Analytical, Nutritional and Clinical Methods

Multivariate characterization of table olives according to their mineral nutrient composition

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Abstract

Different commercial presentations of table olives were characterized by their mineral compositions. Cu, Fe, Mn, Zn, Ca, Mg, Na, K, and P were determined. The processing of table olives affects the mineral content of commercial presentations and significant differences ($p < 0.05$) were found among green (Spanish style), directly brined, and ripe olives. A predictive discriminate analysis showed that the most discriminating elements were Fe, K, Na, Mn, Cu, and P (among styles) and with Ca (among cultivars). A good classification and cross-validation was observed in the case of elaboration styles but discrimination among cultivars was less conclusive. A further analysis of the confusion matrix, according to cultivars, showed that the lower classification efficiency, in this case, was mainly due to misclassification of samples from Manzanilla and Gordal cultivars. The analysis of the confusion matrix can be useful when the assessment of its results is not obvious.
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1. Introduction

The table olive reached a total production of 1,600,000 t in the 2003/2004 season, with Spain being the main producer and exporter with about 500,000 and 250,000 t, respectively. The consumption of table olives in the Mediterranean Basin, is a widespread tradition, which is also reaching other non-producing countries (IOOC, 2005). Fresh olives, as legumes (Iqbal, Khalil, Ateeq, & Khan, 2006), fruits (Sánchez Castillo et al., 1998) or other plants (Guill Guerrero, Jiménez Martínez, & Torija Isasa, 1998) are rich in minerals (Biricik & Basoglu, 2006). However, olives must be processed before eating to remove their natural bitterness (Garrido Fernández, Fernández-Díez, & Adams, 1997). Their elaboration includes several styles (Sánchez Gómez, García García, & Rejano Navarro, 2006). Green olives are treated with lye, washed and fermented; ripe olives, darkened by oxidation after a storage

period, are lye treated, washed several times and packed; other olives are brined directly. All of them use salt in different proportions as the principal preservation agent (Garrido Fernández et al., 1997). The different aqueous treatments may produce some changes in the mineral composition of the processed fruits. Nosti Vega, Vázquez Ladrón, and de Castro Ramos (1979) and De Castro Ramos, Nosti Vega, and Vázquez Ladrón (1979) studied the mineral content of some processed samples from the Spanish cultivars. Ünal and Nergiz (2003) studied Na, K, Ca, Fe, and Zn in Memecik (Turkish cv.). Biricik and Basoglu (2006) reported the mineral content in olive cv. Samanly, Domat, Manzanilla and Ascolano from Turkey. However, none of these investigations used samples from marketed products.

Currently, there is a diversity of commercial presentations on the market, which, in addition to the main processing styles, also differ in their final conditioning, stuffing materials, preservation technologies and cultivars. A survey of the mineral concentration of all of them, is therefore appropriate. Such data will provide information

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on minerals to the olive industry as required by the nutritional labeling in USA (CFR, 2003), Canada (Canadian Food Inspection Agency, 2003), Europe (Council of the European Communities, 1990) or any other country. Data on mineral content in table olives may also be useful for nutritionists or consumers.

Chemometric techniques, appear to be the most powerful tools for characterizing and classifying wines (Arvanitoyannis, Katsota, Psarra, Soufleros, & Kallithraka, 1999), honeys (Nozal Nalda, Bernal Yagüe, Diego Calva, & Martín Gómez, 2005), dairy products (Herrera García et al., 2006), pistachios (Anderson & Smith, 2005) and beer (Alcázar, Pablos, Martín, & Gustavo González, 2002) according to their source, processing conditions, or origin. Discriminant analysis is used to find theoretical values resulting in the best possible discrimination between a priori established groups. Discrimination relies on weighting the theoretical values for each variable in such a way as to maximize between group variance with respect to within group variance. Discriminant analysis models comprise sets of equations that are linear combinations of the independent variables, resulting in the maximum possible separation between groups (Uriel & Ardás, 2005).

The aim of this work was to (i) determine the mineral composition of all marketed Spanish commercial presentations of table olives; (ii) check for possible differences in the concentration of minerals among them; (iii) evaluate the ability of discriminant analysis to classify these commercial presentations according to elaboration styles and cultivars; (iv) test these classifications with respect to those which could be obtained by chance.

2. Materials and methods

2.1. Samples

Samples belonged to the following styles, cultivars and commercial presentations:

2.1.1. Green Spanish-style olives
Gordal: plain, pitted, and seasoned. Gordal stuffed with: red pepper strips, natural red pepper, almond, cucumber, onions, garlic, and jalapeño. A blend of Gordal olives and red pepper strips called "salads". Manzanilla: plain, pitted, sliced, anchovy flavored, and plain seasoned. Manzanilla stuffed with: red pepper strips, anchovy strips, marinated anchovy strips, natural red pepper, almond, almond and red pepper, salmon strips, tuna strips, onions, capers, garlic, hazelnut, hot pepper, hot pepper strips, "piquillo" pepper, lemon paste, ham paste, orange strips, cheese, "jalapeño" strips, and garlic strips. A blend of pitted or slices of Manzanilla olives with red pepper strips called "pitted salads" and sliced "salads", respectively; a blend of Manzanilla olives with slices of carrot added called "gazpachas"; and a blend of Manzanilla olives and capers called "alcaparrado". Carrasqueña: pitted. A blend of pitted Carrasqueña olives and red pepper strips, called "salads"; and a blend of Carrasqueña olives and capers called "alcaparrado". Hojiblanca: plain, pitted, sliced. Hojiblanca olives stuffed with red pepper strips.

