

Determination of mineral elements in fresh olive fruits by flame atomic spectrometry

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Abstract

The mineral element characterisation of olive fruits is acquiring interest to evaluate the link between their nutritional status and the olive oil quality. A method for the analysis of mineral elements in fresh olive fruits is proposed. The presence of mineral elements such as potassium, sodium, calcium, magnesium, zinc, copper, iron, and manganese in olive fruits was quantified by flame atomic absorption spectrometry. The limits of quantification were (expressed in mg kg⁻¹ of dry weight) 1.266, 1.569, 0.272, 0.172, 0.268, 0.316, 1.017 and 0.513 for K, Na, Ca, Mg, Zn, Cu, Fe and Mn, respectively. For all of the mineral elements the precision of the instrumental method was < 2.03%, and that of the analytical procedure was always ≤ 10%. The accuracy of the method was evaluated according to the standard additions method, the recoveries being > 98% for all of the added concentrations, indicating no noticeable interference of the matrix. Finally, the mineral composition of green olive fruits of different cultivars, 'Arbequina', 'Picual', 'Hojiblanca', 'Frantoio', and 'Bella de España' were monitored. The levels of the evaluated mineral elements were within the working range established. The results showed the method is a robust, reliable, and simple analytical procedure for the mineral element characterisation of olive fruit.

Additional key words: cultivars; flame atomic spectrometry; nutritional status; olive oil quality; olive tree.

Resumen

Determinación del contenido de elementos minerales en aceituna mediante espectrofotometría de absorción atómica

La caracterización mineral de las aceitunas es de gran interés por la relación que existe entre el contenido de los elementos minerales en fruto y la calidad del aceite de oliva. En el presente trabajo se describe un método para el análisis de elementos minerales en aceituna. Se ha determinado la concentración de potasio, sodio, calcio, magnesio, cinc, cobre, hierro y manganeso, utilizando un espectrofotómetro de absorción atómica con un sistema de atomización de llama. Los límites de cuantificación obtenidos, expresados en mg kg⁻¹ sobre materia seca, han sido los siguientes: 1,266; 1,569; 0,272; 0,172; 0,268; 0,316; 1,017 y 0,513 para K, Na, Ca, Mg, Zn, Cu, Fe, y Mn, respectivamente. La precisión del método instrumental fue < 2,03% mientras que la determinada para el método analítico resultó ≤ 10% para todos los elementos minerales evaluados. La exactitud del método se evaluó según el método de adición de patrón, obteniendo recuperaciones > 98% para todas las concentraciones evaluadas. Estos resultados indicaron ausencia de interferencias debido a la matriz. Finalmente, se determinó la composición mineral de frutos procedentes de distintos cultivares de olivo. Los niveles de los elementos minerales evaluados estuvieron dentro del rango de trabajo establecido. Los resultados mostraron que este método es un procedimiento analítico reproducible, fiable y sencillo para la caracterización mineral de aceitunas.

Palabras clave adicionales: calidad de aceite de oliva; cultivares; espectrofotometría de absorción atómica; estado nutricional; olivo.

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Abbreviations used: AAS (atomic absorption spectrometry), CV (coefficient of variation), FAAS (flame atomic absorption spectroscopy), SD (standard deviation).

Introduction

The mineral concentration of plant parts, in particular leaves, is used to identify nutrient deficiencies, excesses, or imbalances in a crop. The nutritional status of fruit trees affects crop yield (Sanz *et al.*, 1992; Casero *et al.*, 2004; Nestby *et al.*, 2004). In the specific case of olive trees, leaf-nutrient analysis is the evaluation commonly used for diagnosing tree nutritional status, and is an important tool for determining future fertilisation requirements (Fernández-Escobar, 1997; Fernández-Escobar *et al.*, 2006).

Researchers have investigated the relationships between mineral elements (Golomp and Goldschmidt, 1981; Hartmann *et al.*, 1996) and flower bud formation in different fruit types as well as the olive tree (Ulger *et al.*, 2004). In this last study, the highest macro- and microelement concentrations were found to be for Ca and Fe, respectively. The Na, Ca, Fe, Mn, Zn, and Cu concentrations were highest during the differentiation period, while K and P were highest in induction and initiation periods, respectively. The N, Ca, Fe, Mn, Zn, and Cu levels were highest in leaves, but P and K levels were highest in fruits and nodes, respectively.

