

## Validation of a Method for the Analysis of Iron and Manganese in Table Olives by Flame Atomic Absorption Spectrometry

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There is no generally accepted method for determination of the amounts of iron and manganese in table olives. Application of flame atomic absorption spectrometry to the analysis of both elements has been examined to validate a method that may be used by the industry's quality control laboratory as well as by the laboratories of regulatory agencies. The method has detection limits of 0.106 and 0.022 mg/L and quantification limits of 0.271 and 0.057 mg/L, for Fe and Mn, respectively, referred to the solution to be measured. There was no significant effect due to the matrix, but a slight bias due to the presence of Ca has been detected. Recoveries were excellent, and the method was robust. Influence of operator, HCl and Mg salt compounds, calcination equipment, or dates on results was not found. Relative errors were, in general, below 4% for both cations, and repeatability was below 3.43 and 0.38 mg/kg of olive paste for Fe and Mn, respectively. The method is proposed for the analysis of Fe and Mn in ripe olives and table olives in general.

**KEYWORDS:** Table olives; iron; manganese; calcium; analysis

### INTRODUCTION

World table olive production reached 1,344,000 metric tons in the 1999–2000 season (1). The commercial preparation of so-called ripe olives accounted for ~260,000 metric tons ( $\cong 20\%$ ) of this. Ripe olive production has steadily increased for the past three decades.

The processing of ripe olives involves storage of the fruit for a variable period of time and a stage of darkening or oxidation in an alkaline medium (2). Then a ferrous gluconate or lactate solution is added to fix the color formed (3). Finally, fruits are canned and sterilized. The whole process lasts 5–7 days.

The maximum concentration of Fe permitted by the Unified Qualitative Standard Applying to Table Olives in International Trade (4) is 150 mg of total iron/kg of olive pulp, that is, wet weight basis. The same limit is also established in the European Union (5) and by Spanish legislation (6).

Manganese salts can catalyze the chemical oxidation of *o*-diphenols and the darkening reaction involved in ripe olive processing (7). The effect of Mn salts markedly improved the color obtained in Manzanilla cultivar (*Olea europaea* L) (8). The use of this cation during the desalting and storage steps of pilot and industrial ripe olive processing of Manzanilla, Hojiblanca, Carrasqueña, and Cacereña Spanish cultivars showed a general improvement of color, giving rise to low residues of Mn in the final product (9).

ISO 9526—Fruits, vegetables and derived products: Determination of iron content by flame atomic absorption spectrom-

etry (10)—is the only standard methodology tested for products relatively similar to ripe olives, but it has never been applied to them. The most widely used method for Fe determination in ripe olives is that developed by Albi and Garrido (11). It is based on the colorimetric reaction of ferrous ions with *o*-phenanthroline. Mineralization can be replaced by an extraction with trichloroacetic acid, which allows results to be obtained faster and more easily (12).

Flame atomic absorption spectrometry has also been used (3, 7–9) for the determination of Fe and Mn in ripe olives, but a detailed study of its use in the analysis of both elements in ripe olives has never been undertaken.

The aim of this work was the evaluation and validation of a method, based on flame atomic absorption spectrometry, to be applied for Fe and Mn determination in ripe olives and table olives in general. This method could be used as a reference in the next revision of the Unified Qualitative Standard Applying to Table Olives in International Trade, the International Standards Recommended for Table Olives (COI/CODEX Alimentarius), and the Spanish Quality Standards Applying to the Exportation of Table Olives and by the U.S. FDA and other national or international organizations related to these products. It can also serve as a guide to validate a similar method for other vegetable products.

### MATERIALS AND METHODS

**Samples.** Samples were of Hojiblanca, Manzanilla, and Cacereña cultivars, processed as ripe olives at both pilot and industrial scales.

**Reagents.** All reagents were of analytical purity. Magnesium nitrate 25% (w/v) was obtained by diluting 25 g of  $Mg(NO_3)_2 \cdot 6H_2O$  (Sigma, St. Louis, MO; and Merck, Darmstadt, Germany) to 100 mL with 96%

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**Table 1.** Instrumental Conditions for Fe and Mn Determinations

	element		
	iron	manganese	calcium
lamp current (mA)	10.0	5.0	10.0
wavelength (nm)	248.3	279.8	422.7
band-pass (nm)	0.2	0.2	0.5
flame type	oxidizing	stoichiometric	oxidizing
instrument mode	absorbance	absorbance	absorbance
optimum working range ( $\mu\text{g/mL}$ or $\text{mg/L}$ ), according to apparatus manual	2.0–9.0	1.0–3.6	0.5–14.9

(v/v) ethanol (Panreac, Barcelona, Spain). Hydrochloric acid (6 N) solution was obtained by dilution of concentrated HCl (Panreac; and Fluka, Buchs, Switzerland).