2.1.2. Directly brined olives
Gordal: broken "seasoned" turning colour. Manzanilla: turning colour in brine alone, "seasoned" turning colour, and olives from biologic (or ecologic) production. Hojiblanca: "seasoned" turning colour. Arbequina: "seasoned" turning colour. Aloreña: green "seasoned" broken, prepared from fresh fruits and from stored olives. Verdial: green "seasoned" broken.

2.1.3. Ripe olives (by alkaline oxidation)
Gordal: plain. Manzanilla: pitted. Carrasqueña: plain and pitted. Hojiblanca: plain, pitted, and sliced. Cacereña: plain, pitted, and sliced.

2.2. Reagents

All reagents were of analytical purity (Panreac, Barcelona, Spain). Hydrochloric acid (6 N) solution was obtained by dilution of concentrated HCl (Fluka, Buchs, Switzerland). The stock solutions of Cu, Fe, Mn, Mg, Zn, Ca, and P were obtained from Sigma. Stock solutions of Na and K were purchased from PACISA (Madrid, Spain). The standard solutions were obtained by dilution of the corresponding stock solutions and the addition of HCl in a concentration similar to that obtained in the sample solutions.

2.3. Cleaning of the material

All glassware used for the determination of the minerals was immersed in 6 N HCl overnight and then rinsed several times with distilled, deionized water.

2.4. Sample preparations

Analyses were carried out in triplicate on composite samples from each commercial presentation, which were made up of 3–8 units (cans, jars or plastic pouches), depending on their sizes, and different packing dates, from 1 to 5 elaboration companies, according to their availability on market shelves. Producers kindly supplied those commercial presentations not available in the local markets. Average time from packing was about 3 months.

The pulp of 100 g of sample olives was separated from the pit, when it was necessary, by manual or automatic pitting machine, ground and homogenized. From the resulting paste mentioned above, 5 g olive pulp (2.5 g for ripe olives) of the diverse samples, was exactly weighted in a quartz capsule. The capsule was put in a muffle oven and incinerated at 550 °C. At this point, the temperature was quickly brought to 100 °C and then increased slowly until the calcination temperature was reached, which was maintained for ≈8–10 h. The ashes, white-grayish in colour, were slightly moistened and dissolved with three parts of

2 ml of 6 N hydrochloric acid and filtered, bit by bit, through a filter paper into a 25 ml volumetric flask. After that, the filter was cleaned three times with 3 ml of deionized water, which was also added to the volumetric flask, and it was filled with deionized water until level. Dissolution was aided by slightly heating the capsule after every addition of hydrochloric acid. To make it easier, the filtration was achieved by means of a suction hood. At the same time, a blank was prepared with only the reagents.

Lantane chloride was added both to the acid solutions of the ashes and to the standard solutions in a final proportion of 1% (w/v) to avoid possible interferences in the determination of the minerals (Ca and Mg).

2.5. Analytical methods and apparatus

Cu, Fe, Mn, Ca, Mg, and Zn were determined by atomic absorption spectrophotometry. A GBC model 932 AA (Victoria, Australia) atomic absorption spectrometer equipped with two hollow multi-element cathode lamps, (Cu, Fe, and Mn) (GBC, Victoria, Australia) and (Ca, Mg, Cu and Zn) (Photometry, Victoria, Australia) was used. An air acetylene flame was used. Instrumental conditions for each element were fixed according to the equipment manual (Anonymous, 1994).

Sodium and K were determined by flame photometry. A Meteor Model NAK-1 flame photometer with a scale reading from 0 to 200 meq/l (PACISA, Madrid, Spain), and LIC Instruments model 346 automatic diluter (PACISA) were used. The apparatus was calibrated to express the elements as milligrams per gram of flesh. Instrumental conditions for Na and K were fixed according to the equipment manual (Anonymous, 1990).

The analysis of phosphorus was carried out following the official method of the AOAC no. 970-39 Phosphorus in Fruits and Fruit Products (spectrophotometric molybdovanadate method) (AOAC, 1993). The method is based on the capacity of P (in phosphoric acid form) in an acid solution and in the presence of V^{5+} and Mo^{6+} to form a yellow phosphomolybdovanadate complex whose absorbance can be measured at 400 nm. Measurements were made in a Cary UV/Visible spectrophotometer model 1E (Varian Australia, Mulgrave, Victoria).

2.6. Calibration curves and analytical characteristics

Calibration curves were obtained daily, from successive dilutions of the stock solutions of the elements, with double distilled deionized water, using working ranges recommended by the instructions of the respective pieces of equipment. Linearity, detection and quantification limits, sensitivity and accuracy were previously checked with standard solutions to perform the analytical assays. Precision was obtained from every sample determination. The average of blank signals was subtracted from analytical signals of samples before interpolation on calibration curves. Samples of standard solutions were periodically included within

the samples to control the adequate performance of the procedures and apparatus.

2.7. Statistical analysis

Each olive sample (object) was considered an assembly of nine variables represented by the Cu, Fe, Mn, Zn, Ca, Mg, Na, K, and P concentration in flesh. These variables formed a data vector which represented an olive sample. Data vectors belonging to the same group (elaboration style or cultivar) were analyzed. The group was termed a category. The database from the analysis of minerals was thus arranged in a 201 × 9 (cases × variables). Elaboration styles were coded as 1 (green Spanish style), 2 (directly brined), and 3 (ripe olives); cultivars were also coded as 1 (Gordal, G), 2 (Manzanilla, M), 3 (Carrasqueña, Cr), 4 (Hojiblanca, H), 5 (Arbequina, Ar), 6 (Aloreña, Al), 7 (Verdial, Vr), and 8 (Cacereña, Cc).

