Classification of wines according to several factors by ICP-MS multi-element analysis

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ABSTRACT

Wines from different grape varieties, geographical zones, soil types, foliar N application, SO_2 addition and oak ageing were analyzed by inductively coupled plasma mass spectrometry (ICP-MS). For this purpose, ICP-MS methodology was optimized. The elements which allowed differentiate wines from studied grape varieties were Sr, Ca, Mg and Mn. Geographical zones were classified according to Sr, Ba, Ni, and Cu. Cs and Pb were the main elements to discriminate the wines from the 3 soil types. Wines from several N foliar doses application were classified by Pb, Ni, Mn and Zn. The content of Cs, Mg, Cu and Pb in wines characterized the SO_2 addition. Finally, wines storage in barrels were differentiate by Na and Cs concentration. The discriminant functions classify 100% of the wines, with the exception of grape variety (97.0%) and oak ageing (95.8%). Consequently, ICP-MS can be applied to classified wines according to viticultural and oenological factors.

1. Introduction

Wine is a complex matrix and, it contains low level concentration of mineral elements (the level of the major elements, Ca, K, Mg, Na, and Mg usually ranged between 10 and 1000 mg/L, minor elements such as Al, Fe, Cu, Mn, Rb, Sr, and Zn are present in the range of 0.1 to 10 mg/L and trace elements, including Ba, Cd, Co, Cr, Li, Ni, Pb, and V are in the range of 0.1–1000 μg/L) (Pohl, 2007). Some of these trace elements, such as Fe, Zn, Cu, Cr, and Se, are essential for the human organism in that they form an integral part of one or more enzymes involved in a metabolic or biochemical process (Pan, Tang, Chen, Wu, & Han, 2013). Elements are also important for efficient alcoholic fermentation and for the prosthetic metallo-enzyme activation of yeast (Rodríguez Mozaz, García Sotro, Garrido Segovia, & Ancín Azpilicueta, 1999). In addition, minerals can contribute to stability and clarity in the wine and its color, and they may affect the organoleptic characteristics of the wine, mainly Zn and Fe (Iglesias, Besalú, & Anticó, 2007), or wine conservation, i.e. precipitation of K and Ca tartrates (McKinnon & Scollary, 1997). Wine minerals are useful because of the possibility of toxicological risk, such as Cd and Pb (Lara, Cerutti, Salonia, Olsina, & Martinez, 2005) and food regulations (Frías, Conde, Rodríguez, Dohnal, & Pérez-Trujillo, 2002).

Mineral content in wines depends on diverse factors: i) natural sources, which reflect the vineyard soil geochemistry and represent the uptake of minerals from soils (Kment et al., 2005). For instance, vineyards located near coastal areas may be influenced by wind from sea or ocean (González-Hernández, Hardisson de La Torre, & Arias Léon, 1996; Frías, Pérez Trujillo, Peña, & Conde, 2001; Sauvage, Frank, Stearne, & Millikan, 2002; Frías, Conde, Rodríguez-Bencomo, García-Montelongo, & Pérez-Trujillo, 2003); ii) external contamination of the grapevine during growth (from inorganic pesticides, herbicides, fungicides and fertilizers applications, plant protection practices or from environmental pollution (Álvarez, Moreno, Jos, Cameán, & González, 2007; Fiket, Mikac, & Kniewald, 2011; Kment et al., 2005; Pohl, 2007); iii) contamination during the winemaking process (prolonged contact with the materials from which pipes, casks and barrels are made, use of additives, fining and clarifying substances…) (Jakubowski, Brandt, Stuewer, Eschnauer, & Görtges, 1999; Fiket et al., 2011; Kment et al., 2005; Lara et al., 2005; Rusjan, Strlič, Pucko, Šelih, & Korošec-Koruza, 2006; Pohl, 2007; Kruzlicova, Fiket, & Kniewald, 2013); and iv) grape maturity and variety, and climatic conditions (González-Hernández et al., 1996). Therefore, knowledge of the mineral content in wines is a growing concern for both consumers and producers (Pohl, 2007).

Usually, atomic absorption spectrometry (AAS), atomic emission spectrometry (AES), inductively coupled plasma mass spectrometry (ICP–MS) and inductively coupled plasma optical emission spectrometry (ICP–OES) are the analytical methods used for the determination of elements in foods (Orvini, Speziali, Salvini, & Herborg, 2000). Multi-elemental analysis in wines, ICP-MS is the most versatile technique, which provides high detection power (due to low detection limits) and high selectivity and sensitivity (Eschnauer, Jakob, Meierer, & Neeb, 1989; Thiel & Danzer, 1997; Murányi & Papp, 1998; Murányi & Kovács, 2000; Castiñeira Gómez, Brandt, Jakubowski, & Andersson, 2004; Jos, Moreno, González, Repetto, & Cameán, 2004; Šperková & Suchánek, 2005; Ivanova-Petropulos et al., 2013, 2015). This technique offers the advantage of analyzing several elements that are present in low concentration in wines at the same time thus considerably shortening the length of time taken for analysis (Williams, Jarvis, & Wills, 1992).

Taking into consideration that wine is a complex water-ethanol mixture, containing various inorganic and organic substances at different levels (Álvarez et al., 2007; Pyrzynska, 2007; Gonzálvez, Armenta, Pastor, & De La Guardia, 2008; Moreno et al., 2008; Voica, Dehelean, & Pamula, 2009; Rodrigues et al., 2011), the largest matrix effects of the samples must be eliminated before the ICP-MS analysis is carried out. For this reason, the stage of pre-treatment of samples in wine mineral analysis can be time-consuming, labor-intensive, and prone to potential contamination problems, as it is a very delicate stage and one that must be properly carried out. The preparation of wine samples for mineral elements determination includes microwave-assisted acid digestion, UV-assisted digestion, thermal digestion in an open reactor, sample dilution, dry and wet ashing, and also less common approaches such as de-alcoholization or analyte separation (Gonzálvez et al., 2008; Ivanova-Petropulos et al., 2016). Moreover, when choosing the method of wine sample preparation, one must consider which procedure provides the best results in the shortest time, with minimum losses and contamination risks, consumes the smallest quantities of reagents and samples and generates the smallest amount of residue and waste (Gonzálvez et al., 2008).