The elemental content of fresh olive fruits could be influenced by cultivar, location, environment, packing material, the chemical used during cultivation and production as well as ripening (García *et al.*, 2002; Soares *et al.*, 2006). Regarding the fruit ripening, a non-uniform evolution of mineral elements was observed along this period. While K always increased throughout the development and ripening of fruits, the other mineral elements showed fewer differences, and their evolution has caused more disagreement in the scarce studies carried out (Nergiz and Engezb, 2000; Soyergin and Katkat, 2002; Soyergin *et al.*, 2002), whose variations were attributed to the olive cultivar, the distribution of elements in the soil and environmental and weather conditions during the sampling period.

Besides, the mineral element contents in fruit trees is acquiring interest for their effect on characteristics of the fruits quality in general, and olive oil quality in particular (Jordaó *et al.*, 1990). For instance, some studies in other fruit trees showed correlations among mineral element contents and fruit quality such as orange (Storey and Treeby, 2000), apple (Raese and Drake, 1997; Casero *et al.*, 2004), peach (Raese and Drake, 1997; Tagliavini *et al.*, 2000), mango (Nguyen *et al.*, 2004) between others.

For the particular case of olive tree, the concentration of certain elements were correlated with some para-

meters of quality. Deidda (1968) found that the concentration of N, P and K were a positive correlation with the fat content; just as Jordaó *et al.* (1990) found between the fruit weight and P and B concentration. However, a significant negative correlation was observed between the weight, the fat content, and the level of Ca and linoleic acid, just as the concentration of Mg Mn, Zn and the levels of oleic acid (Jordaó *et al.*, 1990; Aizpurua *et al.*, 1997). These results reinforce the need for adequate nutrition of the olive tree.

For this reason, taking into account the importance of knowing the mineral composition of olive fruits, the aim of the present study was to determine the K, Na, Ca, Mg, Zn, Cu, Fe, and Mn content in fresh olive fruits by flame atomic absorption spectrometry.

Material and methods

Samples were of 'Arbequina', 'Picual', 'Hojiblanca', 'Frantoio', and 'Bella de España' cultivars, harvested as ripe olives and cultivated in the experimental farm of IFAPA *Centro Venta del Llano* in Mengibar (Jaén, Spain) (37.58 N, 3.48 O, 323 m asl).

All reagents were of analytical or chromatographic grade. Triton X-100, hydrochloric acid, and lanthanum (III) oxide were acquired from Panreac (Barcelona, Spain). Caesium chloride, aluminium chloride hexahydrate, standard solutions of K, Na, Ca, Mg, Zn, Cu, Fe, and Mn of 1,000 mg kg⁻¹ concentration were purchased from Merck (Darmstadt, Germany).

Analysis of metals was performed by atomic absorption spectrometry (AAS) (Perkin-Elmer 2280), using flame atomic absorption spectroscopy (FAAS). This system was equipped with a hollow mono-element cathode lamp (Hollow Cathode Lamp, England) for each element analysed (K, Na, Ca, Mg, Zn, Cu, Fe, Mn). An air-acetylene flame was used with a ratio ranging from 3.0 kg cm⁻² to 1.2 kg cm⁻² and from 0.7 kg cm⁻² to 1.2 kg cm⁻² for air and acetylene respectively. The conditions used for each element, with wavelength, band-pass, lamp current, optimum working range, and instrument mode are summarised in Table 1.