Average values for cultivars within elaboration styles were obtained by the general linear model technique (nested ANOVA). Data were also studied by multiple analyses of variance (MANOVA) to test overall differences between groups across the different variable. These tests were carried out using original data. Patter recognition tools used in this work were as follows:

Autoscale, which is the most commonly, used scaling technique (Kowalski & Bender, 1972). The procedure standardizes a variable m according to

$$y_{mj} = \frac{(x_{mj} - \bar{x}_m)}{s_m}$$

where y_{mj} is the value j for the variable m after scaling, x_{mj} is the value of the variable j before scaling, $\bar{x}_m$ is the mean of the variable and s_m is the standard deviation for the variable. The result is a variable with zero mean and a unit standard deviation. The chemometric study was carried out using this standardized data.

2.8. Feature selection

The selection of variables containing the most powerful information for the correct classification of olive samples, of the three (types) or eight (cultivars) categories, was carried out on the basis of the backward stepwise analysis. This first includes all the variables in the model and then at each step, eliminates the variable that least contributes to membership prediction. The process continues until only the important variables that contribute most to discrimination between groups are in the model. The values of probability to enter or to remove were fixed at 0.05 and 0.1, respectively. The number of steps was fixed at 100, the minimum tolerance at 0.01 and no variable was forced to enter in any model (SYSTAT, 2002).

2.9. Linear discriminant analysis (LDA)

This is a supervised technique that provides a classification model, characterized by a linear dependence of the

classification scores with respect to the descriptors (groups previously defined). LDA assumes an a priori knowledge of the class membership of each sample in a training set. Two very distinct purposes and procedures for conducting discriminate analysis exist: discriminate predictive analysis (which involves only the derivation of the linear discriminant functions) and discriminant classification analysis (to evaluate the previous linear functions in order to classify current and future samples). A visualization of the classification possibilities can be obtained by plotting the scores of cases as a function of the first two or three canonical discriminant functions. In addition, to measure the classification power of the analytical data, the number of individuals correctly predicted to belong to the assigned group is calculated, considering that prior probabilities were proportional to the number of samples in each group; this number is expressed as a percentage of the group population. But this is usually overoptimistic because of the use the same samples employed for the deduction of the linear functions used to test its ability. In this work, a leaving-one-out cross-validation procedure was performed for assessing the performance of the classification rule. In this procedure, the sample data minus one observation was used for the estimation of the discriminant functions, and then the omitted variable was classified from them. The procedure was repeated for all samples. Consequently, each sample was classified by discriminate functions which were estimated without its contribution (Rencher, 1995).

2.10. Confusion matrix analysis

The computation of the confusion matrix has traditionally been the final step in the discriminant analysis. However, the confusion matrix, when viewed as a contingency table, may be subject to further analysis, specifically with respect to the observed correct classification (Smith, 2006). In this work, we applied tests for overall classification, group classification (individual rows), and individual cells to compare the predicted classification using the model to that expected from chance alone.

2.10.1. Overall classification

The first test may be accomplished by the conventional chi-square test for a contingency table, in which

$$\chi^2 = \sum_i \sum_j \frac{(o_{ij} - e_{ij})^2}{e_{ij}}$$

where o_{ij} is the observed number of samples classified in the cell ij; $e_{ij} = (n_{i.} \cdot n_{.j})/n$ with $n_{i.}$ the number of samples classified in row i, $n_{.j}$ the number of samples in column j, and n the total number of samples. As usual, the number of degree of freedom is $(i-1)(j-1)$.

2.10.2. Tests of group differences

The Morrison (1969) likelihood analysis provides a criterion that may be used to compare the proportion of correctly classified observations with the proportion expected by chance. The proportion expected by chance, designated the proportional chance criteria or c_{pro}, is expressed as: $c_{pro} = p \cdot \alpha + (1-p)(1-\alpha)$, where p = the true proportion of each type (or cultivar) in the total sample, and α the proportion of each type (cultivar) in the whole sample categorized in that type (cultivar) by the model. This relationship between chance and observed proportions can be tested using a Z statistic of the form:

$$Z = \frac{p_{cc} - c_{pro}}{\sqrt{\frac{c_{pro}(1-c_{pro})}{n}}}$$

where p_{cc} is the overall percent observations correctly classified in the sample.

2.10.3. Classification and misclassification within groups (cells in the confusion matrix)

This test, applied to determine the source of deviation, was conducted using the maximum chance criterion, c_{max}, defined as the minimum expected correct classification for a select group of interest; the computation of c_{max} is based on the assumption that all observations are categorized as coming from that group (Morrison, 1969). A Z statistic is used to test this relationship:

$$Z_{ij} = \frac{o_{cc} - c_{max}}{\sqrt{\frac{c_{max}(1-c_{max})}{n}}}$$

where o_{cc} stands for observed correct (incorrect) classification of the specific cell. The test may be conducted for all the cells in the confusion matrix.

The different statistical techniques used in this work were implemented using STATISTICA, release 6.0 (StatSoft, 2001) and SYSTAT, release 10.2 (SYSTAT, 2002).

3. Results and discussion

3.1. Concentration of minerals in table olives

The concentrations of the different analyzed elements are shown in Tables 1 and 2. Data were studied all together but results (rounded according to their corresponding standard deviation) have been distributed into two tables to facilitate their illustration. The univariate study showed significant ($p < 0.05$) differences among elaboration styles and cultivars within styles (except for Zn). Therefore, processing styles and cultivars are important for determining the final content of metals in table olives.

