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Elemental fingerprint of wines from the protected designation of origin Valencia

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ABSTRACT

Inductively coupled plasma optical emission (ICP-OES), in combination with different chemometric approaches, has been used to verify the origin of different red wine samples from Utiel-Requena, Jumilla, Yecla and Valencia protected designation of origin (PDO). The ability of multivariate analysis methods, such as hierarchical cluster analysis (HCA), principal component analysis (PCA), classification and regression trees (CARTs) and discriminant analysis (DA), to achieve wine classification from their elemental contents has been investigated. The calculations were performed using 38 variables (contents of Al, Ba, Be, Ca, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Gd, Ho, K, La, Li, Lu, Mg, Mn, Mo, Na, Nd, Ni, Pb, Pr, Sc, Se, Sm, Sr, Tb, Ti, Tm, V, Y, Yb and Zn, at mg l^{-1} level, determined by ICP-OES).
Results of HCA and PCA clearly show that Utiel-Requena and Jumilla red wines are very easy to discriminate from the wines of the other PDO in their vicinity. However, wines from Valencia and Yecla PDO cannot be discriminated, based on the aforementioned procedures. Using the CART method, wines from Utiel-Requena and Jumilla can also be easily authenticated, by using only the concentration of Li and Mg, respectively. Wine samples from Valencia PDO sub-regions can be also discriminated with a quite acceptable re-substitution rate. On the other hand, discrimination analysis allows the separation of the entire studied PDO samples, obtaining accuracy results by the re-substitution method of 100%.

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1. Introduction

The identification of the geographical origin of wines is of great interest for wine consumers and producers, since it may provide determinant criteria for wine price and guarantees of quality. It has been observed that the elemental content of wines depend on several factors, including soil characteristics, type of grape, area of production and environmental conditions, allowing the definition of a representative "fingerprint" which is especially important for quality wines produced in specific regions, such as protected designation of origin (PDO) wines. These wines are produced by using particular grape varieties cultivated in specific geographical regions under controlled growing and production conditions. The main purpose of Regulatory Councils is the prevention of fraud, by guaranteeing the origin and quality of wines (Medina, 1996; Ortega, Gonzalez-San Jose, & Beltran, 1999).

The use of the mineral content of samples to determine the wine origin, taking into account the relationship between metals present in the wine and soil composition, has been previously studied. Table 1 summarises papers previously published in the literature, using as the source the science citation index (SCI) database of the institute for scientific information (ISI, Philadelphia, PA) from 1900 to 2007.

As can be seen, atomic spectrometry techniques have been commonly employed to perform multi-elemental determinations in wine samples. Graphite furnace atomic absorption spectrometry (GFAAS) was used in the differentiation of Canary Islands' wines (Moreno et al., 2007), and flame atomic absorption spectrometry (FAAS) has been employed for the identification of some Czech wines (Kment et al., 2005). Nowadays the techniques of choice to obtain the elemental fingerprinting of wine are those with multi-element detection capability; such as inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS), which are suitable for the accurate and fast determination of trace and ultra-trace elements in the same sample. From Table 1 it can be observed that the differentiation of wine from different origins can be carried out by using major, trace and ultra-trace elements.

On considering the different kinds of wines characterised, it can be seen that samples from different countries have been studied. Concerning Spanish wines, Canary Island, Andalusia (including Montilla-Morilles and Jerez), and Catalonia wines were evaluated from their elemental composition. However, there is no precedent on the characterisation of Valencian wines or wines from the Murcia area, like Yecla or Jumilla. So, this study could be considered as the first on the elemental composition of Valencian wines.

Fingerprint techniques, based on chemical composition and multivariate statistical analysis, can be used for characterising or classifying products according to origin, quality, variety, type or

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Table 1
Methods proposed in the literature for the elemental fingerprint characterisation of wine samples