Sample preparation

Fifty olive fruits of each cultivar were selected to assess the mineral element content in fresh olive fruit. The olive fruits were washed with an aqueous solution

Table 1. Instrumental conditions for the mineral element determination in fresh olive fruits

Element	Wavelength (nm)	Band-pass (nm)	Lamp current (mA)	Optimum working range (mg kg ⁻¹)	Instrument mode
K	766.5	2.0	8	2.0-300.0	Emission
Na	589.0	0.7	8	2.0-50.0	Emission
Ca	422.7	0.7	6	1.0-10.0	Absorbance
Mg	285.2	0.7	4	0.5-3.0	Absorbance
Zn	213.9	0.7	10	0.2-10.0	Absorbance
Cu	324.8	0.7	10	0.1-10.0	Absorbance
Fe	248.3	0.2	30	1.0-40.0	Absorbance
Mn	279.5	0.2	20	0.1-10.0	Absorbance

Flame type: air/acetylene. Gas flow: 3.0/0.7 kg cm⁻².

of non-ionic detergent (1% Triton X-100), rinsed several times with normal water, and finally rinsed twice with deionised water. In washing the samples, the least possible amount of water was used to avoid the loss of soluble mineral elements. After washing the fruits, surface moisture was removed using filter paper. Then, the olives were pitted with a manual pitting machine, and flesh and pits were stored separately in plastic bottle. Samples (flesh and pits) were dried in a forced-air drying chamber at 60°C until constant weight. The samples' humidity was determined by difference of weight (at the beginning and after drying the sample). After their trituration in an electric mill, the samples were stored in closed plastic containers in a drying chamber at 60°C, to prevent proliferation of fungus, until later analysis.

For the determination of mineral elements in flesh and pits, it was necessary to ash the samples. Dried, milled sample (0.50 ± 0.01 g) was placed in a porcelain crucible of approximately 15 mL in volume. Then, the sample was ashed in a muffle furnace (Selecta, Spain) at 550°C for ~8 h (until obtaining a whitish ash, indicating the complete elimination of organic material). The cold ash was dissolved in 3 mL concentrated HCl, and the solution was diluted with 2% HCl (v/v) to a final concentration of 0.1 N. The solution of acidified ash was filtered through filter paper, and the consecutive rinses [with 2% HCl (v/v)] from crucibles were collected in a 50 mL volumetric flask. For the measurement of K, Ca, and Mg in flesh, it was necessary to dilute the samples to a final concentration of 8%, while for the determination of Fe, Mn, Cu, and Zn in pits, it was necessary to concentrate the samples by dissolving the ash obtained after the incineration of 0.50 g of sample in a final volume of 5 mL, in order to obtain a sensitivity within the optimum working range specified in Table 1.

Interference due to phosphate in the measurement of Ca, and due to Ca and the ionisation of Na in the measurement of Na, was prevented by the addition of La₂O₃ and Al-Cs respectively, dissolved in 2% HCl to a final concentration of 10% (v/v).

Determination of mineral elements: K, Na, Ca, Mg, Zn, Cu, Fe and Mn

The mineral elements in olive fruits were measured using an atomic absorption spectrometer with flame atomisation (Model 2280 Perkin Elmer, Spain) operating under the working conditions summarised in Table 1. The measurements were made in hold mode with air-acetylene flame, where the air (as oxidant) was maintained at a flow of 50 mL min⁻¹ and the acetylene (as fuel) was maintained at a flow of 20 mL min⁻¹, to reach a flame temperature of 2,600°C. The hollow-cathode lamps were specific for each element analysed. Previously, to achieve maximum sensitivity and precision, the equipment was equilibrated by alignment of the lamp and lighter and adjustment of the selected wavelength. Table 1 shows the instrumental conditions for each mineral element evaluated in the samples.

Analytical procedure

The analytical conditions for the measurement of mineral elements were established using the respective acidified standard solutions as well as the digested samples evaluated independently (flesh and pit). Calibration was carried out with the commercial stock standard of the corresponding nitrate at a concentration of 1,000 mg kg⁻¹, except for the mineral Cu, which was purchased as the chloride. The different concentrations

used for the specific calibration curve of each mineral element were prepared from commercial standards, using 2% HCl (v/v) as solvent, except in the case of Cu, which was dissolved in deionised water. The optimum working range of concentration used for each mineral element is summarised in Table 1. A daily calibration curve was carried out for the quantification of the different mineral elements.

The limit of detection was calculated as $3 s/m$, where s is the standard deviation of 20 blank measurements and m is the slope of the calibration curve. The limit of quantification was calculated as $10 s/m$. The blank was a 2% HCl (v/v) solution.