Na is the only element habitually added during processing (Garrido Fernández et al., 1997) and its concentration was the highest in all commercial presentations. Its daily recommended intake is also high, with a maximum 2400 mg/day in USA (CFR, 2003), but Tables 1 and 2 indicate that olive consumption may account for a significant proportion of Na daily value. This high Na concentration is because the preservation of most olive products still relies on salt. However, there are big differences among elaboration styles. The highest concentrations were found in green

Table 1
Mean values (±standard error) of mineral nutrients in table olives, expressed as mg kg^{-1} e.p, according to processing style and cultivars (green and ripe table olives)

Composites	Green olives				Ripe olives				
	G ($n = 30$)	M ($n = 89$)	Cr ($n = 8$)	H ($n = 12$)	G ($n = 3$)	M ($n = 3$)	Cr ($n = 6$)	H ($n = 9$)	Cc ($n = 8$)
Cu	3.33 (.51)	1.73 (.61)	3.6 (1.1)	2.04 (.34)	4.72 (.36)	3.950 (.066)	3.26 (.78)	3.47 (.53)	2.40 (.41)
Fe	3.88 (.20)	5.40 (.89)	3.49 (.89)	5.23 (.50)	68.5 (6.4)	131.5 (4.2)	58.3 (4.7)	66.7 (4.1)	84 (12)
Mn	0.61 (0.04)	0.45 (.02)	0.539 (.067)	0.526 (.032)	0.717 (.012)	1.280 (.012)	0.872 (.076)	1.539 (.072)	0.953 (.053)
Zn	1.82 (.21)	2.52 (.40)	1.52 (.27)	2.13 (.61)	2.55 (.11)	3.6 (1.5)	2.58 (.86)	3.25 (.72)	3.18 (.70)
Ca	476 (25)	589 (25)	709 (81)	850 (61)	362.7 (2.4)	579 (16)	527 (48)	731 (33)	580 (17)
Mg	133.1 (9.0)	98.6 (3.2)	103.2 (7.7)	146.7 (2.5)	51.7 (4.3)	109.7 (2.3)	72.3 (5.2)	98.0 (5.9)	73.9 (2.6)
Na	17,221 (679)	15,057 (368)	18,144 (962)	14,378 (349)	5706 (453)	9386 (289)	6750 (356)	7194 (468)	7964 (109)
K	538.8 (31)	333 (14)	393 (30)	444 (35)	81.7 (3.3)	190 (77)	176 (20)	223 (29)	168.8 (9.7)
P	118 (11)	96.0 (4.5)	68.8 (4.5)	70.0 (5.1)	72.1 (1.4)	57.0 (2.8)	78.2 (7.0)	91.8 (4.4)	93.3 (5.2)

G, Gordal; M, Manzanilla; Cr, Carrasqueña; H, Hojiblanca; Cc, Cacereña. Mean values obtained by GLM; n = sample number.

Table 2
Mean values (±standard error) of mineral nutrients in table olives, expressed as mg kg^{-1} e.p, according to processing style and cultivars (directly brined olives)

Composites	Directly brined olives					
	G ($n = 3$)	M ($n = 9$)	H ($n = 3$)	Ar ($n = 2$)	Al ($n = 6$)	Vr ($n = 3$)
Cu	5.09 (.50)	5.22 (.66)	3.99 (.59)	10.93 (.70)	4.68 (.48)	4.353 (.064)
Fe	7.7 (1.3)	5.51 (.55)	7.62 (.55)	4.09 (.18)	5.73 (0.75)	4.90 (.31)
Mn	0.240 (.060)	0.554 (.065)	0.920 (.054)	0.290 (.015)	1.040 (.085)	1.093 (.073)
Zn	3.20 (.67)	1.98 (.16)	2.9 (1.7)	1.55 (.14)	2.03 (0.20)	2.94 (.27)
Ca	379 (33)	447 (11)	337 (20)	613.0 (5.7)	691 (19)	684.7 (3.3)
Mg	119.0 (5.9)	143.4 (2.9)	56.1 (4.8)	67.7 (1.2)	53.8 (5.0)	197.3 (2.3)
Na	12,675 (426)	11,490 (1100)	13,797 (1226)	13,152 (465)	16,600 (1305)	16,020 (1305)
K	1176 (69)	750 (87)	684 (83)	571 (32)	902 (252)	766 (110)
P	129.4 (8.0)	121.9 (5.1)	111.90 (.40)	105.4 (6.3)	99 (22)	144.1 (1.4)

G, Gordal; M, Manzanilla; H, Hojiblanca; Ar, Arbequina; Al, Aloreña; Vr, Verdial. Mean values obtained by GLM; n = sample number.

olives followed by directly brined olives and ripe olives. Ripe olives have a moderate Na content because they are preserved by sterilization.