Analytical methods	Sample pre-treatment	Elements employed	Chemometric treatment	Origin	References
GF-AAS	Dry ashing	Ni and Pb	LDA	Canary Island (Spain)	(Moreno et al., 2007)
FAAS	Mineralisation	Ca, Fe, K, Mg, Mn and Na	PCA, FA and cluster	Czech Republic	(Kment et al., 2005)
ICP-OES	Dry ashing	Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Se and Zn	LDA	Canary Island (Spain)	(Moreno et al., 2007)
	Mineralisation	Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P and Sr	LDA and ANN	Andalusian (Spain)	(Álvarez, Moreno, Jos, Cameán, & González, 2007)
	Open vessel digestion	Al, Ca, Cu, Fe, Mg, Mn, Sr and Zn	PCA and LDA	Catalonia (Spain)	(Iglesias, Besalú, & Anticó, 2007)
	Dry ashing	Ca, Fe, K, Mg and Na	PCA and DA	Czech Republic	(Sperková & Sichánek, 2005)
	Direct, decomposition	Al, B, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, Rb, Sr, V and Zn	PCA, cluster, DA, fisher DA and ANN	–	(Sun, Danzer, & Thiel, 1997)
	Decomposition	Al, B, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, Rb, Sr, V and Zn	DA	Germany	(Thiel & Danzer, 1997)
	Acid digestion	Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, Sr and Zn	PCA	Montilla-Morilles (Spain)	(Álvarez et al., 2007)
	Dry ashing	Al, B, Ba, Cu, Fe, Mn and Zn	PCA, HCA and RDA	Slovenia and Abulia (Italy)	(Brescia, Kosir, Caldarola, Kidric, & Sacco, 2003)
	Mineralisation	Al, B, Ba,Cu, Fe, Mn and Zn	PCA and HCA	Abulia (Italy)	(Brescia, Calderola, De Giglio, Benedetti, Fanizzi, & Sacco, 2002)
ICP-MS	Mineralisation	Ag, Al, As, Ba, Be, Cd, Co, Cr, Cs, Cu, Li, Ni, Pb, Rb, Sb, Sr, Tl, U, V and Zn	PCA, FA and cluster	Czech Republic	(Kment et al., 2005)
	UV irradiation	Al, B, Ba, Be, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, Li, Mn, Mo, Nb, Ni, Pb, Rb, Sb, Sc, Sr, Th, Ti, Tl, U, V, W, Y and Zn and Zr, REEs	Pearson's correlation	Portugal	(Marisa, Almeida, & Vasconcelos, 2003)
		Al, As, Ba, Cd, Ce, Co, Cr, Cs, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ge, Ho, La, Li, Lu, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Rh, Ru, Sb, Sm, Sn, Sr, Ta, Tb, Th, Ti, Tl, Tm, U, V, W, Yb, Zn and Zr	DA	England and Spain	(Baxter, Crews, Dennis, Goodall, & Anderson, 1997)
	Dilution	As, Be, Cd, Co, Cs, Ga, Li, Mo, Nb, Ni, Rb, Sb, Te, Ti, Tl, U, W and Y, Zr, REEs	PCA	Germany	(Thiel, Geisler, & Blechschmidt, 2004)
	Open vessel digestion	Al, Ba, Cd, Co, Li, Ni, Pb, Sb and V	PCA and LDA	Catalonia (Spain)	(Iglesias et al., 2007)
	Dry ashing	Al, As, Ba, Ca, Ce, Co, Cr, Cs, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Rb, Sb, Sn, Sr, Th, U, V, Y and Zn	PCA and DA	Czech Republic	(Sperková & Sichánek, 2005)
	Dilution	Ag, As, Ba, Bi, Cd, Ce, Co, Cs, Cu, La, Li, Mo, Ni, Pb, Rb, Sr, Th, U, V and Zn	MDS	Canada	(Greenough, Mallory-Greenough, & Fryer, 2005)
	–	Cd, Co, Cs, Ga, Mn, Ni, Pb, Rb and Sr	LDA	New Zealand	(Angus, O'Keeffe, Stuart, & Miskelly, 2006)
	Dilution	11B/10B	–	(Stellenbosch, Robertson and Swartland) South Africa, France (Bergerac) and Italy (Valpolicella)	(Cortezee & Vanhaecke, 2005)
	–	Al, B, Ba, Cl, Cs, Ga, La, Li, Mg, Mn, Nb, Ni, Rb, Sc, Se, Si, Sr, Tl, U and W	DA	(Stellenbosch, Robertson and Swartland) South Africa	(Cortezee & Vanhaecke, 2005)
	Dilution	Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, Hf, Li, Mn, Mo, Nb, Ni, Pb, Rb, Sb, Sc, Sr, Th, Ti, Tl, U, V, W, Y, Zn and REEs	QDA	Portugal	(Almeida & Vasconcelos, 2003)
	Without preleminary treatment	Al, As, B, Ba, Be, Ca, Cd, Ce, Co, Cr, Cs, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ge, Hf, Ho, I, K, La, Li, Mg, Mn, Mo, Na, Nb, Nd, Ni, P, Pb, Pd, Pr, Rb, Rh, Sb, Si, Sm, Sn, Sr, Tb, Te, Th, Ti, Tl, Tm, U, V, W, Y, Yb, Zn and Zr	PCA and LDA	Province of Cuneo (Piedmont)	(Marengo & Aceto, 2003)
	Dilution	Ag, Al, As, Ba, Be, Bi, Br, Ca, Cd, Ce, Cl, Co, Cs, Cu, Fe, La, Li, Mg, Mn, Mo, Ni, P, Pb, Rb, Sb, Se, Sr, Th, Ti, Tl, U, V and Zn	PCA and DA	Canada	(Taylor, Longerich, & Greenough, 2003)
	Dilution	As, Au, Ba, Be, Cd, Co, Cu, Cs, Ga, Li, Ni, Pb, Pd, Pt, Rb, Re, Sb, Sn, Sr, Te, Ti, Tl, V, W, Zr and REEs	SIMCA	Canary Islands (Spain)	(Barbaste, Medina, Sarabia, Ortiz, & Perez-Trujillo, 2002)
	UV irradiation/dilution/cation-exchange chromatographic separation	Sr-87/Sr-86	–	Portugal and France	(Almeida & Vasconcelos, 2001)

Sample treatment	Elements	Pattern recognition method	Country	Reference
Microwave digestión/ High pressure ashing	REE		Germany	(Jakubowski, Brandt, Stuewer, Eschnauer, & Gotges, 1999)
Dilution	B, Ca, Co, Cs, Fe, Li, Mg, Mn, Pb, Rb, Sr, V and Zn	DA	Germany	(Gomez, Feldemann, Jakubowski, & Adersson, 2004)
AES	Cellulose ion Al, B, Co, Sr, Fe, Mg, Mn, Ni, P, Pb, Ti and Zn		–	(Borszeki, Koltay, & Inczedy, 1983)

GFAAS: graphite furnace atomic absorption spectrometry, FAAS: flame atomic absorption spectrometry, ICP-MS: inductively coupled plasma mass spectrometry and AES: atomic emission spectrometry, ICP-OES: inductively coupled plasma optical emission spectrometry, PCA: principal component analysis, FA: factor analysis, DA: discriminant analysis, LDA: linear discriminant analysis,REEs: rare earth elements,RDA: regularised discriminant analysis, ANN: artificial neural network; MDS: multidimensional scaling, QDA: quadratic discriminant analysis and SIMCA: soft independent modelling class analogy, HCA: hierarchical clustering analysis.

other features (Benincasa, Lewis, Perri, Sindona, & Tagarelli, 2007; Jurado, Alcazar, Pablos, Martin, & Gonzalez, 2005). The most common procedures used for pattern recognition purposes include hierarchical cluster analysis (HCA), employed to discover natural groupings of samples, principal component analysis (PCA), used to establish the relationships and differences between variables, and discriminant analysis (DA), applied to select the most important and significant variables (Korenovska & Suhaj, 2005). Artificial neural networks (ANNs), multidimensional scaling (MDS) and soft independent modelling class analogy (SIMCA) have been used to discriminate the origin of wine samples.

This article examines another multivariate approach, which has been rarely used for the authentication of the origin of wine samples, namely classification and regression trees (CARTs) (Capron, Smeyers-Verbeke, & Massart, 2007).