The instrumental precision was evaluated by measuring the absorbance signals 20 times in the same digested sample under the established instrumental conditions. For the evaluation of the precision of the analytical procedure, readings were made of 20 different digested solutions of the same olive fruit sample for each analyte.

The accuracy of the method was determined by standard addition analysis. Three different concentrations

of metal standard solutions were added to the sample (six replicates for each concentration) and submitted to the overall procedure. After the quantification of mineral elements, the respective recoveries were calculated. The results are expressed in mg kg^{-1} .

Statistical analysis

Results are expressed as means \pm standard deviation of four determinations for the analytical determination. The data were subjected to a one-way analysis of variance (ANOVA) using Statistix 8.0 program for Windows. The level of significance was $p < 0.05$.

Results

Calibration curves for absorbance *versus* mineral element concentration were plotted (Fig. 1). Taking into account that a linear fit is not appropriate in flame

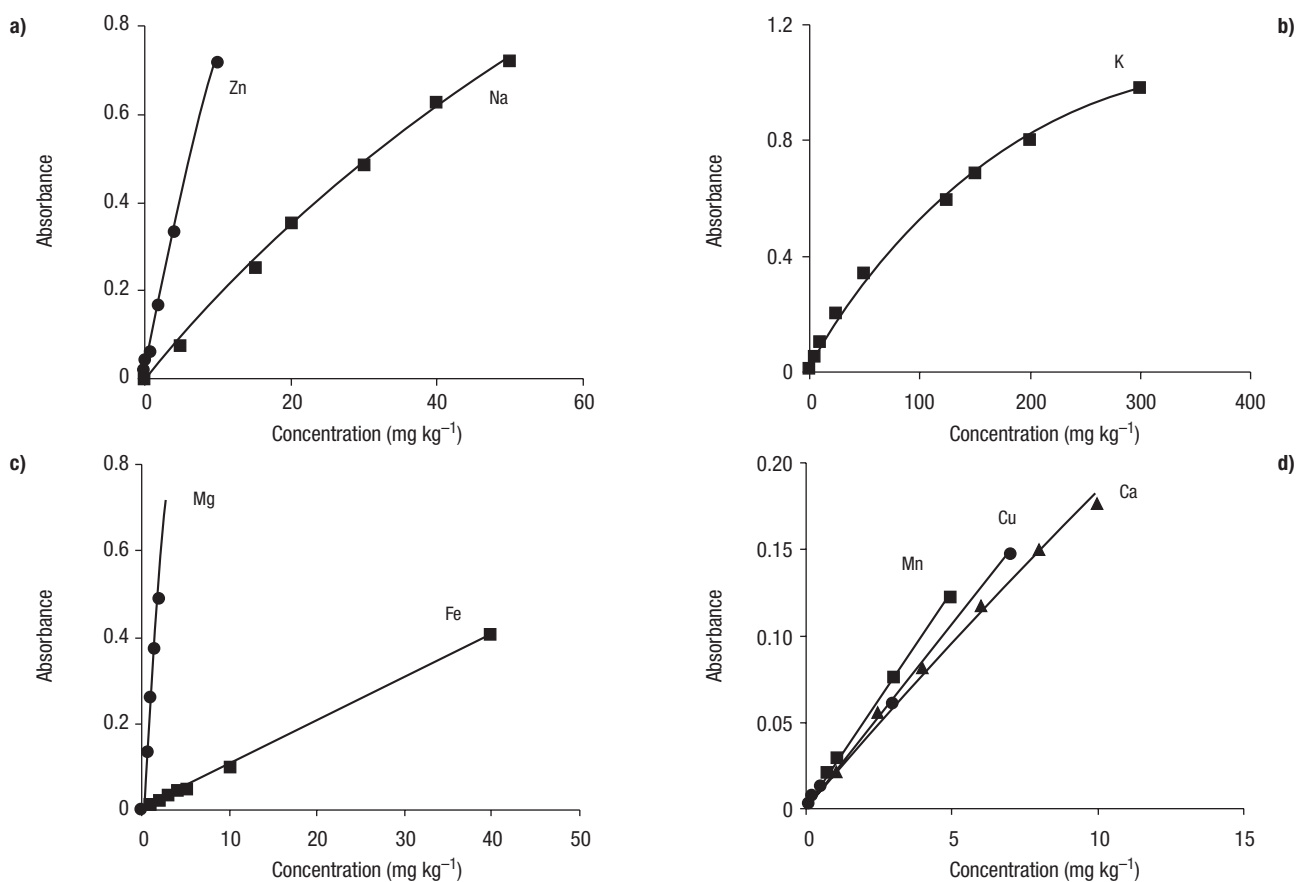


Figure 1. Calibration curves for absorbance *versus* mineral element concentration: (a) Zn and Na, (b) K, (c) Mg and Fe, (d) Mn, Cu and Ca.

Table 2. Linear or parabolic regression analysis data for the relationship between absorbance (A) and concentration (mg kg⁻¹) for mineral elements

Metallic element	a ± SD	b ± SD	c ± SD	R ²	n	p (r = 0)
K ¹	-8 · 10 ⁻⁶ ± 1 · 10 ⁻⁶	0.0056 ± 0.0003	0.04 ± 0.01	0.995	27	<0.0001
K ²		0.0033 ± 0.0003	0.1 ± 0.4	0.952	27	<0.0001
Na ¹	-1.1 · 10 ⁻⁴ ± 0.2 · 10 ⁻⁴	0.0207 ± 0.0009	-0.02 ± 0.01	0.999	18	<0.0001
Na ²		0.0143 ± 0.0008	0.04 ± .02	0.994	18	<0.0001
Ca ¹	-0.0005 ± 0.0001	0.0223 ± 0.0008	-0.00049 ± 0.00007	0.999	18	<0.0001