Calcium concentration in fresh table olives is high (Garrido Fernández et al., 1997). In addition, Ca is used in the preparation of the stuffing strips from green olives and sometimes added during the storage phase of ripe olive processing, so its high concentration in these elaboration styles may be justified. However, its concentration in directly brined olives, in which it is not intentionally incorporated, is also high. In general, olive flesh can absorb Ca and retain it. This Ca is not released during the lye or water washing treatments involved in processing (Garrido Fernández et al., 1997). The presence of Ca is very effective in preventing olive softening (De Castro, Garcia, Romero, Brenes, & Garrido, 2007). The concentration of Ca in green, directly brined and ripe olives, ranged from 476 to 850, 337 to 691, and 363 to 731 mg kg^{-1}, respectively. This concentration is comparable to those found in dairy products (Herrera García et al., 2006). Table olives may contribute to the daily intake (1000 mg) of this element (CFR, 2003), especially in those countries where its consumption is habitual. Other values of Ca concentration in olives from the literature are 422–850 mg kg^{-1} in green Turkish olives (Biricik & Basoglu, 2006); 460–860 mg kg^{-1} in the green Spanish cultivar (De Castro Ramos et al., 1979); 270–450 mg kg^{-1} or 110–230 mg kg^{-1} for Kalamata or natural black olives after fermentation, respectively (Ünal & Nergiz, 2003); and 960 mg kg^{-1} in marinated green olives (Souci, Fachman, & Kraut, 1994).

As also occurs in any vegetable product, potassium is the most abundant element in fresh olives (Garrido Fernández et al., 1997); however, this monovalent metal is not as strongly fixed as Ca in the pulp of the olive and is progressively lost during processing. Its concentration is higher in directly brined olives (especially in Gordal and Aloreña, 1176 and 902 mg kg^{-1}, respectively), which are not subjected to lye treatments. Successive immersions in lye or washing waters partially removes this element (mainly in ripe olives), although its final concentrations, even in ripe olives, were still outstanding (82–223 mg kg^{-1}) (Tables 1 and 2). Values reported by De Castro Ramos et al. (1979) for green olives ranged from 640 to 1090 mg kg^{-1}. Ünal and Nergiz (2003) found 560–1130 mg kg^{-1}, 1140–1820 mg kg^{-1}, and 3260–3760 mg kg^{-1} for green, Kalamata and natural black olives, respectively. Biricik and Basoglu (2006) reported a concentration of 4230–7410 mg kg^{-1}. However, the Food Composition and Nutrition Tables (Souci et al., 1994) reported an average value of 430 mg kg^{-1} for marinated olives. Some of these values are within the range found in this study but others are higher. Such differences may be due to the fact

that the last ones did not come from packed final products or because of cultivar effects. Daily values for K are high (3.500 mg); fruits and vegetables may be considered its main source for daily intake but olives should also be considered in light of their concentration.

Magnesium concentration ranged from 51 to 197 mg kg^{-1} (Tables 1 and 2). Its wide interval of concentration reflects that its presence may be greatly affected by processing. Magnesium is, like Ca, a divalent element, but no research on its role in table olives has yet been carried out. De Castro Ramos et al. (1979) found 60–400 mg kg^{-1} in green olives and Biricik and Basoglu (2006) 47–360 mg kg^{-1}. The Food Composition and Nutrition Tables (Souci et al., 1994) recorded an average of 190 mg kg^{-1} for this element in green marinated olives. The daily value for magnesium is 400 mg (CFR, 2003). Olives may then contribute to fill its requirements.

Phosphorous had a concentration that ranged from 57 to 144 mg kg^{-1}. Its highest average concentration was found in directly brined olives. So, this element may also be partially lost during processing. Its marked concentration in green and directly brined Gordal may be responsible, at least in part, for the ability of this cultivar for lactic acid fermentation (Garrido Fernández et al., 1997). The content of P in olive products is similar to that found in honey (Fernández-Torres et al., 2005). Other values in the literature were 120–210 (De Castro Ramos et al., 1979) and 170 mg kg^{-1} (Souci et al., 1994).

Iron is used for fixing the final colour of ripe olives (Garcia, Brenes, Romero, & Garrido, 2001). So its concentration in this style can be high. The maximum concentration allowed is 150 mg kg^{-1} (IOOC, 2004) but processors hardly reach it. In this study, its range was from 58 to 131 mg kg^{-1}. Ripe olives may, therefore, be a good source of Fe for which the daily recommended value is 18 mg. The presence of Fe in green and directly brined olives is unfavourable because it may cause browning due to the formation of complexes with the olive polyphenols (Garrido Fernández et al., 1997); so, Fe concentrations in these styles are relatively low (3.49–7.70) (Tables 1 and 2). Values reported by other authors were 6.4–10.9 mg kg^{-1} (green olives) (De Castro Ramos et al., 1979), 3.23–15.10 mg kg^{-1} for Turkish cultivars (Biricik & Basoglu, 2006), and 18 mg kg^{-1} (Souci et al., 1994).

The highest Cu concentration was found in directly brined olives. This element is included in some chemical products used to treat olive trees. Apparently, a part of it may be absorbed by the fruit. The processing of fruits such as green or ripe olives, which requires vigorous lye and washing treatments, slightly reduces its presence. So Cu seems to be strongly retained in the flesh and, apparently, is involved in the green spots sporadically observed in the olive surface of the green fermented Gordal cultivar. (Gallardo Guerrero, Gandul-Rojas, & Mínguez-Mosquera, 1999). Zinc concentrations were always low and insignificant ($p < 0.05$) differences among cultivars within elaboration types were found. Manganese content ranged from

0.24 to 1.5 mg kg^{-1}. Manganese may be used as catalytic element during the oxidation process of ripe olives (Romero, García, Brenes, & Garrido, 2001). The concentrations found in this style were slightly higher that those in green olives but similar to some directly brined olives. So, the addition of Mn in ripe olive darkening seems to have a limited, if any, use on an industrial scale. Concentrations of Cu, Zn, and Mn found in this study were of the same order of those given by other authors (De Castro Ramos et al., 1979; Biricik & Basoglu, 2006; Souci et al., 1994).