Several studies have looked at the use of spectroscopic methods, such as AAS or ICP-MS, for elemental characterization of wines or for testing wine authenticity, in the case of, for example investigating fraud or adulteration (Brescia et al., 2002). In this study, major, minor and trace elements of 34 AOC Rioja wines (red and white) were determined by ICP-MS. Different calibration methodologies (standard addition and external calibration) and two different approaches for the pre-treatment of samples (direct acidic dilution of the wines and digestion procedures) were compared. Moreover, the element content was used in order to classify the wines according to different grape varieties, geographical zones, soil types, foliar nitrogen application, with or without SO₂ addition and oak ageing.

2. Material and methods

2.1. Reagents and materials

All reagents used were of analytical grade. For sample dilution and preparation of standards, ultrapure water was used (18.2 mΩ, Milli-Q, Millipore-Merck, Darmstadt, Germany). Nitric acid Suprapur® grade (65.0%, Merck) was used for wine digestion, dilutions and for standard solution preparation. Multi-element certificate standard solution ICP-MS-68B-A (100 mg/L) was from High-Purity Standards (Charleston, SC, USA). Tuning solution (ICP-MS Tuning solution), containing 10 µg/L each of Ce, Co, Li, Tl, and Y in a matrix of 2% HNO₃ (Agilent Technologies, Palo Alto, CA, USA), was used for ICP-MS instrument optimization in order to achieve $CeO^+/Ce^+ < 2\%$ and $Ce^{2+}/Ce^+ < 3\%$. The signal was maximized while maintaining 0.9–1.1% CeO^+/Ce^+ during tuning. Ru, Rh, and Ir were used as internal standards for possible instrumental drifts and matrix effects corrections.

2.2. Wine samples

A total of 34 wine samples (8 whites and 26 reds) were collected from AOC Rioja, Spain. All the wines were elaborated in the same cellar following traditional procedures used in AOC Rioja (Spain) and came from the 2016 vintage, except those aged in barrels, which were elaborated in 2015. The white grape varieties were: Viura and Tempranillo blanco. Tempranillo blanco grapes came from five AOC Rioja grapevine growing zones with different edaphoclimatic conditions (La Grajera (2 wines), Valdegón (1 wine), Cenicero (1 wine), Corera (1 wine), and Alfaro (1 wine)), while the Viura grapes came from La Grajera (1 wine). Moreover, a Tempranillo blanco wine from La Grajera was aging in barrels. The red samples were elaborated with Tempranillo, Garnacha, Maturana, and Graciano grape varieties. Tempranillo red wines were from different soils, classified as *Fluventic haploxerepts* (FH), *Typic calcixerepts* (TC), and *Petrocalcic palexeroll* (PP), according to the Soil Survey Staff (2010) classification, and located in Uruñuela (La Rioja). Three wines were elaborated from each of the three soil types (a total of 9 wines). Moreover, urea foliar applications were carried out according to Pérez-Álvarez, Garde-Cerdán, García-Escudero, and Martínez-Vidaurre (2017), and the treatments were made in triplicate (9 wines in total). In addition, wines from Tempranillo, Graciano, Maturana, and Garnacha grape varieties were produced from La Grajera (4 wines). The wines of Tempranillo and Graciano were also aged in barrels (2 wines). Fermentations were carried out without the addition of SO₂ in two Tempranillo wines (2 wines), one of which was aged in barrels.

2.3. Sample preparation

Two different procedures were assayed over the 34 wine samples: acidic wine dilution and acidic digestion. The perfluoroalkoxy (PFA) beakers used for storing and treating the samples were cleaned to avoid contamination by any traces of metal. The containers were treated with 5% HNO₃ and then washed with Milli-Q water.

2.3.1. Dilution

Samples were diluted 10 fold for minor and trace elements (Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Cd, Cs, Ba, and Pb) and 100 fold for major elements (Na, Mg, P, K, and Ca) with 2% HNO₃, without any prior preparation.

2.3.2. Digestion

Wine digestion was carried out in the PFA beakers adding 5 mL of each wine and 2 mL of 65% HNO₃; then, the beakers were put on a hotplate between 50 and 70 °C until the samples became colorless because of the digestion of the acid. Once the digestion step had been completed, the solutions were cooled to room temperature. The tempered samples were transferred to PFA volumetric flasks and fulfilled with Milli-Q water up to 50 mL.

2.4. ICP-MS analysis

Multi-element determination was performed on an Agilent 8800 Triple Quadrupole ICP-MS, equiped with a Micromist nebulizer. In accordance with the analytes of interest, the collision/reaction cell was in "no-gas mode", "He mode", "O₂ mode" and "NH₃ mode".

Plasma gas flow rate was 15 L/min and collision and reaction gases flow rates were 4 mL/min, 0.5 mL/min and 1.5 mL/min for He, O₂ and NH₃, respectively. Analyses were optimized at 1550 W forward power and 1.1 L/min carrier gas flow with no dilution or makeup gas. Sampling depth (10 mm) and lens parameters were optimized for highest signal and optimum peak shape while maintaining low oxides.