The standard solutions of Fe, Mn, and Ca were obtained by dilutions of the corresponding stock solutions (Sigma) and the addition of HCl in a concentration similar to that obtained in the sample solutions (1.44 M). Na, K, and Ca added in the interference experiments were also from stock solutions (Sigma). All reagents were of AA grade.

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

**Sample Preparations.** The pulp of 100 g of brined olives was separated from the pit by manual or automatic pitting machine, ground, and homogenized. From the resulting paste, 2 g was exactly weighted in a quartz capsule containing 1 mL of magnesium nitrate solution, which has the double function of facilitating the combustion; the small ashes were retained and placed in a capsule, avoiding possible losses due to the adsorption of the vessel walls. The capsule was put over an electrical heating plate and the temperature increased gradually to 350 °C. The capsule was then put in a muffle oven and incinerated at 550 °C. To this end the temperature was quickly brought to 250 °C and then increased slowly until the calcination temperature had been reached, which was maintained for ~8–10 h.

The ashes, white-grayish in color, were slightly moistened and dissolved with three parts of 2 mL of 6 N hydrochloric acid and filtered, part by part, 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 completed with deionized water until level. The dissolution can be aided by heating slightly the capsule in every addition of hydrochloric acid. To make it easier, the filtration can be made by means of a suction hood. At the same time a blank was prepared with only the reagents.

**Apparatus.** A GBC model 932 AA (Victoria, Australia) atomic absorption spectrometer equipped with two hollow multielement cathode lamps, (Cu, Cr, Co, Fe, Mn, and Ni) (GBC, Victoria, Australia) and (Ca, Mg, Cu, Zn) (Photrompty, Victoria, Australia), was used. An air–acetylene flame was used. Other working conditions are given in **Table 1**.

**Calibration Curve and Analytical Characteristics.** In the evaluation tests, calibration curves for Fe and Mn were obtained, daily, from successive dilutions of the standard solutions of elements with double-distilled deionized water, using working ranges from 0 to 12.480 mg/L and from 0 to 4.848 mg/L, respectively. In standard addition assays, recovery experiments, analysis of commercial samples, and Ca determinations, analyses were performed following the apparatus' instructions (**Table 1**). Fe and Mn calibration plots were visually and statistically evaluated. Linearity, best fitting, and comparison between calibration curves were obtained using separate solutions of Fe and Mn or only one containing both analytes. Between-day deviations of calibration curves were also tested. For this purpose, only a polynomial ( $\text{abs} = a + b \times \text{concn} + c \times \text{concn}^2$ ) and a rational function of the type  $\text{abs} = \text{concn}/(a + b \times \text{concn})$ , similar to that used by the apparatus as an evaluation function, were used. Values of  $a$ ,  $b$ , and  $c$  were estimated in each case by iteration using  $(\text{observed} - \text{predicted})^2$  as loss function and Hooke–Jeeves and Quasi Newton and quasi-Newton estimation methods (StatSoft, 1999).

The quality coefficient of fitting (QC) proposed by Knecht and Stork (13) was defined as  $\text{QC} = 100 \times [\sum_{i=1}^N (y_i - \hat{y})/y_i / (N - 1)]^{1/2}$ , where  $y_i$  is the measure for each calibration solution,  $\hat{y}$  is the value predicted from the fitting function, and  $N$  is the number of calibration solutions, not including the zeroing solution. Good fittings must yield QC values within a predefined criterion, for example, 5%.

The only evaluation function used was that automatically estimated by the apparatus because, very possibly, this (or any other similar function) would be the only one used by analysts, with this or any other instrument.

The detection and quantification limits were calculated according to IUPAC rules (14), using 13 independent measurements of Fe and Mn blanks.