Ca ²		0.0172 ± 0.0006	0.010 ± 0.004	0.997	18	<0.0001
Mg ¹	-0.013 ± 0.002	0.270 ± 0.009	0.001 ± 0.007	0.999	18	<0.0001
Mg ²		0.224 ± 0.006	0.03 ± 0.01	0.999	18	<0.0001
Zn ¹	-0.0017 ± 2 · 10 ⁻⁴	0.088 ± 0.002	0.002 ± 0.003	0.999	18	<0.0001
Zn ²		0.071 ± 0.002	0.017 ± 0.009	0.998	18	<0.0001
Cu ¹	0.0002 ± 0.0002	0.019 ± 0.002	0.003 ± 0.002	0.999	18	<0.0001
Cu ²		0.0207 ± 0.0004	0.002 ± 0.001	0.999	18	<0.0001
Fe ¹	1.0 · 10 ⁻⁵ ± 2 · 10 ⁻⁶	0.0094 ± 0.0001	0.0025 ± 4 · 10 ⁻⁴	0.999	18	<0.0001
Fe ²		0.01004 ± 3 · 10 ⁻⁵	0.0013 ± 5 · 10 ⁻⁴	0.999	18	<0.0001
Mn ¹	-0.0004 ± 3 · 10 ⁻⁴	0.026 ± 0.001	0.001 ± 0.001	0.999	18	<0.0001

¹ The regression equation is $A = a^2 \times b + c$, where a (concentration, mg kg⁻¹), b (concentration, mg kg⁻¹), and c are the parabolic function parameters; r is the linear regression coefficient, and p the probability that $r = 0$. ² The regression equation is $A = b + c$, where b (concentration, mg kg⁻¹) is the slope and c is the intercept; r is the linear regression coefficient, and p the probability that $r = 0$.

atomic absorption spectrometry, as was corroborated by Mandel's test (Galeno-Díaz *et al.*, 2006), a polynomial function was applied in the quantification of the samples assayed, and the result was compared with that obtained after the application of a linear function for the fit of absorbance *versus* mineral element concentration.

Table 2 summarises the parameters for each function of first and second order, corresponding to the plot of absorbance versus mineral element concentration. The coefficient of determination (R^2) was included, in order to demonstrate the quality of the fit. Different samples submitted to the overall treatment were evaluated and quantified using both alternative equations, parabolic and linear function (Table 3). The results for instrumental and analytical procedure precision are summarised in Table 4.