Values on the mineral concentrations from the Spanish cultivars given in Tables 1 and 2 are of general interest because Spanish table olives represent about 30% of the word production and more than 50% of the international table olive trade. Such data may be used for the industry to prepare nutrition facts (USA and Canada) or nutritional labeling in general. Nutritionists may also find in them valuable data to estimate mineral intake, especially in those countries where the consumption of table olives is a wide spread habit.

3.2. Chemometric analysis

A previous study of the means showed significant differences between styles (except for Zn and Ca) and cultivars (except Zn); apparently, the data were appropriate to be used in a chemometric analysis. The correlation matrix only showed a slight relationship ($r = 0.373$) between K and Na. This fact discouraged the application of procedures for achieving a reduction of dimensions (principal components analysis). Sodium concentration was markedly greater than that of any other element. So, the data were autoscaled in order to achieve independence in the different scale factors of element concentrations. Linear discriminate analysis was applied to this standardized matrix.

The selection of the minimum number of variables to reach a correct classification according to styles, was achieved by choosing the features which contain the most discriminant information for the classification. The selection of a small number of key variables increases the reliability of the mathematical classification, eliminates features with minor information and allows a visual examination of the data set by a two-dimensional plot of the key features. The most discriminant elements for categorizing samples into elaboration styles were Fe, K, Na, Mn, Cu, and P (Table 3), according to F to remove. Tolerance was markedly high for all variables in the model. The multiple correlation of each variable with all others already in the model can be estimated as 1-Tolerance. This means that the redundant information introduced by the respective variable was fairly limited. This was in agreement with the low values of the correlation matrix.

The coefficients of the canonical discriminant functions, standardized by the variance within groups are also shown in Table 3. These coefficients may be interpreted in a similar way as the β in multiple regressions. Thus, the most outstanding contribution to discrimination in the first function

Table 3
Discriminant analysis (elaboration styles)

Variable/element	F to remove	Tolerance	Function 1	Function 2
Cu	13.29	0.96	0.020	0.493
Fe	134.18	0.99	0.806	−0.098
Mn	30.72	0.66	0.641	0.106
Na	40.41	0.74	−0.558	−0.497
K	45.40	0.80	−0.061	0.872
P	9.84	0.70	−0.364	−0.165

Elements retained in the model and standardized canonical functions.
Wilks lambda in the selection process: approximately $F = 114.2$ (df = 12, 376), $p = 0.000$.

was obtained from Fe, Mn, Na, and P whereas for the second, it was obtained from K, Na, and Cu. Applying these functions to the metal composition of the different olive samples, the corresponding scores for each function may be calculated. A plotting of them versus the canonical functions visualizes their ability to discriminate among elaboration styles (Fig. 1). Samples of ripe olives were characterized by low values of Function 2 and positive values of Function 1. All of them were clearly separate from the rest. Green and directly brined olives were characterized by negative values of Function 1 (approximately from −2 to 0) and mainly negative values of Function 2 (green) or positive (directly brined); however, some green olive samples were situated in the region of directly brined olives.

The confusion matrix associated with the discriminant analysis between styles showed good specificity and sensibility. The overall success of classification reached was 94% (Table 4). The best characterized style was ripe olives with 100% specificity and sensibility. The second class,

from the point of view of its classification, was green olives with a very high specificity (97.1%); only four samples of directly brined olives were misclassified as green, and sensibility (94.3%), with only eight green samples misclassified as directly brined olives. Directly brined olives still had high specificity, 74.2%, with eight green olives misclassified as directly brined; they had slightly higher sensibility, 85.2%, because four directly brined samples were predicted to belong to green. The confusion between green and directly brined olives, could be due to the use of similar brine concentrations in both styles. Cross-validation, showed practically the same results. So the deduced discriminate functions were able to detect different mineral concentrations among styles, but also to classify further unknown samples. However, it must emphasize that the study focused mainly on the demonstration that the elaboration styles introduce differences in the mineral content of

Table 4
Confusion matrix of discriminant analysis (elaboration styles) according to mineral concentration

Current olive style	n_i	Predicted olive style			Sensibility (%)
		Green	Directly brined	Ripe	
Green	140	132 (131)	8 (9)	0 (0)	94.3
Directly brined	27	4 (4)	23 (23)	0 (0)	85.2
Ripe	29	0 (0)	0 (0)	29 (29)	100.0
Specificity (%)		97.1	74.2	100.0	

In parenthesis, classification obtained in the cross-validation.
Note: Overall correct classification, 94%; overall correct classification after cross-validation, 93%.
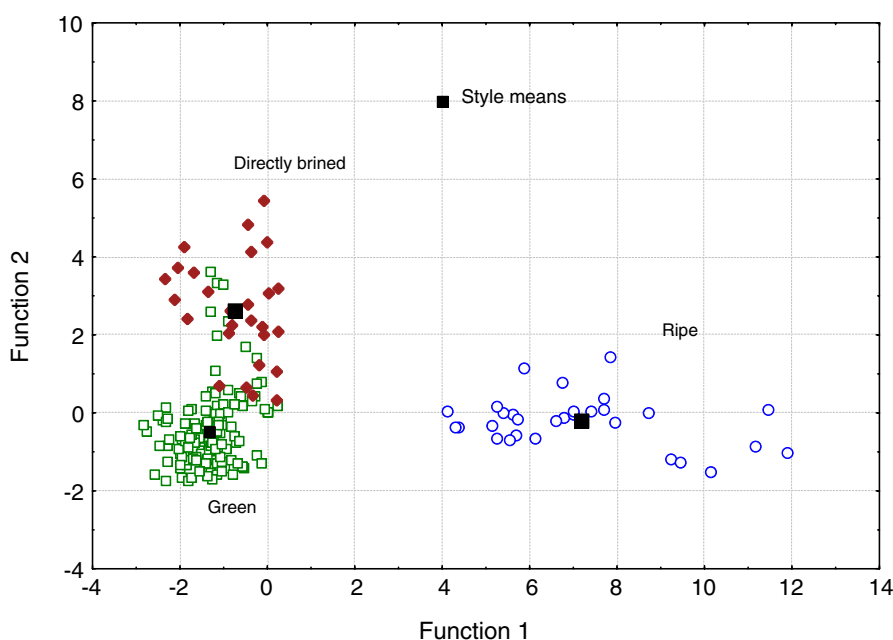