The sensitivity, in terms of absorbance, was evaluated from calibration curves as the first derivative of the fitted polynomial function. Precision estimations and standard deviations (SD) were obtained in every experiment because all of them were carried out in triplicate. Such estimations were later used to calculate pooled precisions. Accuracy was checked with recovery essay tests by adding known amounts of analyte (20.2, 60.6, and 101.0 mg/kg and 10.1, 225.25, and 45 mg/kg of Fe and Mn, respectively) to different samples prepared from a paste of canned ripe olives. Repeatability, defined as the value below which the absolute difference between test results can be expected to lie within 95% confidence limits when results have been obtained, under repeatability conditions, was determined by using the formula  $r = t2^{-1/2}s$ , where  $r$  = repeatability,  $t$  = Student's  $t$ , and  $s$  = standard deviation (15).

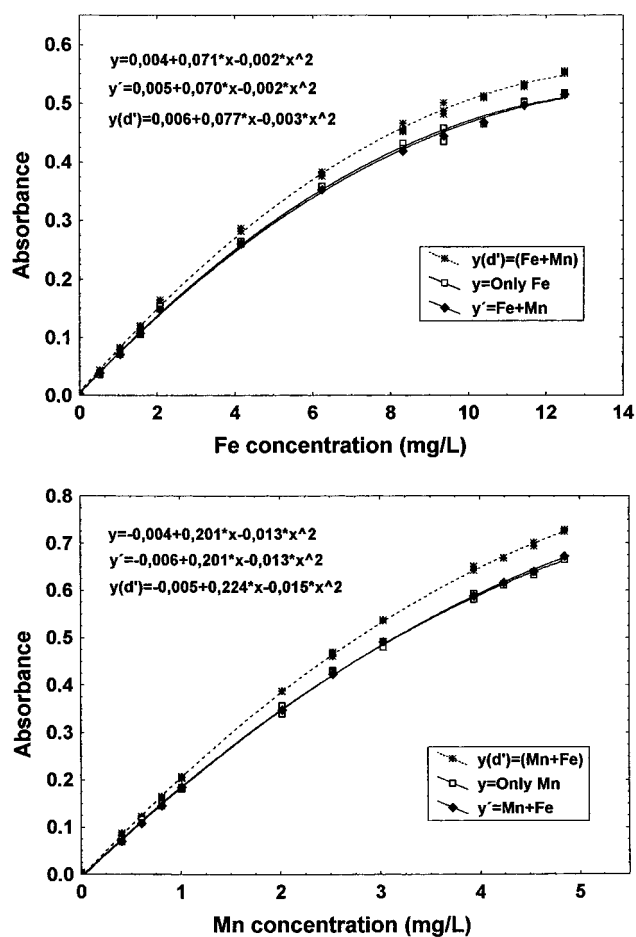
The ruggedness of the method was checked by a half-factorial experimental design (16), using HCl source, Mg salt source, oven, time, and analyst as variables.

**Statistical Analysis.** The different statistical analyses (curve fitting, graphs, significant test, etc.) were performed using the Statistica software package (17).

## RESULTS AND DISCUSSION

**Calibration Curves.** Curves for absorbance versus Fe and Mn concentrations are shown in **Figure 1**. In the range of concentrations used in this study, the plot of absorbance versus Fe or Mn concentrations was never a straight line. This situation is usual because there is always a part of the radiation that cannot be absorbed, because either it is an unresolved nonabsorbing line adjacent to the absorbing line or the light path does not completely go through the flame or strays or is scattered. In the typical situation Lambert–Beer's law becomes absorbance =  $\log[I_0/(I + K)]$ , where  $I_0$  is the absorbable radiation and  $K$  is the nonabsorbable component. Typically, results differ more as the absorbance increases. In spectroscopy, the best precision is obtained at an absorption of 0.434 (36.8% T), and the optimum working range recommended by the instruction manuals for our apparatus is 0.2–0.9 absorbance unit, where gross curvature and possible errors are minimized (18). However, in our case gross curvature was already evident at the higher limits tested (0.500 and 0.700 absorbance values for Fe and Mn, respectively).

There are numerous algorithms used for curve fitting (19), but a systematic comparison of them is beyond the scope of this work. Only a linear, polynomial, and rational function, similar to that used by the apparatus's evaluation function, have been compared. Linear fitting is not appropriate, and it was always corroborated by Mandel's test (20). Furthermore, the application of it to successively shorter intervals of concentrations showed that significant improvement, with respect to the linear one, was always achieved by fitting a rational or quadratic function. This is a circumstance that must be taken into account in flame spectrometric analysis of these elements because many references in the literature use the linear fitting, based on the correlation coefficient, but without any further checking of



**Figure 1.** Typical plot of absorbance versus Fe and Mn concentrations, using different standard solutions and two consecutive days. Curves with (d') distinguish data obtained on different dates. Fe and Mn concentrations ranged from 0 to 12.48 mg/L and from 0 to 4.85 mg/L, respectively.

goodness of fit. Only in the bracketing technique, where a small section of the calibration curve is involved, will this linear approach be taken into consideration (21).