Because there is no certified reference material for metals in olive fruits, the accuracy studies were performed by the standard additions method, submitting the spiked samples to the overall procedure and measuring the metals in the samples, digested as described in the material and methods section. This study was carried out with three different concentrations of each mineral

Table 3. Differences in the mineral elements present in olive fruits, determined by the two regressions indicated in Table 2. Mean values of mineral elements in olive fruits expressed as mg kg⁻¹

Metallic element	Parabolic regression	Linear regression	Difference (%)
'Arbequina'			
K	6,205	7,479	-20.5
Na	852	778	8.7
Ca	1,302	1,328	-2.0
Mg	579	594	-2.6
Zn	6.6	5.6	15.2
Cu	7.7	7.5	2.6
Fe	23	24.2	-5.2
Mn	8.1	8.0	1.2
'Picual'			
K	22,702	27,256	-20.1
Na	1,102	1,192	-8.2
Ca	2,512	2,406	4.2
Mg	775	792	-2.2
Zn	7.5	6.5	13.3
Cu	18.2	17.9	1.6
Fe	20	21.4	-7.0
Mn	7.6	7.6	0.0

Table 4. Performance characteristics of the method explained

	Precision (CV %)		Working range (mg kg ⁻¹)	Detection limit (mg kg ⁻¹)	Quantification limit (mg kg ⁻¹)
	Instrumental	Analytical procedure			
K	2.03	6.25	2.0-300.0	0.380	1.266
Na	1.18	7.65	2.0-50.0	0.471	1.569
Ca	0.39	6.28	1.0-10.0	0.082	0.272
Mg	1.29	7.15	0.5-3.0	0.052	0.172
Zn	0.86	6.25	0.2-10.0	0.080	0.268
Cu	0.18	8.97	0.1-10.0	0.095	0.316
Fe	0.62	10.69	1.0-40.0	0.305	1.017
Mn	0.79	5.62	0.1-10.0	0.154	0.513

element, and the recoveries obtained were always >98% for all of the mineral elements evaluated, as shown in Table 5, except Fe, whose recovery ranged from 95.0% to 95.5%. The results obtained showed that there was no contamination or loss during the pre-treatment procedure for any of the mineral elements analysed.

On the basis of 0.50 g of dry sample in a final volume of 50 mL, the limits of detection were 0.380, 0.471, 0.082, 0.052, 0.080, 0.095, 0.305 and 0.154 mg kg⁻¹, and the limits of quantification were 1.266, 1.569, 0.272, 0.172, 0.268, 0.316, 1.017 and 0.513 mg kg⁻¹, for K, Na, Ca, Mg, Zn, Cu, Fe, and Mn, respectively (Table 4).

Finally, the levels of the evaluated mineral elements were measured in fresh olive fruits of different cultivars: 'Arbequina', 'Picual', 'Hojiblanca', 'Frantoio' and 'Bella de España', the results being expressed as mg kg⁻¹ of dry weight (Table 6).

Discussion

Assuming the increasing interest of mineral composition content characterization in fresh olive fruit for their effect on its quality as well as olive oil quality, evaluation of sample preparation and flame atomic absorption spectrometry technique has been addressed in the present manuscript.

After optimization of conditions for the treatment of samples, a detailed evaluation of algorithms previously used for the quantification of the samples (Bysouth and Tyson, 1986; García *et al.*, 2002), was carried out. In this sense, taking into account that a linear fit is not appropriate in flame atomic absorption spectrometry (Mandel, 1964), a comparison between polynomial function and linear functions for the fit of

absorbance versus mineral element concentration has been evaluated. The obtained results showed differences lower than 10% for all elements except K and Zn, for which the parabolic function is recommended for the quantification. As the element Na showed a marked difference in the two functions, although without reaching the 10% limit of difference, the parabolic function was preferred for its quantification. In view of the results obtained, and in the range of concentration used, it can be concluded that the use of a linear function for the quantification of the mineral nutrient present in olive fruits was appropriate in all cases except K, Zn, and Na, whose quantification should be dealt with using the parabolic second-order function.