CART yields a classification tree by splitting the data into subsets, called nodes, which are more homogeneous with respect to the classes than the initial set. The splitting process starts by the division of the principal node, containing all available samples, in two. The procedure is recursive, since the two child nodes obtained from the principal node are then treated as parent nodes and split again into two subsets. The splitting of nodes continues until terminal nodes are obtained. These are nodes that can be considered sufficiently homogeneous (all samples in the node belong to the same class) or a small number of objects in the terminal nodes is reached.

In the present work, wines with Valencia PDO have been comprehensively studied using several of the aforementioned chemometric treatments. Valencia PDO comprises a vast region located across the province of Valencia (Spain). At present, this PDO shelters four separated and different sub-zones; namely: (i) Alto Turia, (ii) Valentino, (iii) Moscatel and (iv) Clariano (see Fig. 1). Taking into consideration that several PDO such as Utiel-Requena, Jumilla and Yecla are located in the vicinity of the Valencia PDO, the elemental composition of the wines was used to differentiate Valencia PDO wines from the other PDO present in the region and also to verify the coherence of such a geographically diverse PDO.

2. Experimental

2.1. Reagents and apparatus

Stock solutions of a multi-elemental standard solution (100 µg ml^{-1}) containing 26 elements (Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Se, Sr, Ti, Tl, V and Zn) dissolved in 5% HNO$_3$ from Scharlau (Barcelona, Spain), and a multi-elemental (100 µg ml^{-1}) solution containing 16 lanthanides dissolved in 5% HNO$_3$ from Alfa Aesar (Karlsruhe, Germany) were employed for calibration.

HNO$_3$ 69% (w/v) from J.T. Baker (Deventer, The Netherlands), H$_2$O$_2$ 35% reagent grade from Scharlau and nanopure water with a maximum resistivity of 18.2 MΩ cm^{-1}, obtained from a Milli-Q Millipore system (Bedford, MA, USA) were used for sample treatment and sample dilution.

A microwave laboratory system, Ethos SEL from Millestone (Sorisole, Italy), equipped with an optical fibre sensor, for automatic temperature control, and an automatic gas detector, operated at a maximum exit power of 1000 W, was employed for microwave-assisted digestion of wine samples introduced inside ten high pressure vessels of 100 ml inner volume; samples were treated simultaneously.

An ultrasound water bath from Selecta (Barcelona, Spain), of 350 ml volume, with 50 W power and 50 Hz frequency, was employed for sample sonication.

Measurements by ICP-OES were carried out using a Perkin Elmer Model Optima 5300 DV spectrometer (Norwalk, CT, USA),
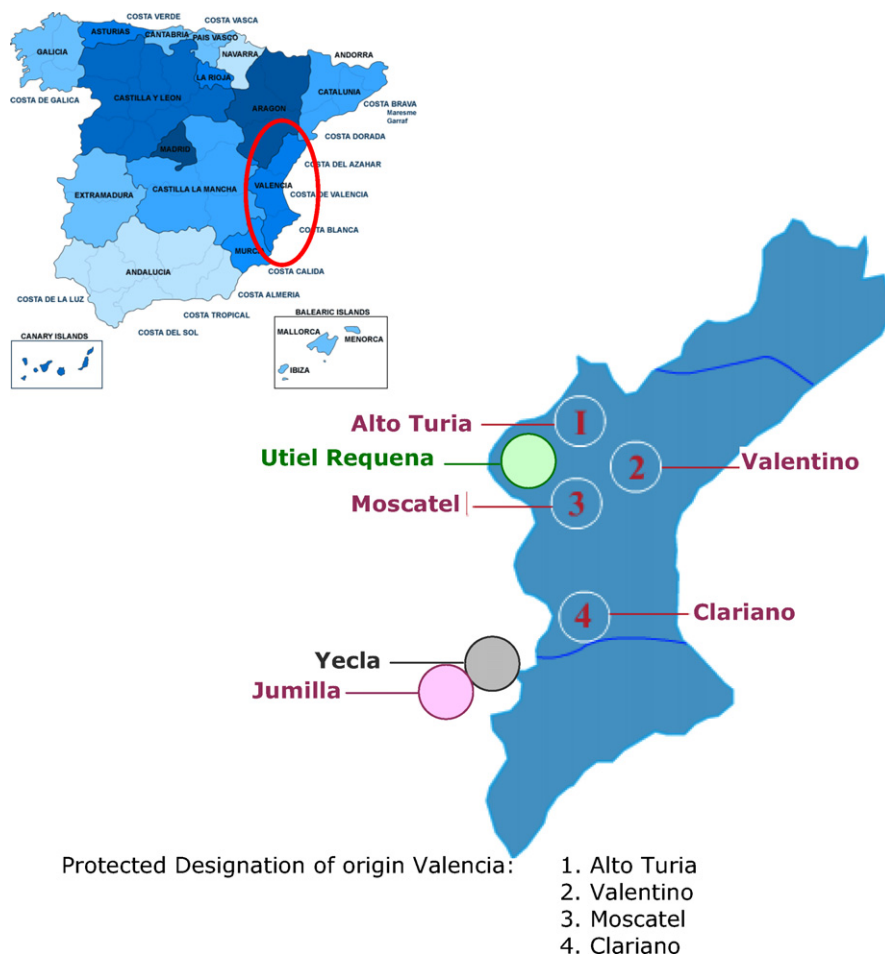
Fig. 1. Map of Spain indicating the Valencian region and its viticulture areas and registered PDO.

Table 2
Instrumental conditions employed in ICP-OES for the elemental analysis of samples

Parameter	Value
RF power	1400 W
Gas	Argon
Plasma Gas	15 l min^{-1}
Auxiliary Gas	0.2 l min^{-1}
Nebuliser gas	0.8 l min^{-1}
Sample aspiration rate	1.1 ml min^{-1}
View	Axial
Background correction	2-Point
Number of replicates	1
Nebuliser	Meinhard

equipped with an autosampler, AS 93-plus, and a Meinhard nebuliser. Argon C-45 (purity higher than 99.995%), supplied by Carburos Metálicos (Barcelona, Spain), was employed as plasmogen and carrier gas.