Calibration curves (Figure 1) for Fe or Mn, obtained from standard solutions containing one of the respective cations in solution or both, were coincident, but they differed from curves obtained on successive days under the same conditions (Figure 1). Statistical comparisons among calibration curves were achieved by fitting the same type of equation to these curves (rational or polynomial). Both fittings gave similar results. To simplify, only polynomial results will be commented on. There were no significant differences between values of  $c$  or  $a$  from different Fe calibration curves, irrespective of the conditions of the standard solution used. Similar behavior was observed also in Mn calibration curves. However, significant differences between  $b$  values of the calibration curves on different dates (batches) were obtained (Figure 2). The fact that calibration curves prepared with only one cation or both were the same makes the analyses easier because only one standard solution with both metals must be prepared. However, calibration must be made for each analysis series. Furthermore, recalibration within a series of measurements is also recommendable, if evidence of deviations is observed (18).

The application of the quality coefficient of fitting (QC) proposed by Knecht and Stork (13) to different Fe and Mn intervals using the rational and polynomial functions is shown in Figure 3. In general, the intervals used in this work are wider than those recommended by the manual of the apparatus. Using

the predefined criterion value of 5%, the rational and polynomial fitting can be judged as appropriate for the whole interval used in this work (excluding the zero to avoid division by this figure), except the two widest intervals of Fe. However, differences among QC indices from diverse curve fitting functions and intervals were evident. In general, polynomial fitting (black points and continuous line in Figure 3) gave better QC values than the rational function. Irrespective of the function fitted, the QC values mainly depended on the lower limits of the intervals. As those diminished there was a shift in the QC indices, which yielded lower values (better fitting). Lower limits of 0.118 and 0.283 for Fe and 0.150 and 0.183 for Fe and Mn, respectively, yielded very similar and low QC indices, which, in turn, were almost independent of the upper limits. This indicates that lower limits are more critical than upper absorbance limits. QC indices obtained from the rational equation always diminished as the upper limit was lowered, yielding always the best fitting at values of about those recommended by the apparatus, with values progressively lower as the lower limit is raised. However, values from the polynomial fitting in manganese slightly increased as the upper interval decreased. It must be emphasized that the quality (QC) of calibration curves using polynomial fitting was systematically better than that from the rational algorithm used by the apparatus. In fact, it was good enough ( $p < 0.05$ ) even using standard solutions from 0 to 12.48 and 4.848 mg/L of Fe and Mn, respectively. Similar QC indices for rational and polynomial fitting were obtained only in the tighter intervals recommended by the apparatus.

**Detection Limits and Sensitivity.** Limits of detection and quantification were determined as established under Materials and Methods, following the IUPAC (14) recommendations. Their values for Fe and Mn are shown in Table 2, for both absorbencies and Fe and Mn concentrations, referred to the solution to be measured.

Sensitivities can change slightly from calibration to calibration. In terms of absorbance, they may be obtained as the derivative of the polynomial fitted equation. Estimations of them (expressed in mg/L) are  $S_{Fe} = 0.071 - 0.004 \times \text{concentration}$  for Fe and  $S_{Mn} = 0.201 - 0.026 \times \text{concentration}$  for Mn, respectively.

**Evaluation Function and Working Range.** To obtain the concentration as a function of the absorbance, most instruments use rational functions (19, 22). In our case, the function used by the apparatus is as follows:  $\text{concentration} = \text{abs}/(a + b \times \text{abs})$  (18). As shown before, polynomial fitting could be more appropriate because it usually yields lower QC. However, it could be rare that any analyst will prefer his own estimations against those just obtained by direct readings from the apparatus, especially using the interval recommended by the manual where QC indices are very similar. Relative errors obtained in the calculation of Fe and Mn concentrations for different calibration intervals are shown in Figure 4. The shape of the curves is typical of this kind of representation (21). Above 2 mg/L of any cation, errors are  $<4\%$ , except some cases of Fe determinations, irrespective of the interval used for calibration. These results show that the upper limit of calibration concentrations can be expanded further than those recommended by the apparatus's manual without increasing the measurements' errors. Thus, the working range can be from 2 mg/L to the upper limits used in this work (12.48 and 4.848 mg/L for Fe and Mn, respectively), in the standards or solution to be measured. These working ranges are wider than those recommended by the apparatus but, as has been shown, can be used without significant increase in errors.