In "no-gas mode", selected masses at Q1/Q2 were 23/23 (Na), 24/24 (Mg), 39/39 (K), 101/101 (Ru), 103/103 (Rh), 111/111 (Cd), 115/115 (In), 133/133 (Cs), 137/137 (Ba), 139/139 (La), 140/140 (Ce), 141/141 (Pr), 146/146 (Nd), 147/147 (Sm), 153/153 (Eu), 157/157

(Gd), 166/166 (Er), 169/169 (Tm), 172/172 (Yb), 193/193 (Ir), 208/208 (Pb), and 209/209 (Bi). In "He mode", selected masses at Q1/Q2 were 23/23 (Na), 24/24 (Mg), 27/27 (Al), 39/39 (K), 45/45 (Sc), 51/51 (V), 52/52 (Cr), 55/55 (Mn), 56/56 (Fe), 57/57 (Fe), 59/59 (Co), 60/60 (Ni), 63/63 (Cu), 66/66 (Zn), 78/78 (Se), 88/88 (Sr), 101/101 (Ru), 103/103 (Rh), 111/111 (Cd), 115/115 (In), 133/133 (Cs), 137/137 (Ba), 193/193 (Ir), 208/208 (Pb), and 209/209 (Bi). In "O_2 mode", selected masses at Q1/Q2 were 31/47 (P), 75/91 (As), 101/101 (Ru), 103/103 (Rh), and 193/193 (Ir). And in "NH_3 mode", selected masses at Q1/Q2 were 42/42 (Ca), 101/101 (Ru), 103/103 (Rh), and 193/193 (Ir). All the operation modes were with the MS/MS scan type. The number of replicates was 3 and sweeps per replicate were 10.

Two calibration modes were carried out: external calibration and standard addition calibration. The external calibration was prepared from high purity standards (ICP-MS-68B-A) diluted in synthetic wine (12% ethanol and 4 g/L tartaric acid) and 2% HNO_3, in order to obtain 10 different concentration solutions: 0, 1, 10, 50, 100, 200, 500, 1000, 1500, and 3000 mg/L. The standard addition calibration was different for minor, trace and for major elements. In the first case, 3 mL of one of the wine samples were mixed with 2% HNO_3 and the necessary amount of the high purity standards (ICP-MS-68B-A) up to 5 mL in order to obtain six different concentration solutions: 0, 50, 100, 200, 400, and 800 µg/L. For the major elements, also 3 mL of one of the wine samples were mixed with 2% HNO_3 and the necessary amount of the high purity standards (ICP-MS-68B-A) up to 5 mL in order to obtain five different concentration solutions: 0, 500, 1000, 2000, and 4000 mg/L. Both calibration modes, external and standard addition, were employed for dilution and digestion sample procedures. The limits of detection (LOD) and quantification (LOQ) of the different elements analyzed are shown in Tables S1 and S2.

2.5. Statistical analysis

A statistical analysis on mineral elements (major, minor, and trace) of red, white and all wines together was performed using variance analysis (one-way ANOVA) using SPSS Version 21.0 (Chicago, USA). Differences between averages were compared using the Duncan test at 0.05 probability level. Discriminant analyses were carried out on data showing the concentration of elements found in the wines in order to classify them according to several factors: red and white wines from different grape varieties, geographical zones, soil types, foliar nitrogen application, with or without SO_2 addition, and oak ageing.

3. Results and discussion

3.1. Optimization of sample preparation and quantification method

Table S3 shows the mean, the minimum and the maximum contents of the major elements obtained in the 34 wine samples. Each of these wines underwent both sample preparations, dilution and digestion, and both calibration methods. The results indicated that the digestion sample preparation with the standard addition (GSA) was the best for Mg, K, and Ca, due to their content in the wines was the highest; and also for P but without significant differences in the dilution sample preparation with standard addition (LSA). In the case of Na, there were no significant differences between the four approaches. Considering only the white samples (Table 1), GSA was the best methodology for Mg, and Ca determination. In the case of P concentration, GSA was better than dilution sample preparation with the external calibration (LEC) but without significant differences with respect to the digestion sample preparation with the external calibration (GEC) and LSA. Potassium content was higher in GSA than in LEC and LSA, but with no differences with GEC approach. For Na concentration, there were no significant differences between the four approaches. Regarding the major elements in red wines (Table 2), in all the elements the best approach was GSA, except for P, in which GSA methodology was

Table 1
Results of the major elements (mg/L) in white wines (n = 8).

		LEC	GEC	LSA	GSA
Na	Mean	12.76 a	17.84 a	17.68 a	20.45 a
	SD	12.46	13.01	12.99	15.02
	Minimum	0.90	4.91	5.16	6.72
	Maximum	35.50	41.90	41.12	48.12
Mg	Mean	84.82 a	90.28 a	87.23 a	114.64 b
	SD	10.08	10.70	11.47	14.68
	Minimum	69.50	73.24	75.04	99.80
	Maximum	103.88	109.23	111.52	144.38
P	Mean	29.13 a	48.55 ab	55.25 ab	62.26 b
	SD	14.13	24.14	26.77	30.01
	Minimum	12.23	20.24	23.30	25.64
	Maximum	53.97	89.69	101.29	114.33
K	Mean	539.62 a	586.51 ab	485.91 a	688.60 b
	SD	104.90	119.64	92.57	135.80
	Minimum	463.04	481.92	420.09	574.59
	Maximum	780.57	836.23	693.96	967.33
Ca	Mean	51.54 a	49.13 a	53.65 a	63.98 b
	SD	8.98	9.40	8.93	12.15
	Minimum	35.47	34.32	39.78	44.52
	Maximum	62.44	61.55	64.49	79.46

LEC: sample dilution and element quantification by external calibration. GEC: sample digestion and element quantification by external calibration. LSA: sample dilution and element quantification by standard addition. GSA: sample digestion and element quantification by standard addition. SD: standard deviation. For each element, different letters indicate significant differences between sample preparation procedures and quantification methods (p ≤ 0.05).