Fig. 1. Separation of green (Spanish style), directly brined, and ripe olives by the first two canonical functions (Function 1 and Function 2) for the different commercial presentations studied.

products. Then, depending on circumstances, the consumption of one style or another could be more appropriate.

For a classification of the samples into cultivars, all the elements, except Zn were selected. Seven standardized canonical functions, and the score of the different samples relative to them were deduced. However, the two or three dimensional plotting of the score versus the corresponding canonical functions did not lead to a net separation of cultivars. This was also reflected on the confusion matrix (Table 5). One hundred percent sensibility was obtained for Verdial and Arbequina; however, their specificities were 60% and 50%, respectively, due to the misclassification of one sample of Gordal and Manzanilla as Verdial and two of Gordal and one of Carrasqueña as Arbequina. Hojiblanca obtained a good classification in sensibility (90.5%) and specificity (82.6). Cacereña also had high sensibility (75.0%) but its specificity was rather low (37.5). Manzanilla showed moderate sensibility due to the classification of many of its samples into Gordal, Carrasqueña, Hojiblanca, Verdial and Cacereña but its specificity was good because only 11 samples of Gordal, 3 of Carrasqueña and 2 of Hojiblanca were predicted to belong to Manzanilla. The rest of the cultivars had rather low sensibility and specificity. Results relative to some cultivars must be assessed with caution because of their low number of samples but the commercial presentations in the market were also reduced. Cross-validation leads to only slightly worse results. So the prediction ability of the model to differentiate among cultivars was worse than that observed to classify elaboration styles. In principle, elaboration styles had a greater effect on mineral concentrations than cultivars.

This is a case where the evaluation of the confusion matrix is difficult. An analysis of results with respect to those expected by chance may be of interest. A first test could assess the overall ability of the model for classification. The overall correct classification observed was 62.2%, $(18 + 63 + 7 + 19 + 3 + 3 + 3 + 6)/196$. The calculus of the expected cases (e_{ij}) per cell and the overall χ^2

leads to a value of 456.41 with $p = 0.000$ (49 df). Then, it must be concluded that the model performance yielded a better classification into cultivars than those expected just by chance.

The test based on the likelihood ratio defined by Morrison (1969) can be applied to evaluate expected classification of specific cultivars (rows). The proportional chance criteria c_{pro} can give an estimation of the expected correct classification by chance. The values of $p_{i.}$ and $\alpha_{.j}$, necessary for the calculus of c_{pro}, are given in Table 5. The estimated c_{pro} may be compared with the overall correct classification by the Z_i score obtained for each group (cultivar) according to the formula defined in Material and Methods. The Z_i values for the respective cultivars are shown in Table 6. Except for Manzanilla, the classification obtained using the model is significantly lower than expected by chance. This is because samples of some cultivars were classified by the model as belonging to others. This overall test of significance suggests that further analysis should be conducted to determine the source of the divergence from chance expectations. Divergence may be present in any of the confusion matrix cells and thus each may be tested to determine whether its proportion differs from chance. The Z_{ij} values of this comparison and its associated probabilities are shown in Table 6. The correct classification of Gordal was higher than that expected by chance but the model also misclassified Gordal samples as Manzanilla more than expected by chance (model discrimination between these two cv. was then poor). There are also other samples of Gordal classified as other cultivars but this misclassification was lower than by chance. Manzanilla was better classified by the model than by chance and was always misclassified in lower proportion than that expected by chance. The model also confused Carrasqueña with Gordal, Manzanilla, Arbequina, Aloreña and Cacereña, since all their cells had significantly higher values than expected by chance. The model was fairly good for Hojiblanca because it only showed better proportion than by

Table 5
Confusion matrix of discriminant analysis (cultivar) according to mineral concentration