2.2. Samples

Red wine samples from Spain were categorised into different classes as a function of their declared origin; six samples from Utiel-Requena PDO, two from Yecla PDO and three from Jumilla PDO were employed to verify the correct classification of Valencia PDO samples based on their elemental composition. Fifty-six samples from Valencia PDO, of which three were from Alto Turia, two from Valentino, six from Moscatel and 45 from Clariano regions;

were considered. A total of 67 red wine samples, with ethanol content ranging from 11 to 14.8% v/v and vintage from 1999 to 2006, were analysed.

The plastic containers used for storing and treating the samples were cleaned to avoid contamination of samples with traces of any metal. Containers were treated with nitric acid and were then washed with Milli-Q water.

Once opened, wine samples were treated according to the following procedures.

2.3. Sample pre-treatment

Samples (2 ml) of wine were weighed inside Teflon digestion vessels, and 8 ml of concentrated nitric acid were added. Once the vessels were capped, they were placed in the microwave oven and the following program was run: step 1, 3 min to reach 85 °C; step 2, 12 min to reach 145 °C; step 3, 6 min to reach 180 °C; step 4, 15 min at 180 °C and step 5, cooling down. After cooling to ambient temperature, the reactors were opened and sonicated to eliminate the nitrous vapours. The resultant solution (25 ml) was transferred into a plastic flask.

Teflon digestion vessels were previously cleaned in nitric acid solution to avoid cross-contamination.

2.4. ICP-OES determination

The operating conditions of the ICP-OES equipment are summarised in Table 2. The calibration standards were prepared from a

Table 3
Elemental composition of red wine samples according to their geographical origin

	Yecla				Utiel-Requena				Jumilla				Valencia			
	Max	Min	Mean	SD	Max	Min	Mean	SD	Max	Min	Mean	SD	Max	Min	Mean	SD
Al	16	1	6	9	0.11	<LOD	<LOD	<LOD	1.28	1.05	1.15	0.12	2.4	<LOD	0.8	0.2
Ba	1.31	1.04	1.20	0.14	0.011	<LOD	<LOD	0.001	0.17	0.09	0.12	0.04	1.38	<LOD	0.12	0.13
Be	0.031	0.028	0.030	0.001	0.0033	0.0032	0.0032	0.0001	0.036	0.035	0.035	0.001	0.049	0.009	0.021	0.008
Ca	1010	67	382	544	8	5	6	1	57	38	47	10	231	<LOD	51	18
Cd	0.33	0.03	0.13	0.17	0.005	0.004	0.004	0.001	0.051	0.049	0.050	0.001	0.068	<LOD	0.019	0.012
Ce	<LOD				0.007	0.005	0.006	0.001	0.002	<LOD	0.001	0.001	0.33	<LOD	0.08	0.06
Co	0.07	<LOD	0.02	0.04	0.002	0.001	0.001	0.0001	0.004	<LOD	0.001	0.002	<LOD			
Cr	0.014	0.003	0.008	0.006	0.017	0.009	0.012	0.003	0.139	0.109	0.120	0.016	0.18	<LOD	0.05	0.03
Cu	0.8	0.1	0.3	0.4	<LOD				0.11	0.06	0.08	0.02	0.44	<LOD	0.07	0.03
Dy	<LOD				0.0038	0.0032	0.0036	0.0002	0.011	0.010	0.011	0.0002	<LOD			
Er	0.355	0.025	0.136	0.190	0.012	0.006	0.007	0.002	0.044	0.035	0.039	0.004	0.100	<LOD	0.031	0.008
Eu	0.035	0.030	0.032	0.003	0.0037	0.0027	0.0031	0.0003	<LOD				0.046	<LOD	0.031	0.015
Fe	7	3	5	3	0.08	<LOD	<LOD	<LOD	4	1	2	2	30	<LOD	3	5
Gd	<LOD				0.0034	0.0029	0.0032	0.0002	0.018	0.016	0.017	0.001	<LOD			
Ho	<LOD				0.0034	0.0030	0.0032	0.0001	0.0176	0.0173	0.0174	0.0001	<LOD			
K	1048	1012	1025	20	778	764	772	5	832	821	826	6	1396	499	741	41
La	0.059	0.012	0.029	0.026	0.0045	0.0042	0.0043	0.0001	0.006	0.004	0.005	0.001	0.089	<LOD	0.032	0.019
Li	0.222	0.208	0.215	0.007	0.015	0.013	0.014	0.001	0.119	0.106	0.113	0.007	0.33	0.12	0.24	0.03
Lu	0.0065	0.0061	0.0062	0.0001	0.00288	0.00285	0.00286	0.00001	0.0087	0.0083	0.0084	0.0002	0.037	<LOD	0.010	0.005
Mg	1614	134	628	854	8.8	7.9	8.5	0.4	44	24	32	11	192	<LOD	75	23
Mn	7	1	3	4	<LOD				1.5	0.6	0.9	0.5	1.09	<LOD	0.41	0.13
Mo	0.034	0.018	0.025	0.008	0.007	0.005	0.006	0.001	0.052	0.045	0.048	0.003	0.35	<LOD	0.05	0.03
Na	322	26	124	171	4	1	3	1	49	15	30	17	101	<LOD	21	8
Nd	0.30	0.02	0.11	0.16	0.005	0.003	0.004	0.001	0.012	0.007	0.010	0.003	0.05	<LOD	0.02	0.02
Ni	4	<LOD	1	2	<LOD				0.051	0.045	0.048	0.003	<LOD			
Pb	<LOD				<LOD				0.057	0.038	0.050	0.011	<LOD			
Pr	<LOD				0.006	0.004	0.005	0.001	0.024	0.014	0.018	0.005	<LOD			
Sc	<LOD				0.0064	0.0064	0.0064	<LOD	0.119	0.106	0.113	0.007	0.09	<LOD	0.07	0.02
Se	1.8	1.0	1.2	0.4	0.050	0.017	0.039	0.012	0.792	0.569	0.698	0.116	1.05	0.07	0.45	0.09
Sm	0.27	<LOD	0.09	0.15	0.004	0.002	0.003	0.001	0.007	0.005	0.006	0.002	<LOD			
Sr	1.265	1.262	1.263	0.001	0.110	0.054	0.090	0.019	0.76	0.52	0.60	0.13	4.1	<LOD	0.8	0.4
Tb	<LOD				0.0029	0.0020	0.0024	0.0004	0.011	0.005	0.009	0.003	<LOD			
Ti	0.058	0.048	0.051	0.006	0.017	0.007	0.010	0.003	0.089	0.072	0.079	0.009	0.141	0.003	0.048	0.013
Tm	0.0070	0.0068	0.0069	0.0001	0.00324	0.00314	0.00317	0.00004	0.030	0.029	0.029	0.001	0.044	<LOD	0.015	0.009
V	0.678	0.025	0.281	0.348	<LOD				0.11	0.03	0.06	0.04	0.098	<LOD	0.022	0.019
Y	<LOD				0.004	0.004	0.004	0.001	0.009	0.008	0.008	0.001	<LOD			
Yb	0.0061	0.0061	0.0061	0.0001	0.00366	0.00358	0.00361	0.00003	0.0046	0.0042	0.0044	0.0002	0.046	<LOD	0.012	0.006
Zn	0.41	0.30	0.36	0.06	<LOD				0.405	0.189	0.288	0.109	6.1	<LOD	0.3	0.4