Table 2
Results of the major elements (mg/L) in red wines (n = 26).

		LEC	GEC	LSA	GSA
Na	Mean	3.20 a	6.45 a	6.43 a	7.33 a
	SD	6.19	6.98	6.94	8.22
	Minimum	< LOQ	1.99	1.90	2.00
	Maximum	22.14	26.81	26.04	31.25
Mg	Mean	78.74 a	80.40 a	80.15 a	104.97 b
	SD	21.87	18.95	21.57	26.17
	Minimum	47.97	55.55	52.02	69.28
	Maximum	131.69	116.60	132.55	155.13
P	Mean	69.32 a	110.86 b	129.58 bc	142.07 c
	SD	25.05	35.17	46.64	44.77
	Minimum	33.94	56.91	66.18	72.51
	Maximum	134.73	164.22	261.54	210.97
K	Mean	1112.46 ab	1167.60 b	988.09 a	1371.67 c
	SD	282.52	224.87	233.56	268.57
	Minimum	859.71	735.18	739.67	904.25
	Maximum	2125.45	1762.73	1776.94	2159.68
Ca	Mean	67.72 b	59.89 a	66.81 b	77.52 c
	SD	12.58	10.42	13.76	12.67
	Minimum	36.93	35.01	37.46	46.03
	Maximum	92.01	76.25	98.50	94.86

LEC: sample dilution and element quantification by external calibration. GEC: sample digestion and element quantification by external calibration. LSA: sample dilution and element quantification by standard addition. GSA: sample digestion and element quantification by standard addition. SD: standard deviation. LOQ: limit of quantification. For each element, different letters indicate significant differences between sample preparation procedures and quantification methods (p ≤ 0.05).

similar to the LSA one. In the case of Na, significant differences between the four approaches were not found.

Only 15 minor and trace elements were found in the wines out of a total of 29 minor and trace elements searched for (Table S4), as 14 elements were not found in the wines: Sc, V, In, La, Ce, Pr, Nd, Sm, Eu, Gd, Er, Tm, Yb, and Bi. For Al, the best approach was the digestion of

Table 3
Results of the minor and trace elements (μg/L) in white wines (n = 8).

		LEC	GEC	LSA	GSA
Al	Mean	32.81 a	85.91 a	53.41 a	87.76 a
	SD	76.04	64.92	83.08	105.51
	Minimum	< LOQ	41.24	< LOQ	< LOQ
	Maximum	219.28	219.93	255.72	330.13
Cr	Mean	6.53 a	8.58 ab	10.57 b	8.66 ab
	SD	2.47	2.09	3.02	3.01
	Minimum	2.89	6.50	6.85	4.42
	Maximum	9.15	12.88	14.81	13.53
Mn	Mean	666.74 a	579.72 a	687.47 a	662.25 a
	SD	193.51	125.44	152.17	147.34
	Minimum	414.73	381.09	438.59	429.03
	Maximum	1030.30	792.69	947.13	916.11
Fe	Mean	293.97 a	415.39 a	410.20 a	385.74 a
	SD	362.48	340.40	323.32	309.28
	Minimum	< LOQ	152.42	135.67	119.47
	Maximum	1048.01	1086.96	1056.56	984.89
Co	Mean	1.19 a	1.35 a	0.78 a	0.61 a
	SD	1.21	0.99	0.82	0.95
	Minimum	0.35	0.50	0.08	< LOQ
	Maximum	3.92	3.42	2.62	2.51
Ni	Mean	7.96 ab	6.28 a	8.52 b	6.84 ab
	SD	1.33	1.81	2.16	1.76
	Minimum	6.30	3.88	5.80	4.94
	Maximum	9.97	8.20	11.28	9.59
Cu	Mean	101.04 a	91.16 a	97.74 a	93.80 a
	SD	56.38	46.61	50.70	48.25
	Minimum	12.96	13.25	12.77	13.68
	Maximum	176.31	151.60	162.40	154.26
Zn	Mean	304.55 a	286.86 a	322.83 a	305.82 a
	SD	127.47	87.03	116.55	98.77
	Minimum	164.39	168.16	158.07	181.83
	Maximum	597.56	459.68	568.32	516.12
As	Mean	10.18 c	2.41 a	6.77 b	3.57 a
	SD	3.45	1.76	1.72	1.26
	Minimum	6.42	1.32	5.70	2.41
	Maximum	16.54	6.45	10.91	6.54
Se	Mean	11.10 b	2.83 a	4.19 a	1.28 a
	SD	7.84	5.08	3.69	2.44
	Minimum	2.09	< LOQ	< LOQ	< LOQ
	Maximum	22.99	11.10	11.50	6.17
Sr	Mean	1314.35 a	1316.32 a	1270.31 a	1245.70 a
	SD	596.93	599.55	574.63	573.84
	Minimum	644.16	697.90	692.27	644.25
	Maximum	2558.77	2631.17	2546.37	2527.01
Cd	Mean	0.21 a	0.36 b	0.06 a	0.09 a
	SD	0.14	0.20	0.11	0.11
	Minimum	< LOQ	0.20	< LOQ	< LOQ
	Maximum	0.40	0.71	0.30	0.32
Cs	Mean	3.10 a	2.69 a	2.56 a	2.82 a
	SD	2.35	2.29	2.16	2.39
	Minimum	0.19	0.02	0.04	< LOQ
	Maximum	6.72	6.61	5.64	6.58
Ba	Mean	38.74 a	23.55 a	42.24 a	35.67 a
	SD	20.09	17.69	22.27	22.47
	Minimum	18.68	7.13	20.51	13.77
	Maximum	72.95	55.00	86.70	77.72
Pb	Mean	3.75 a	4.68 a	3.07 a	4.12 a
	SD	2.73	2.26	2.62	2.69
	Minimum	1.44	2.42	0.93	1.20
	Maximum	10.06	9.27	8.76	9.57