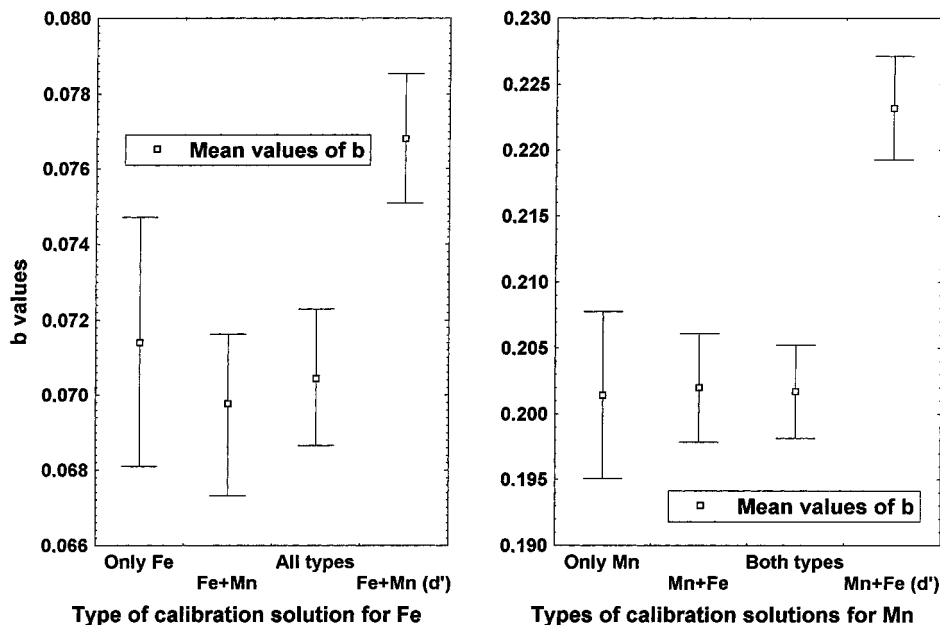


Figure 2. Values of *b* and their confidence limits ( $p < 0.05$ ) in the quadratic equation adjusted to absorbance versus Fe or Mn concentrations (represented in Figure 1), using different standard solutions and two consecutive days. *d'* distinguishes data obtained on different dates.

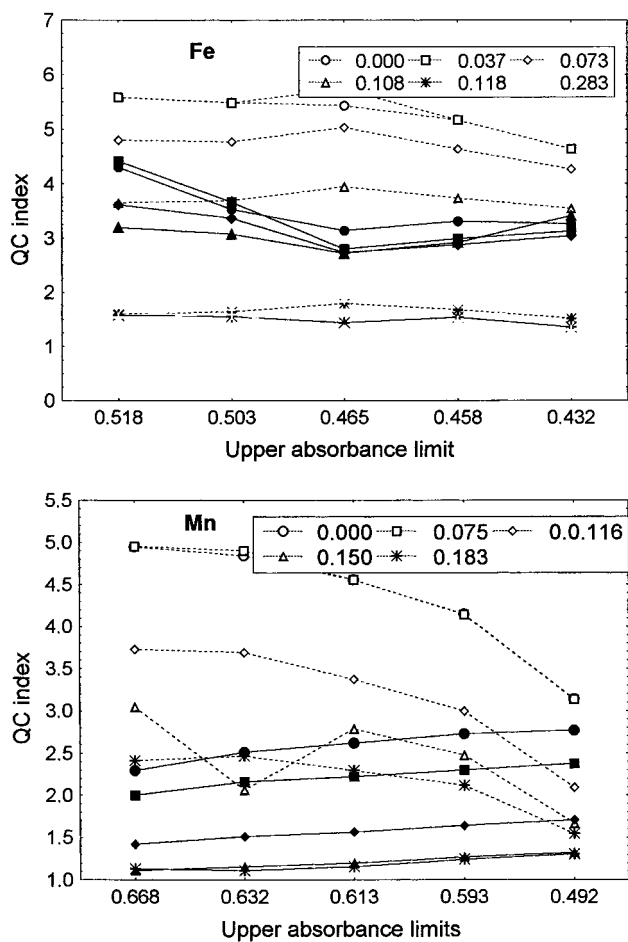


Figure 3. Effect of the absorbance interval on the QC index of curve fitting. Black points and continuous lines correspond to polynomial fitting, the others, to rational fitting.