Note: max: maximum; min: minimum; SD: standard deviation and LOD: limit of detection.
Limit of detection values: Al, 0.08 µg l⁻¹; Ba, 0.02 µg l⁻¹; Be 0.1 µg l⁻¹; Ca, 0.13 µg l⁻¹; Cd, 0.1 µg l⁻¹; Ce, 0.1 µg l⁻¹; Co, 0.01 µg l⁻¹; Cr, 0.11 µg l⁻¹; Cu, 0.4 µg l⁻¹; Dy, 0.1 µg l⁻¹; Er, 0.1 µg l⁻¹; Eu, 0.1 µg l⁻¹; Fe, 0.07 µg l⁻¹; Gd, 0.1 µg l⁻¹; Ho, 0.1 µg l⁻¹; K, 0.12 µg l⁻¹; La, 0.1 µg l⁻¹; Li, 0.07 µg l⁻¹; Lu, 0.1 µg l⁻¹ Mg, 0.10 µg l⁻¹; Mn, 0.03 µg l⁻¹; Mo, 0.1 µg l⁻¹; Na, 0.12 µg l⁻¹; Nd, 0.1 µg l⁻¹; Ni, 0.1 µg l⁻¹; Pb 11 µg l⁻¹Pr, 0.1 µg l⁻¹; Sc, 0.05 µg l⁻¹; Se, 6 µg l⁻¹; Sm, 0.1 µg l⁻¹; Sr, 0.03 µg l⁻¹; Tb, 0.1 µg l⁻¹; Ti, 0.09 µg l⁻¹; Tm, 0.1 µg l⁻¹; V, 0.05 µg l⁻¹; Y, 0.1 µg l⁻¹; Yb, 0.1 µg l⁻¹ and Zn, 0.07 µg l⁻¹.

multi-elemental standard solution and a lanthanide standard solution in 0.5% nitric acid. The calibration range for all the 41 elements evaluated (Al, As, Ba, Be, Bi, Cd, Ca, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Gd, Ho, K, La, Li, Lu, Mg, Mn, Mo, Na, Nd, Ni, Pb, Pr, Sc, Se, Sm, Sr, Tb, Ti, Tl, Tm, V, Y, Yb and Zn) was from 0.01 to 0.5 mg l⁻¹, and for the highly concentrated elements (Ca, Fe, K, Mg, Mn and Na) up to 25 mg l⁻¹.

2.5. Statistical analysis

Evaluation and analysis of data were carried out by means of the following software packages: PLS Toolbox software version 2.1 (Eigenvector Research, Wenatchee, WA) in MATLAB computer and visualisation environment (the Mathworks, Natick, MA). Cluster analysis according to Ward, discriminant analysis (DA) and principal component analysis (PCA) were performed.

Although 41 elements were evaluated in the 67 red wine samples, the calculations were performed using the 38 variables (contents of Al, Ba, Be, Ca, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Gd, Ho, K, La, Li, Lu, Mg, Mn, Mo, Na, Nd, Ni, Pb, Pr, Sc, Se, Sm, Sr, Tb, Ti, Tm, V, Y, Yb and Zn in mg l⁻¹, determined by ICP-OES). The concentration of As, Bi and Tl were lower than the limit of detection (LOD) of the technique in all the samples analysed and were not used in the statistical analysis of data.

3. Results and discussion

3.1. Mineral content of wine samples

Table 3 shows the maximum, minimum and mean values of Al, Ba, Be, Ca, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Gd, Ho, K, La, Li, Lu, Mg, Mn, Mo, Na, Nd, Ni, Pb, Pr, Sc, Se, Sm, Sr, Tb, Ti, Tm, V, Y, Yb and Zn for samples, as a function of their regions of origin (Yecla, Utiel-Requena, Jumilla and Valencia) expressed in milligrams per litre.

From the aforementioned table, it can be seen that elemental composition of wine samples can effectively discriminate regions of production.

It must be noted that, for data treatment, values found lower than the LOD for some samples, were treated by assuming these LOD values in the corresponding calculations.