Current cultivar	Predicted cultivar								Sensibility (%)	$n_{i.}$	$p_{i.} = n_{i.}/n$
	G	M	Cr	H	Ar	Al	Vr	Cc			
G	18 (16)	11 (12)	1 (1)	0 (0)	2 (2)	0 (0)	1 (2)	3 (3)	50.0	36	0.1836
M	18 (17)	63 (62)	12 (13)	4 (4)	0 (0)	0 (0)	1 (2)	3 (3)	62.4	101	0.5153
Cr	1 (1)	3 (3)	7 (3)	0 (2)	1 (2)	2 (3)	0 (0)	4 (4)	38.9	18	0.0918
H	0 (0)	2 (4)	0 (1)	19 (16)	0 (0)	0 (0)	0 (0)	0 (0)	90.5	21	0.1071
Ar	0 (0)	0 (0)	0 (0)	0 (0)	3 (3)	0 (0)	0 (0)	0 (0)	100.0	3	0.0153
Al	0 (0)	0 (0)	3 (3)	0 (0)	0 (0)	3 (3)	0 (0)	0 (0)	50.0	6	0.0306
Vr	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (3)	0 (0)	100.0	3	0.0153
Cc	0 (0)	0 (0)	2 (3)	0 (0)	0 (0)	0 (0)	0 (0)	6 (5)	75.0	8	0.0408
Specificity (%)	48.65	79.74	48.00	82.61	50.00	60.00	60.00	37.5	–	–	–
$n_{.j}$	37	79	25	23	6	5	5	16	–	$n = 196$	–
$\alpha_{.j} = n_{.j}/n$	0.1888	0.4031	0.1276	0.1173	0.0306	0.0255	0.0255	0.0816	–	–	–

In parenthesis, classification obtained in the cross-validation.
Note: Overall correct classification, 62%; overall correct classification after cross-validation, 57%.
G, Gordal; M, Manzanilla; Cr, Carrasqueña; H, Hojiblanca; Ar, Arbequina; Al, Aloreña; Vr, Verdial; Cc, Cacereña.

Table 6
Analysis of the confusion matrix

Current cultivar	$Z_{i.}$	Predicted cultivar							
		G	M	Cr	H	Ar	Al	Vr	Cc
G	−2.27	9.99	4.41	−5.63	−6.63	−4.63	−6.63	−5.63	−3.62
	(0.011)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
M	3.51	−9.44	3.038	−11.11	−13.33	−14.44	−14.44	−14.16	−13.60
	(0.000)	(0.000)	(0.001)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
Cr	−6.41	−3.97	3.62	14.40	4.45	−3.97	0.93	−4.45	6.32
	(0.000)	(0.000)	(0.001)	(0.000)	(0.000)	(0.000)	(0.176)	(0.000)	(0.000)
H	−6.61	−4.85	−0.54	−4.85	36.10	−4.85	−4.85	−4.85	−4.85
	(0.000)	(0.000)	(0.295)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
Ar	−22.47	−1.75	−1.75	−1.75	−1.75	112.29	−1.75	−1.75	−1.75
	(0.000)	(0.041)	(0.041)	(0.041)	(0.041)	(0.000)	(0.041)	(0.041)	(0.041)
Al	−19.91	−2.95	−2.95	−2.95	−2.95	−2.95	38.15	−2.95	−2.95
	(0.000)	(0.002)	(0.002)	(0.002)	(0.002)	(0.002)	(0.000)	(0.002)	(0.002)
Vr	−24.04	−1.75	−1.75	−1.75	−1.75	−1.75	−1.75	112.29	−1.75
	(0.000)	(0.041)	(0.041)	(0.041)	(0.041)	(0.041)	(0.041)	(0.000)	(0.041)
Cc	−26.48	−2.89	−2.89	14.80	−2.89	−2.89	−2.89	−2.89	50.18
	(0.000)	(0.002)	(0.002)	(0.000)	(0.002)	(0.002)	(0.002)	(0.002)	(0.000)

Values of Z and probability (in parenthesis) for the analysis of cultivars ($Z_{i.}$), rows, and cells.
Overall $\chi^2 = 456.41$ with 49 df, (8-1)(8-1), ($p = 0.000$).

chance in the classification of this cultivar; in all other cases the proportion was significantly lower than by chance. Similar results were obtained for Arbequina, Aloreña, and Verdial in which the only significantly higher proportion was observed for the cell corresponding to their own cultivars. Cacereña, apart from its own cell, showed a higher than expected proportion in the cell of Carrasqueña. This confusion may be explained by the close relationship between both cultivars which, in addition, are from the same region. The model was fairly good for discriminating Hojiblanca, Arbequina, Aloreña, and Verdial from other cultivars. The highest degrees of misclassifications were observed among Manzanilla, Carrasqueña, and Cacereña. This must be due to the close relationship between these cultivars, which come from the same origin but adopt slightly different botanical characteristics when grown in different places.

4. Conclusions

This work provides the mineral composition (elements included in the nutrition labeling) of table olives according to cultivars and elaboration styles. As correspond to a brined product, Na was the most abundant element (18,144–5706 mg kg^{-1}). However, olives can also be a good source of Ca (337–850 mg kg^{-1}), K (82–1180 mg kg^{-1}), Mg (51–197 mg kg^{-1}), and P (57–144 mg kg^{-1}). Fe concentrations were also high in ripe olives (58–131 mg kg^{-1}) but significantly lower in green and directly brined (3.5–7.7 mg kg^{-1}). Microelements Cu (1.7–11.0 mg kg^{-1}), Zn (1.5–3.6 mg kg^{-1}), and Mn (0.2–1.5 mg kg^{-1}) had concentrations similar to other plants. Processing affects the mineral content of the commercial presentations and different significant ($p < 0.05$) means, except for Ca and Zn, among green (Spanish style), directly brined, and ripe olives were found. Significant differences ($p < 0.05$) among cultivars

were also observed, except for Zn. A predictive discriminant analysis showed that the most discriminating elements were Fe, K, Na, Mn, Cu, and P (between styles) and these plus Mg and Ca (for cultivars). A good classification and cross-validation was observed in the case of elaboration styles but discrimination among cultivars was less conclusive. A further analysis of the confusion matrix, according to cultivars, disclosed the causes of the lower classification efficiency in this case. This analysis may be useful when the assessment of the confusion matrix is not obvious.

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