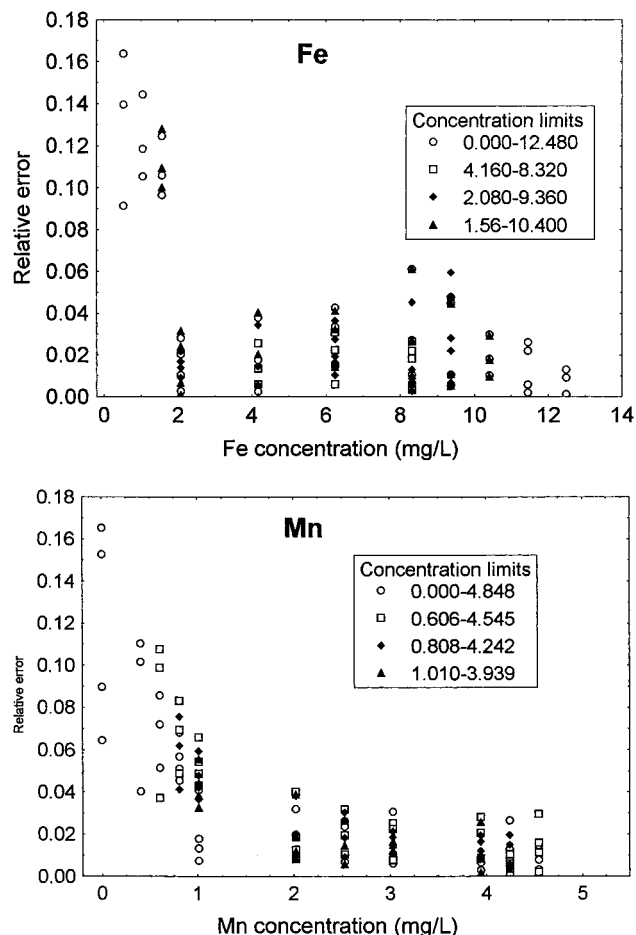
**Interferences.** The effect of matrix was tested by the method of standard addition, adding progressive amounts of Fe and Mn to different subsamples of ripe olive paste, and slopes of the regressions between Fe and Mn found versus added were

Table 2. Limits of Detection and Quantification of Fe and Mn Determination by Flame Atomic Spectrometry in Ripe Olives, Referred to the Solution To Be Measured

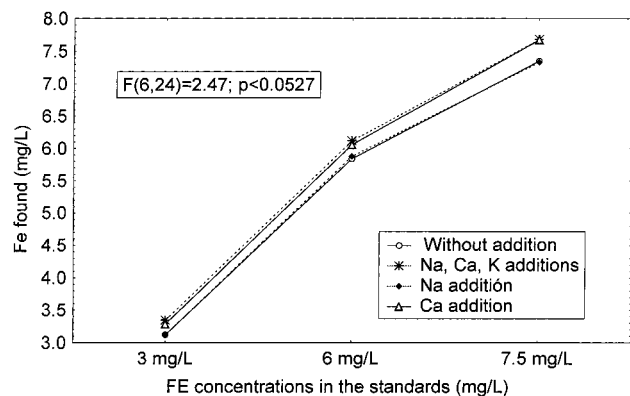
element	limit of detection		limit of quantification	
	absorbance	concn (mg/L)	absorbance	concn (mg/L)
Fe	0.009	0.106	0.023	0.271
Mn	0.005	0.022	0.011	0.057

calculated. Slopes were not statistically different from 1. Thus, there is no proportional error due to matrix (paste of table olives).