3.2. Statistical procedures for wine classification

In the present study, the discrimination between regions of wine origin has been based on variations of the measured concentrations of elements. In this sense, mineral contents were used as chemical descriptors in the statistical methods, in order to establish differences between PDO wine samples, for classification and authentication purposes. HCA, PCA, CART and DA were employed
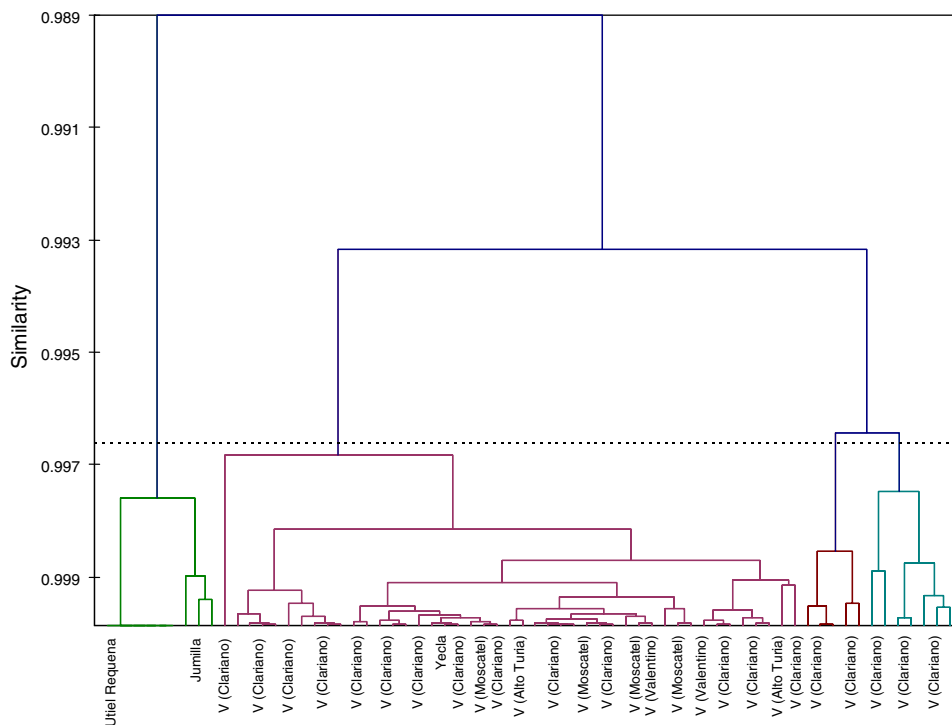
Fig. 2. Dendrographic classification of the 67 red wine samples considered from different PDO (Yecla, Jumilla, Utiel-Requena and Valencia) using the euclidian distance, based on their contents of Al, Ba, Be, Ca, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Gd, Ho, K, La, Li, Lu, Mg, Mn, Mo, Na, Nd, Ni, Pb, Pr, Sc, Se, Sm, Sr, Tb, Ti, Tm, V, Y, Yb and Zn in mg l^{-1}, determined by ICP-OES.
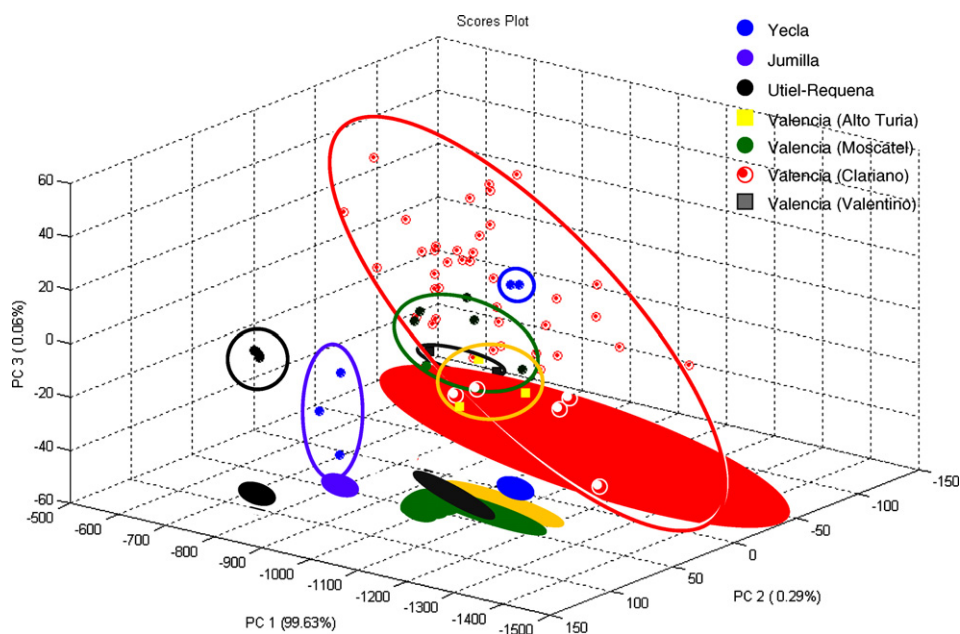

Fig. 3. PCA scores of Yecla, Jumilla, Utiel-Requena and Valencia PDO red wine samples.

for chemometric treatment and data found compared, in order to look for the best analytical tool.

3.2.1. Hierarchical cluster analysis

In HCA, the similarity between samples is established using the distance concept, calculated from mathematical relationships of numerical properties of the samples (Marengo & Aceto, 2003). In an iterative procedure, each sample was linked to the closest sample or group of samples and a characteristic distance was used to

describe this union. The distance between groups of samples (commonly called classes or clusters) can be evaluated in different ways and is the main difference among common linkage methods. The group formation can be represented graphically in a dendrogram, which shows the different groups at a normalised or rescaled distance of each kind of samples from the others, when it is read from right to left.