LEC: sample dilution and element quantification by external calibration. GEC: sample digestion and element quantification by external calibration. LSA: sample dilution and element quantification by standard addition. GSA: sample digestion and element quantification by standard addition. SD: standard deviation. LOQ: limit of quantification. For each element, different letters indicate significant differences between sample preparation procedures and quantification methods (p ≤ 0.05).

the samples. In the case of Cr, the best was the standard addition but with no significant differences between GEC and GSA. Concentration of Co, Se, and Cd was higher when external calibration was carried out as opposed to standard addition, with the exception of LEC for Co that did not show differences with the standard addition. The concentration of As in the wines presented differences between the four approaches, being from the best to the worst methods LSA, LEC, GSA and GEC. The GEC methodology showed the lowest concentration of Ba, with no differences between the other three approaches. The concentration of the other 8 minor and trace elements found in the wines (Mn, Fe, Ni, Cu, Zn, Sr, Cs, and Pb) did not show differences between the four approaches studied (Table S4).

Regarding the concentration of minor and trace elements in the white wines (Table 3), LSA showed the highest Cr and Ni content but without significant differences with GEC and GSA in the case of Cr and with LEC and GSA in the case of Ni. Arsenic content presented higher values in dilution sample preparation than in digestion. LEC was the best method for Se determination, while DEG was the best for Cd quantification. The concentration of the other 10 minor and trace elements found in the white wines (Al, Mn, Fe, Co, Cu, Zn, Sr, Cs, Ba, and Pb) did not show differences between the four approaches studied (Table 3).

With respect to the red wines (Table 4), Al concentration had the highest values for digestion. The standard addition was the best method for Cr determination although with no significant differences with respect to GEC approach. Dilution sample preparation was the best in the case of As concentration. The best method for Se and Cd quantification was the external calibration. GEC was the worst approach for Ba determination. The concentration of the other 9 minor and trace elements found in the red wines (Mn, Fe, Co, Ni, Cu, Zn, Sr, Cs, and Pb) did not show differences between the four approaches studied (Table 4).

Iglesias et al. (2007) also observed significant differences in element concentration between seven techniques used, except in the case of Cu concentration. They argued that after direct measurements of the wine sample, the calibration graphs obtained with aqueous standards are higher than the results obtained with other treatments, confirming the existence of a matrix effect in the wines. In the same way, Cocchi et al. (2004) show the influence of the organic components when direct method is used, although this could be avoided by adding ethanol.

3.2. Multi-element wine content

Among the major elements, K was the most concentrated in wines (Table S3), ranging from 420.09 mg/L in white wine (Table 1) to 2159.68 mg/L in red wine (Table 2); whilst Na was the major element with the lowest concentration in wines (Table S3), ranging from < LOQ in red wine (Table 2) to 48.12 mg/L in white wine (Table 1). These results were in agreement with Frías et al. (2001) and Jos et al. (2004). The remaining major elements were found at intermediate concentrations (Tables S3, 1 and 2). Regarding the difference between white and red wines, all major elements, except Mg, showed significant differences (p ≤ 0.05) with a higher concentration in red wines, except Na, where content was higher in white wines.

With regard to the minor and trace elements, Sr was the element that presented the highest concentration in wines (Table S4), with a range between 157.88 μg/L in red wine and 3,818.88 μg/L also in red wine (Table 4); the element found in lowest concentration was Co (Table S4), where concentration ranged between < LOQ, in both, red and white wines (Tables 3 and 4), and 3.92 μg/L in white wine (Table 3). Comparing red and white wines, the Co content was significantly higher in white wines than in red (p ≤ 0.05), whilst the Al, Fe, Ni, Ba, and Pb concentration was higher in red wines than in white (p ≤ 0.05). The remaining minor and trace elements did not show significant differences between either types of wine (p ≤ 0.05).

Orescanin, Katunar, Kutle, and Valkovic (2003) and Coetzee, van Jaarsveld, and Vanhaecke (2014) observed higher concentration of

Table 4
Results of the minor and trace elements (µg/L) in red wines (n = 26).