Table olives are rich in several mineral elements, Na, Ca, and K being the most important (2). To study the effect of the presence of these metals in the Fe and Mn determinations, different standard solutions with diverse Fe and Mn concentrations were prepared. Four series were amended with Na, K, and Ca, separately and as a pool of all these metals. All them were prepared from the corresponding stock AA grade solutions. They were added at 1200, 240, and 56 mg/L, respectively, the maximum approximate concentrations that could be found in the solutions to be analyzed considering their table olive contents (15.0, 3.0, and 0.7 g/kg of flesh, respectively) (23). Analyses were performed in each of the above-prepared solutions to determine the concentrations of Fe and Mn. Concentrations found were plotted versus theoretical concentrations of Fe and Mn. Statistical analysis showed no effect of the presence of any of the assayed metal on Mn determinations (data not shown). However, the presence of metals produced a slight increase in the concentrations of Fe found. The separate assays of each metal showed that Na, which is the most abundant element in table olives, and K did not produce any interference. Instead, the presence of Ca produced a systematic bias in the Fe determination (Figure 5) that the statistical analysis showed to be very close to being significant ( $p < 0.0527$ ). To study the possible effect of the Ca concentrations, standard solutions of Fe (or Fe and Mn) with Ca concentrations in the range of 20–60 mg/L, which correspond to the levels that could be obtained when a 2 g olive sample containing 250–700 mg/kg of Ca in flesh was analyzed, were introduced into the AA apparatus.



**Figure 4.** Relative errors versus concentrations in the estimation of Fe and Mn, using different intervals of concentration in the calibration curve and the rational evaluation equation fitted by the apparatus.



**Figure 5.** Interference on Fe determinations of different elements present in olives and their ashes. Concentrations of Na, K, and Ca were 1200, 240, and 56 mg/L, respectively. Fe calibration curve was obtained from a standard solution containing only Fe.

Results were compared with those obtained from standard solutions of only Fe (or Fe plus Mn). The same effect, similar to that shown in **Figure 5**, was observed at all levels within the interval checked (data not shown). Thus, it is advisable that calibration curves for Fe determination be prepared from Fe (or Fe and Mn) standard solutions containing at least 20 mg/L of Ca to prevent this systematic constant slight error due to the usual presence of Ca in all of the commercial table olive samples and their ashes.

**Table 3.** Standard Addition

matrix	Fe		Mn	
	$B$	$SD_B$	$B$	$SD_B$
1	0.9948	0.0055	0.9910	0.0059
2	0.9947	0.0058	1.0016	0.0053
3	0.9958	0.0047	0.9902	0.0088

<sup>a</sup> Values of slopes and their standard deviations for three different samples (matrices). In all cases,  $n = 12$  and  $t_{(10,0.05)} = 1.81$ .

**Table 4.** Effect of Ca (500 mg/L) in the Standard Solution and To Obtain the Calibration Curve To Determine Fe in Different Commercial Samples and Their Ca Content<sup>a</sup>

sample	Ca content <sup>b</sup> (mg/kg)		Fe <sup>b</sup> (mg/kg) standard solution without Ca		Fe <sup>b</sup> (mg/kg) standard solution with Ca	
	mean	SD	mean	$SD^c$	mean	$SD^c$
1	859.97	10.29	80.63	1.49	79.45	1.43
2	812.58	20.72	80.38	0.24	79.95	0.93
3	771.79	4.93	89.13	2.06	88.16	0.54
4	571.93	17.64	71.04	2.66	68.69	1.87
5	708.82	15.94	98.89	0.51	96.33	1.60
6	581.10	5.76	67.54	2.03	66.35	1.85
7	518.56	5.98	7.00	1.36	6.87	1.22
8	702.14	4.27	129.00	0.09	127.64	0.35
9	743.25	6.78	86.67	0.53	85.57	0.83
10	805.03	13.77	186.27	1.92	182.45	0.68

<sup>a</sup> Each sample was obtained from three replications [ $t_{(20,0.05)} = 1.7247$ ]. <sup>b</sup> Wet weight basis. <sup>c</sup> Pooled SD for Fe determination, 1.10 and 0.88 mg/kg (20 degrees of freedom) without and with Ca in the standard, respectively.

An evaluation of the deviations that may be obtained in the analysis of commercial samples is shown in **Table 4**. Fe concentrations found using the usual Fe standard solution for calibration were always higher than levels obtained when the calibration solution contained not only Fe but also 500 mg/L Ca. Differences were never statistically significant, but values from calibrations without correction were always higher than those found with Ca correction in the calibration standard. No significant influences of the presence of Ca on the standard deviation values was detected, and the standard deviation pooled values for Fe determination were  $\sim 1.00$  in both cases. **Table 4** also shows the Ca content in the olive flesh of such olives, which, as expected, was very high in comparison with Fe.

With respect to the interference of Ca in the Fe analysis, one may conclude that there is a tendency to give slightly higher values, not statistically significant, when the calibration standard contains only Fe or Fe plus Mn in contrast to when it is added with Ca. Thus, in general, it is advisable to make the correction.