As can be seen from Fig. 2, HCA shows a clear discrimination between samples from Utiel-Requena, Jumilla and the rest of the
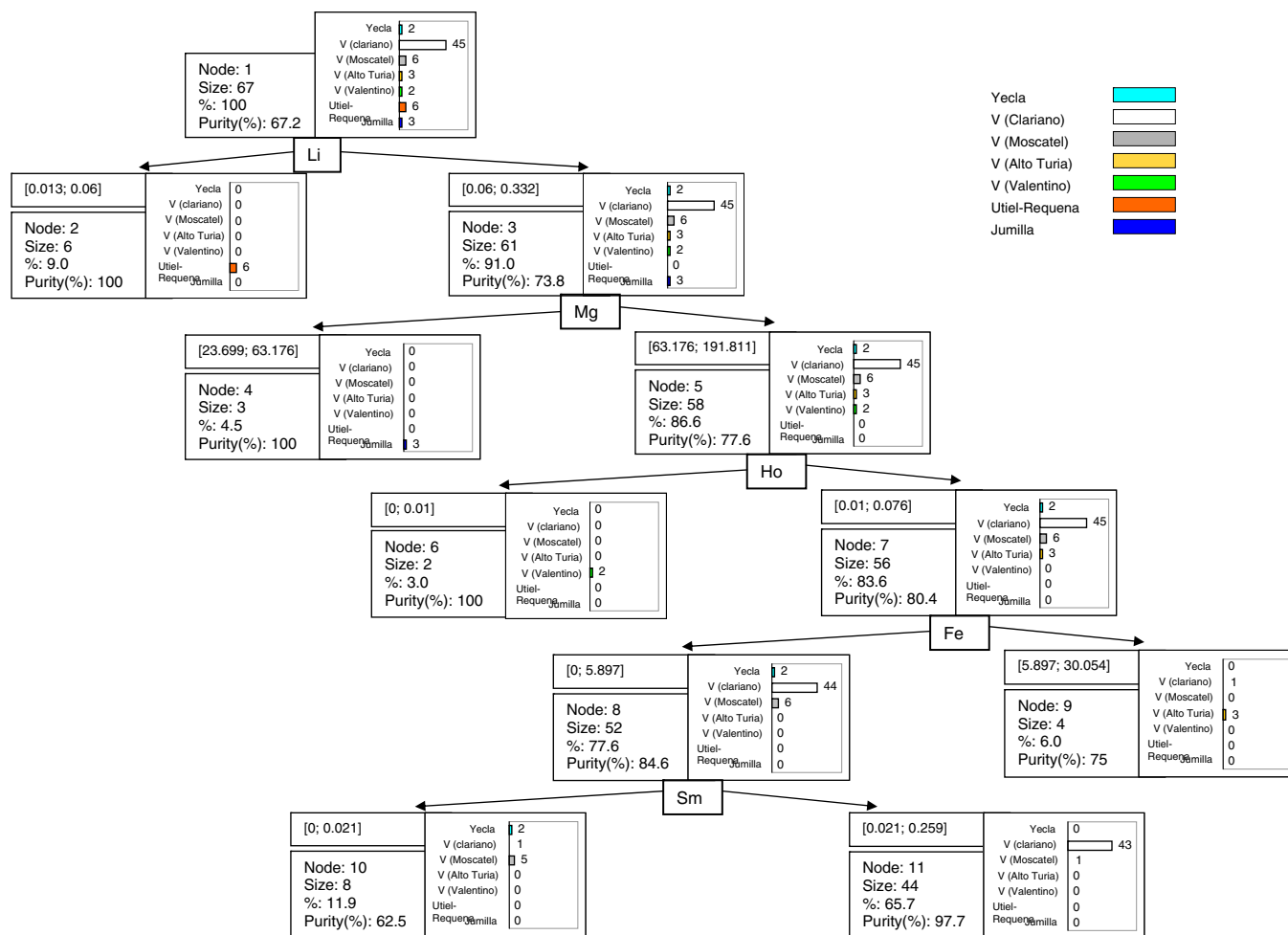
Fig. 4. Classification trees obtained from CART for red wine samples from the four different PDO considered: Yecla, Jumilla, Utiel-Requena and Valencia.

wine PDO studied. However, samples from Valencia and Yecla PDO could not be differentiated. It is easy to explain this fact due to the heterogeneity of the Valencia PDO. As has been aforementioned, this designation comprises a vast region where the weather, the soil and the variety of grapes are not similar, resulting in the Yecla PDO wines being closer to the Clariano area than the Alto Turia, Valentino or Moscatel.

3.2.2. Principal component analysis

PCA reduces the data dimension to some principal components, providing a new set of variables obtained as the best linear combination of the original parameters, which accounts for more of the variance than any other combination. At the same time, PCA enables the extraction of the differences between samples and the main variables. Fig. 3 shows the classification pattern as obtained according to 38 variables (contents of Al, Ba, Be, Ca, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Gd, Ho, K, La, Li, Lu, Mg, Mn, Mo, Na, Nd, Ni, Pb, Pr, Sc, Se, Sm, Sr, Tb, Ti, Tm, V, Y, Yb and Zn in mg l^{-1}, determined by ICP-OES).

The main conclusions that can be drawn from this PCA analysis are as follows: (i) the origin of samples is important and, in fact, it is the most influenced factor in the chemical composition of samples; (ii) Utiel-Requena and Jumilla PDO wines are very easy discriminated, according to obtained data, from the rest of studied PDO and (iii) Valencia and Yecla PDO show overlapping regions. Although, we expect discrimination of Valencia and Yecla PDO wines, the heterogeneity of the Valencia PDO wines and the vicin-

ity of the two regions make their differentiation a difficult task through the use of PCA.

3.2.3. Classification and regression trees

CART method applied to the 67 red wine samples and the 38 available parameters provided a model to discriminate between the different PDOs studied throughout the paper. The tree derived from the CART model is represented in Fig. 4.

The resulting tree clearly confirms the first conclusions obtained from PCA; Utiel-Requena and Jumilla PDO samples are very easy to discriminate from the other evaluated PDOs. Indeed, the correct re-substitution rate achieved by CART for Utiel-Requena is 100% and uses only the value of a single element, Li. The box plot of this parameter (see Fig. 4) shows that Utiel-Requena wines are characterised by a low value of this element as compared to the rest of samples. Regarding Jumilla PDO samples, it should be highlighted that the correct re-substitution rate achieved is also 100%, characterised by high values of Li, but low concentrations of Mg.