		LEC	GEC	LSA	GSA
Al	Mean	115.08 **a**	215.34 **b**	138.06 **a**	203.48 **b**
	SD	71.08	78.77	66.96	86.81
	Minimum	3.78	82.47	34.31	87.76
	Maximum	308.61	375.71	346.15	451.32
Cr	Mean	6.07 **a**	7.66 **ab**	10.02 **b**	9.64 **b**
	SD	3.57	3.77	3.95	5.14
	Minimum	1.32	3.06	5.48	4.17
	Maximum	14.71	17.38	19.73	23.40
Mn	Mean	660.91 **a**	704.57 **a**	684.22 **a**	753.02 **a**
	SD	326.52	409.36	344.30	405.85
	Minimum	350.70	335.13	375.54	396.75
	Maximum	1552.81	1813.01	1671.29	1916.57
Fe	Mean	677.89 **a**	854.98 **a**	820.21 **a**	967.35 **a**
	SD	453.48	482.79	485.46	595.90
	Minimum	227.51	275.62	382.42	291.69
	Maximum	2170.66	2406.52	2401.55	2915.01
Co	Mean	0.47 **a**	0.55 **a**	0.24 **a**	0.27 **a**
	SD	0.58	0.57	0.43	0.63
	Minimum	< LOQ	< LOQ	< LOQ	< LOQ
	Maximum	2.55	2.52	1.81	2.76
Ni	Mean	8.94 **a**	9.79 **a**	8.64 **a**	10.14 **a**
	SD	3.97	5.45	4.01	5.08
	Minimum	4.67	4.07	4.41	4.29
	Maximum	20.60	25.78	21.26	25.18
Cu	Mean	87.05 **a**	102.93 **a**	82.64 **a**	100.64 **a**
	SD	53.43	59.39	48.80	50.06
	Minimum	18.35	25.30	18.75	24.92
	Maximum	216.22	241.21	200.11	237.68
Zn	Mean	249.18 **a**	293.85 **a**	261.90 **a**	285.68 **a**
	SD	383.78	437.07	396.77	422.25
	Minimum	1.59	33.86	13.13	28.31
	Maximum	1476.76	1712.87	1560.78	1694.71
As	Mean	5.24 **c**	2.65 **a**	7.62 **d**	4.00 **b**
	SD	0.70	0.75	1.63	0.71
	Minimum	4.26	1.14	5.10	2.42
	Maximum	6.66	4.64	11.15	5.38
Se	Mean	5.59 **b**	4.96 **b**	1.62 **a**	1.03 **a**
	SD	3.82	4.04	2.81	1.95
	Minimum	< LOQ	< LOQ	< LOQ	< LOQ
	Maximum	12.54	13.67	11.50	8.22
Sr	Mean	899.69 **a**	895.48 **a**	881.54 **a**	965.93 **a**
	SD	1072.81	1070.37	1062.51	1180.36
	Minimum	161.32	165.93	157.88	180.20
	Maximum	3423.07	3390.85	3428.44	3818.88
Cd	Mean	0.18 **b**	0.34 **c**	0.03 **a**	0.03 **a**
	SD	0.18	0.19	0.06	0.09
	Minimum	< LOQ	0.04	< LOQ	< LOQ
	Maximum	0.68	0.95	0.26	0.37
Cs	Mean	3.41 **a**	3.48 **a**	2.83 **a**	3.81 **a**
	SD	2.62	2.86	2.68	3.19
	Minimum	1.14	0.93	0.75	1.01
	Maximum	12.51	13.14	12.09	14.50
Ba	Mean	65.07 **b**	53.14 **a**	70.10 **b**	71.41 **b**
	SD	14.86	16.27	16.72	19.15
	Minimum	37.13	19.30	39.29	34.42
	Maximum	110.38	99.58	119.43	128.68
Pb	Mean	7.37 **a**	8.70 **a**	7.20 **a**	8.54 **a**
	SD	9.72	9.22	10.15	10.89
	Minimum	1.29	2.48	0.49	1.15
	Maximum	41.72	39.17	40.39	44.28

LEC: sample dilution and element quantification by external calibration. GEC: sample digestion and element quantification by external calibration. LSA: sample dilution and element quantification by standard addition. GSA: sample digestion and element quantification by standard addition. SD: standard deviation. LOQ: limit of quantification. For each element, different letters indicate significant differences between sample preparation procedures and quantification methods (p ≤ 0.05).

trace elements in red wine than in white. They argued that this is because the grapeskin contact is longer in the red winemaking process and the skins contain a higher a concentration of the trace element than must.

All the samples presented levels for toxic elements such as As, Cd, Cu, Pb and Zn below the maximal concentration allowed for them in wines (As: 0.2 mg/L, Cd: 10 µg/L, Cu: 1 mg/L, Pb: 0.15 mg/L and Zn: 5 mg/L (Ivanova-Petropulos et al., 2016)).

3.3. Classification of wines according to several factors by their multi-elemental content determined using ICP-MS

3.3.1. ICP-MS classification of wines according to their grape variety

To classify the wines produced with different grape varieties (Tempranillo blanco, Tempranillo, Viura, Graciano, Maturana, and Garnacha), discriminant analysis was performed on data and then expressed as the concentration of the different elements determined by ICP-MS (independent variables). The results are shown in Fig. 1a. Function 1 explained 46.9% of the variance and function 2 explained 35.9% of the variance, therefore the total of variance explained by these two functions was 82.8%. The variables that contributed the most to the discriminant model were Sr, Ca, and Co positively, and Zn, and Ba negatively (function 1) and Mn, K, Ba, and Cs, positively, and Mg, negatively (function 2). The two discriminant functions showed a good separation between wines from white and red grape varieties (Fig. 1a). These discriminant functions allowed us to correctly classify 98.5% of the studied samples.

3.3.2. Aging in oak versus young wines

Fig. 1b showed the discriminant carried out with the data of the different element content from 2015 wines aged in oak barrels *versus* young wines produced in 2016 under the same conditions but without aging. Function 1 explained 62.9% of the variance and function 2 explained 29.0% of the variance, therefore the total of variance explained by these two functions was 91.9%. The variables that most contributed to the discriminant model were Na, positively, and Cs, negatively (functions 1 and 2). The two discriminant functions showed a good separation between Graciano wines, aged & young, but there was no difference between aged & young wines of Tempranillo varieties (Fig. 1b). These discriminant functions allowed us to correctly classify 100% of the studied samples.

3.3.3. Tempranillo wines produced with or without SO_2

The influence of the addition or not of SO_2 to the wines was also studied (Fig. 1c). Function 1 (89.4%) discriminated the samples according to SO_2 addition whereas function 2 (9.5%) discriminated the wines according to the storage in oak barrels, therefore the total of variance explained by these two functions was 98.9% (Fig. 1c). The elements that most contributed to the discriminant model were Mg, Pb, and Cu, positively, and Cs, and Zn, negatively (function 1) and Pb, positively, and Cu, negatively (function 2). These discriminant functions allowed us to correctly classify 100% of the studied samples.