**Trueness, Precision, and Repeatability.** There is no certified material for Fe and Mn content in table olives. Trueness was judged by recovery experiments, made as described under Materials and Methods. Results are shown in **Table 5**. Recoveries were excellent at all levels of Fe and Mn assayed.

Precision was evaluated in all of the experiments carried out in this work because practically all of them were carried out in triplicate. **Table 4** shows the standard deviations obtained in the study of the Ca interference on iron analysis. A pooled standard deviation obtained from all of them gave 1.10 and 0.90 mg/kg of olive paste for values using calibration standards with and without Ca added, respectively. Bearing in mind that  $t_{(20,0.05)} = 1.7241$ , it could represent a relative error of  $\sim 2\%$  for Fe determinations, which coincides with that obtained in the evaluation function section.

**Table 5.** Average Recovery (Percent) of Fe on Mn Added for the Different Samples (Matrices) of Ripe Table Olives<sup>a</sup>

matrix	Fe recovery (%) for amount of Fe added (mg/kg)			Mn recovery (%) for amount of Mn added (mg/kg)		
	20.20	60.60	101.00	10.10	25.25	45.45
1	100.05	99.75	99.46	99.21	99.09	99.2
2	98.71	99.08	99.38	98.35	99.64	100.07
3	99.00	99.86	99.65	99.42	99.72	98.90

<sup>a</sup> For each test,  $n = 3$ .**Table 6.** Ruggedness Check<sup>a</sup>

run	exptl design					Fe <sup>b</sup> (mg/kg)		Mn <sup>b</sup> (mg/kg)	
	operator	HCl	Mg <sup>2+</sup> <sup>c</sup>	oven	date	mean	SD <sup>d</sup>	mean	SD <sup>d</sup>
1	1	1	1	1	1	119.61	1.62	11.24	0.17
2	-1	1	1	1	-1	119.21	3.16	11.18	0.22
3	1	-1	1	1	-1	119.97	1.73	11.17	0.10
4	-1	-1	1	1	1	119.23	2.66	11.35	0.23
5	1	1	-1	1	-1	119.50	0.56	11.21	0.46
6	-1	1	-1	1	1	119.85	1.32	11.14	0.13
7	1	-1	-1	1	1	119.33	2.06	11.08	0.09
8	-1	-1	-1	1	-1	119.43	1.17	11.11	0.17
9	1	1	1	-1	-1	119.10	0.50	11.06	0.12
10	-1	1	1	-1	1	119.33	2.31	11.15	0.27
11	1	-1	1	-1	1	119.67	0.93	11.08	0.28
12	-1	-1	1	-1	-1	119.98	1.80	11.19	0.19
13	1	1	-1	-1	1	119.80	1.82	11.01	0.17
14	-1	1	-1	-1	-1	119.60	0.98	11.17	0.12
15	1	-1	-1	-1	-1	118.76	0.89	11.01	0.25
16	-1	-1	-1	-1	1	118.97	1.05	11.16	0.28

<sup>a</sup> Experimental design, average values obtained, and standard deviations in each run. Each run was tested in triplicate. <sup>b</sup> Wet weight basis. <sup>c</sup> Pooled standard deviations (degrees of freedom = 32): Fe, 1.20 mg/kg; Mn, 0.16 mg/kg. <sup>d</sup> Mg<sup>2+</sup> = Mg salt source.

Results from the ruggedness experiments (Table 6) gave a standard deviation of 1.6963 ( $t_{(32; 0.05)} = 1.6939$ ) for iron determination, giving an average error of 2.4%.

Repeatability in iron determinations was calculated as described under Materials and Methods and, depending on the standard deviation values used, ranged from 2.88 to 3.43 mg/kg of olive paste.

Precision and repeatability from ruggedness experiments gave a pooled standard deviation of 0.1578 ( $t_{(32; 0.05)} = 1.6939$ ) for Mn, which means an average relative error of 2.38% and a repeatability of 0.38 mg/kg of olive paste.

**Ruggedness.** Experimental design, average results, and standard deviations in the determination of Fe and Mn are shown in Table 6. Statistical analysis showed nonsignificant effects of the different variables under study, so the method can be considered to be robust.

This work has made an exhaustive study of the parameters required for in-house method validation. Results have permitted the establishment of the performance characteristics of an AA method as applied to Fe and Mn determinations in ripe olives and table olives in general.

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