As mentioned in the introduction, Valencia PDO comprises a vast region and it shelters four separate and different sub-zones. CART method can discriminate samples from the different subzones. Using the concentration value of Ho as a discriminating criterion, red wine samples from the Valencia (Valentino) PDO can be differentiated. This sub-group of samples is characterised by low concentrations of Ho. By using the concentration of Fe, CART discriminate samples of Valencia (Alto Turia) PDO from the rest, with a correct re-substitution rate of 100%. Finally it can be seen, that
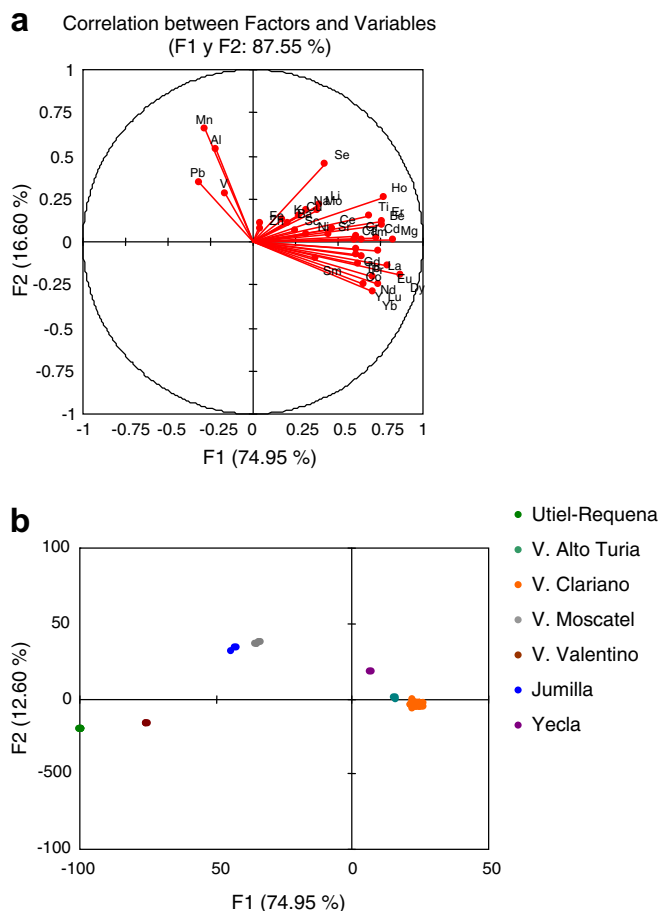
Mo, Na, Nd, Ni, Pb, Pr, Sc, Se, Sm, Sr, Tb, Ti, Tm, V, Y, Yb and Zn in mg l^{-1}, determined by ICP-OES) can be grouped in the three first discriminant functions, which are shown to account for 74.9, 12.6 and 8.2% of the total discriminating power. The variables included in the analysis are determined with a stepwise-LDA, using a Wilk's Lambda selection criterion and an F-statistic factor, to establish the significance of changes in Lambda when a new variable is tested. The prediction capacity of the discriminant models was studied by re-substitution, in order to determine the stability of the model. In this sense, the classification results were based on the computation of the aforementioned three first discriminant functions.

To identify the most significant variables (elements in this case) contributing to the calculation of the discriminant functions, the relevant standardised function coefficients were derived. Results obtained (see Fig. 5a) demonstrate that all the elements studied significantly account for the classification of red wines according to their regional origin.

Once the discriminant functions were obtained, the relevant discriminant scores were calculated for each sample under research. Visualisation of classification and between-group differentiation results was achieved by projecting the set of discriminant scores on the two-dimensional space defined by the first and second discriminant functions (Fig. 5b). The highest differentiation potential was shown to be linked to the direction of the first discriminant function, enabling the complete resolution between the different PDOs.

The apparent classification accuracy of results obtained by the re-substitution method is shown in Table 4. The percentage of correct classification reached 100% for all the studied PDO.

4. Conclusion

On using inductively coupled plasma optical emission analysis and different chemometric approaches, the discrimination of different red wine samples from Utiel-Requena, Jumilla, Yecla and Valencia PDO can be achieved.

Results of hierarchical cluster and PCA found, using the contents of Al, Ba, Be, Ca, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Gd, Ho, K, La, Li, Lu, Mg, Mn, Mo, Na, Nd, Ni, Pb, Pr, Sc, Se, Sm, Sr, Tb, Ti, Tm, V, Y, Yb and Zn as variables, clearly show that Utiel-Requena and Jumilla red wines are very easy to discriminate from the rest of PDO in their vicinity. However, possibly due to the heterogeneity of the red wines from Valencia PDO, wines from this PDO cannot be discriminated by using the aforementioned classification tools.

It should be highlighted that using the CART method wines from Utiel-Requena and Jumilla can be easily authenticated by using the concentration of Li and Mg, respectively, being not necessary a multi-elemental determination. In this case, it can be seen that wine samples from Valencia PDO sub-regions can be also discriminated with a quite acceptable re-substitution rate.

Fig. 5. DA plots of Valencian area red wines: (a) contribution of each variable (element) to the calculation of the discriminant functions and (b) discriminant scores for the four PDO studied (Yecla, Jumilla, Utiel-Requena and Valencia) on the space defined by the two first canonical functions.

samples from the Valencia subset Clariano can also be differentiated, using the concentration of Sm as a discriminating criterion. The correct re-substitution rate achieved in this case is 95.6%.

In the same sense as suggested by PCA the similarities between Yecla and Valencia (Moscatel) PDO red wines make their differentiation difficult.

3.2.4. Discriminant analysis

For the integration of the classifying information individually provided by the metal composition, a multivariate statistical analysis approach, based on discriminant analysis (DA), was also employed. The discriminant information associated with the function derived from metal content (concentration of Al, Ba, Be, Ca, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Gd, Ho, K, La, Li, Lu, Mg, Mn,

Table 4
Discriminant analysis results for Valencian red wines: percentage degree of correct classification (re-substitution analysis) according to regional origin

Origin (PDO)[a]	Jumilla	Utiel-Requena	Valencia (Valentino)	Valencia (Alto Turia)	Valencia (Moscatel)	Valencia (Clariano)	Yecla	Total	% Correct Classification
Jumilla	3	0	0	0	0	0	0	3	100%
Utiel-Requena	0	6	0	0	0	0	0	6	100%
Valencia (Valentino)	0	0	2	0	0	0	0	2	100%
Valencia (Alto Turia)	0	0	0	3	0	0	0	3	100%
Valencia (Moscatel)	0	0	0	0	6	0	0	6	100%
Valencia (Clariano)	0	0	0	0	0	45	0	45	100%
Yecla	0	0	0	0	0	0	2	2	100%
	3	6	2	3	6	45	2	67	100%

[a] Protected designation of origin (PDO).

On the other hand, discrimination analysis performed using the same variables, allows the entire studied PDOs to be separated, obtaining accuracy results by the re-substitution method of 100%. It should be highlighted that the aforementioned models have been developed using red wines with not only different origins, but also different vintages and grape varieties.

Acknowledgements

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