3.3.4. Wines from different zones of the AOC Rioja all produced using Tempranillo blanco

Fig. 2a showed the discriminant carried out using data of the different elements from Tempranillo blanco wines from five AOC Rioja zones (La Grajera, Valdegón, Cenicero, Corera, and Alfaro). Function 1 explained 86.5% of the variance and function 2 explained 7.7% of the variance, therefore the total of variance explained by these two functions was 94.2%. The variables that most contributed to the discriminant model were Sr, and Ba, positively, and Ni, and Pb, negatively (function 1); and Ni, Pb, and Ba, positively, and Cu, negatively (function 2). Function 1 showed a good separation among wines from Cenicero zone and samples from the other four areas (Fig. 2a). These discriminant functions allowed us to classify correctly 100% of the studied
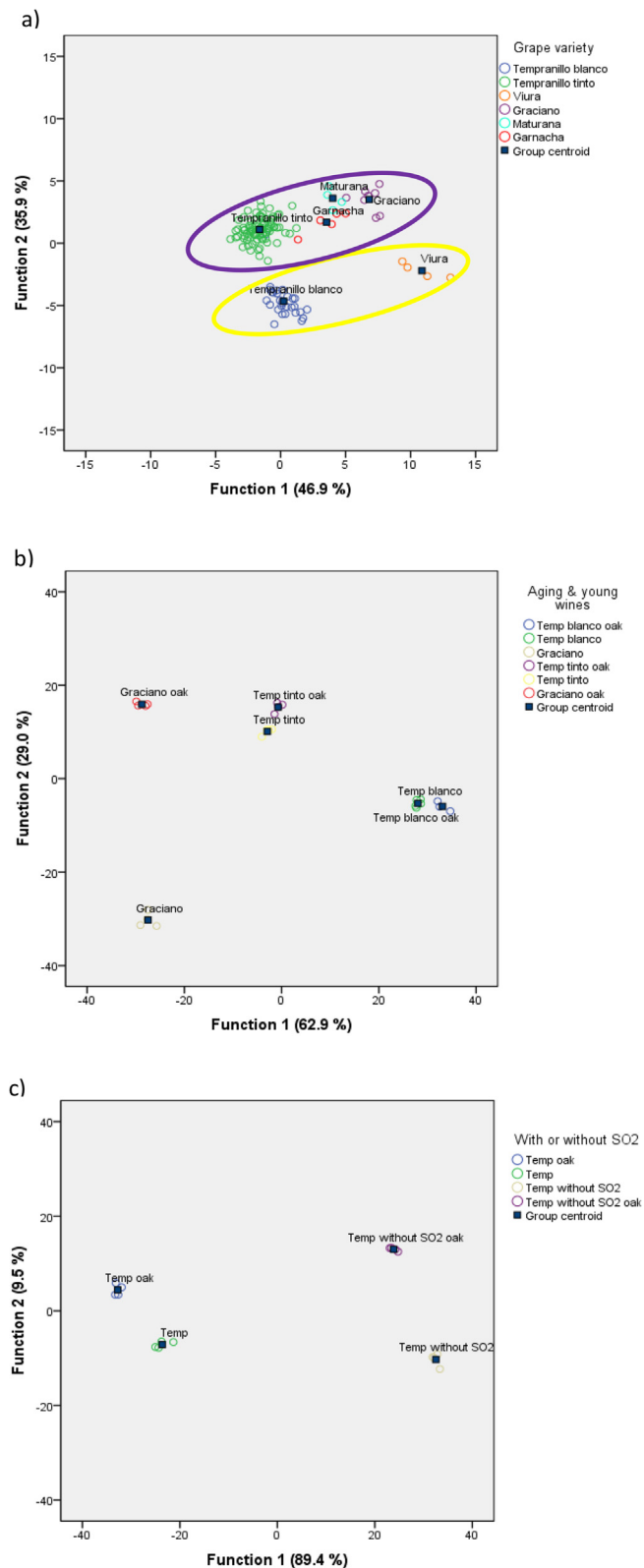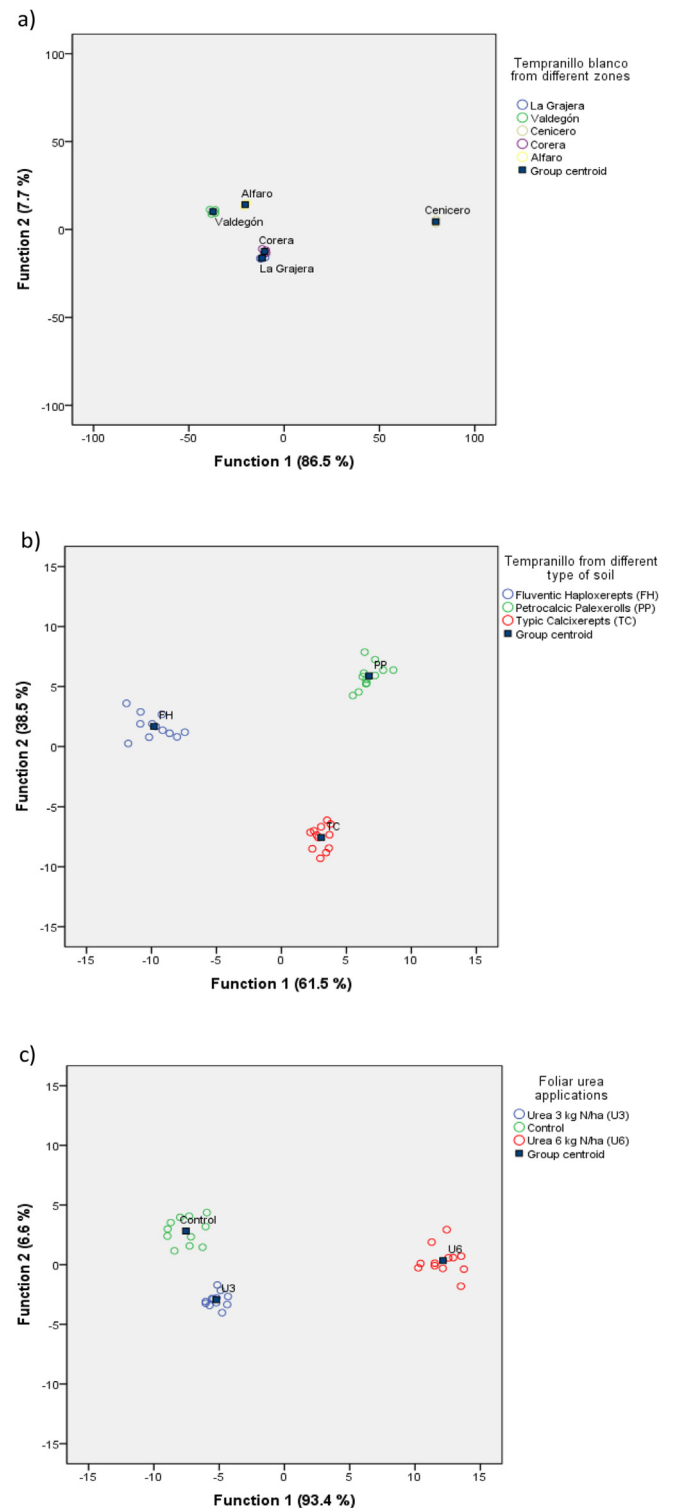
Fig. 1. Discriminant analysis performed on data expressed as concentration of the different elements determined by ICP-MS. a) Data of the different elements from wines produced using different grape varieties (Tempranillo blanco, Tempranillo, Viura, Graciano, Maturana, and Garnacha), b) wines produced in 2015 and aged in oak barrels *versus* young wines produced in 2016 under the same conditions as 2015 but without aging, c) wines with or without the addition of SO$_2$.

