (PCA) for classification according to geographical origin. *Food Chem.* **2001**, *73*, 501−514.

(16) Pérez-Magariño, S.; Ortega-Heras, M.; González-San José, M. L. Multivariate classification of rosé wines from different Spanish protected designations of origin. *Anal. Chim. Acta* **2002**, *458*, 187−190.

(17) Spranger, M. I.; Clímaco, M. C.; Sun, B.; Eiriz, N.; Fortunato, C.; Nunez, A.; Leandro, M. C.; Avelar, M. L.; Belchior, A. P. Differentiation of red winemaking technologies by phenolic and volatile composition. *Anal. Chim. Acta* **2004**, *513*, 151−161.

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