The instrumental precision was lower than 2.03% for all of the analysed mineral elements. With regard to the analytical procedure precision, the values obtained were slightly higher, being lower than 10% (or equal to 10% in the particular case of Fe).

Regarding the mineral composition determined in fresh olive fruits of cultivars such as 'Arbequina', 'Picual', 'Hojiblanca', 'Frantoio' and 'Bella de España', the K levels were found to be highest, followed by Ca, Na, and Mg. Of the minority elements, Fe had the highest level, followed by Zn, Cu, and Mn in agreement with the results published by Nergiz and Engez (2000) or the described mineral content in fresh olive fruits (Jordaó *et al.*, 1990). On the other hand, the mineral characterization provides useful information about the synthesis of fatty acids, by the relationship found between both parameters (Nergiz and Engez, 2000).

Finally, although the results showed wide differences between the different cultivars evaluated, the actual method resulted adequate for the mineral composition characterization of the selected samples, permitting us to discriminate between different culti-

Table 5. Recovery from expected values for the metals obtained in the matrix interference study. The white space corresponding with the samples without added of patron. Results are expressed as mean values of six assays and CV is lower than 10%

Mineral nutrient evaluated	Amount added of each mineral element (mg kg ⁻¹)	Mineral element content (mg kg ⁻¹)	Recovered amount (mg kg ⁻¹)	Recovery for amount of the mineral element added (%)
K	0	6,150.0		
	10	6,159.9	9.9	98.5
	125	6,273.9	123.9	99.1
	250	6,398.7	248.7	99.5
Na	0	800.0		
	2	802.0	2.0	100.0
	25	824.8	24.8	99.2
	45	844.8	44.8	99.6
Ca	0	1,300.0		
	2.5	1,302.6	2.6	104.0
	6	1,305.9	5.9	98.3
	8	1,308.0	8.0	100.0
Mg	0	526.0		
	1	527.0	1.0	100.0
	2	528.0	2.0	100.0
	2.5	528.4	2.5	100.0
Zn	0	6.2		
	0.8	7.0	0.8	100.0
	6	12.1	5.9	98.3
	8	14.1	7.9	98.8
Cu	0	7.1		
	0.3	7.4	0.3	100.0
	5	12.0	4.9	98.0
	7	14.0	6.9	98.6
Fe	0	21.0		
	2	23.0	1.9	95.0
	20	40.7	19.1	95.5
	30	50.6	28.5	95.0
Mn	0	8.5		
	0.5	9.0	0.5	100.0
	3	11.5	3.0	100.0
	4	12.6	4.1	102.5

Table 6. Determination of mineral element content in fresh fruits of five olive cultivars. Results are expressed as mg kg⁻¹ of dry weight. Mean values ± SD (n = 4)

	'Arbequina'	'Frantoio'	'Hojiblanca'	'Bella de España'	'Picual'
K	6,016 ± 376	15,901 ± 796	23,390 ± 895	35,457 ± 925	23,507 ± 870
Na	783 ± 50	1,104 ± 63	1,162 ± 65	1,029 ± 58	1,389 ± 72
Ca	1,291 ± 75	1,795 ± 87	2,572 ± 101	1,611 ± 95	2,688 ± 125
Mg	549 ± 38	691 ± 28	864 ± 35	820 ± 41	807 ± 37
Zn	6.3 ± 0.4	6.4 ± 0.3	7.7 ± 0.5	6.1 ± 0.3	7.4 ± 0.4
Cu	7.4 ± 0.6	7.8 ± 0.6	6.6 ± 0.5	33 ± 2	17.2 ± 0.9
Fe	22 ± 2	25 ± 2	18 ± 2	23 ± 1	20 ± 2
Mn	8.1 ± 0.4	8.2 ± 0.4	8.7 ± 0.5	7.1 ± 0.3	7.7 ± 0.2

vars as recently López *et al.* (2008) has found after the mineral composition characterization of olive fruits.

In summary, it can be concluded that the determination of mineral elements by flame atomic spectrometry is a reliable and simple analytical procedure for fresh olive fruit mineral characterisation.

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