Fig. 2. Discriminant analysis performed on data expressed as concentration of the different elements determined by ICP-MS. a) Data of the different elements from Tempranillo blanco wines from five AOC Rioja zones (La Grajera, Valdegón, Cenicero, Corera, and Alfaro), b) wines produced at vineyards with different types of soil: *Fluventic haploxerepts* (FH), *Typic calcixerepts* (TC), and *Petrocalcic palexeroll* (PP), c) wines produced from three different concentrations (Control: 0; U3: 3 and U6: 6 kg N/ha) of urea applied on grapevine foliar tissues.

samples. However, Šelih, Šala, and Drgan (2014) explained the unsatisfactory classification of the white samples using the PCA, where the first three principal components of the essay explained less than 50% of the variance, mainly due to the small distances between Slovenian wine-growing regions.

3.3.5. Tempranillo wines from different types of soil

The elements composition was used to distinguish between wines from vineyards with different types of soil: FH, TC, and PP, classified according to the Soil Survey Staff (2010). Function 1 (61.5%) and function 2 (38.5%) discriminated the samples according to the three types of soils, therefore the total of variance explained by these two functions was 100% (Fig. 2b). The elements that most contributed to the discriminant model were Cs, and Pb, positively (functions 1 and 2) and As, negatively (function 2). These discriminant functions allowed us to correctly classify 100% of the studied samples. Despite the complexity of the soil type distribution in Rioja region, successful classification from this small geographical vineyard area (the same municipality) was achieved by means of the ICP-MS procedures, such as in Coetzee et al. (2014). These authors also demonstrated the applicability of ICP-MS analysis for intraregional classification of wines, which came from different South African States. Therefore, the variability of trace element composition of the soils, depends on the distribution of soil types in the area.

3.3.6. Tempranillo wines produced after the application of different nitrogen foliar treatments

Finally, the effects of two different concentrations (3 and 6 kg N/ha) of the urea foliar applications on the wine mineral composition, were studied. Fig. 2c showed the discriminant carried out with the different elements content in the wines. Function 1 explained 93.4% of the variance and function 2 explained 6.6% of the variance, therefore the total of variance explained by these two functions was 100%. The variables that most contributed to the discriminant model were Pb, Ni, and Zn, positively, and Mn, negatively (function 1); and Mn, and Ni, positively, and Zn, Ba, and Fe, negatively (function 2). These discriminant functions allowed us to correctly classify 100% of the studied samples.

4. Conclusions

In order to analyze major, minor, and trace elements in red and white wines using inductively coupled plasma mass spectrometry (ICP-MS), the best method was digestion with internal standard calibration for major elements; whilst the most of the minor and trace elements did not present significant differences between the four methods examined (standard addition and external calibration, with dilution and digestion). The content of these elements in the wines allowed their differentiation according to several factors. Thus, wines from the studied grape varieties were classified by Sr, Ca, Mg, and Mn concentration. The wines from different geographical zones were differentiated according to Sr, Ba, Ni, and Cu. Cs and Pb were the main elements to discriminate the wines from the three soil types studied. Wines produced after several grapevine nitrogen foliar doses applications were classified by Pb, Ni, Mn, and Zn. The content of Cs, Mg, Cu, and Pb in wines characterized the SO_2 use in winemaking. Finally, wines aged in oak barrels were differentiated by Na and Cs concentration. The discriminant functions allowed us to classify correctly 100% of the studied wines with the exception of grape variety (97.0%) and oak ageing (95.8%). Therefore, ICP-MS analytical methodology could be a useful tool to fingerprint wines according to viticultural and oenological parameters.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.foodchem.2018.07.087.

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