Multielement Determination Using On-line Emulsion Formation and ICP-MS/FAAS for the Characterization of Virgin Olive Oils by Principal Component Analysis

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ABSTRACT

Several virgin olive oil samples from different Spanish origin denominations were analyzed for the multielement determination of Al, Ba, Bi, Ca, Cu, Mg, Mn, Na, Pb, and Sn with on-line emulsion formation by flow injection analysis and determination by ICP-MS and flame atomic spectroscopy. The data obtained from the automated multielement determination were treated with Principal Components Analysis to assess the feasibility of using the concentration of the different elements in the olive oil for classifying the oils according to geographical origin and the type of olive used. The statistical analysis carried out showed that Al, Ba, and Mn have a differentiating ability which permits separation of the olive oils analyzed according to their geographical origin and the type of olive employed, whereas Bi, Cu, Sn, and Pb are related to contamination and do not display differentiating capacity.

INTRODUCTION

The interest shown by consumers in the geographical origin and/or variety of foodstuffs has increased in recent years because these factors are generally thought to be a better guarantee of quality and authenticity.

The European Economic Council (EEC) regulations 2881/91 and 2082/91 are both of a general nature and pertain to every origin denomination (O.D) of virgin olive oils. These regulations take into account consumer expectations and, in particular, the expectations of producers with regard to better protection of virgin olive oils which, coming from areas especially suitable for olive growing, have peculiar characteristics from an organoleptic or compositional point of view. Some of the compounds present in olive oil, like fatty acids and un-saponifiable compounds (n-alkanes), depend on the type of olive used because genetics is a determining factor in the biosynthetic reaction which leads to the lipidic synthesis. Factors such as climate and altitude, storage conditions of the olives, transport, and the oil extraction procedure may modify the chemical composition of the oils. As an example, olive oils from areas with warm climates are richer in fatty acids than those from colder areas.

It is well known that the presence of certain trace elements (Cu and Fe in particular) in edible oils accelerates oxidation processes which negatively affects oil quality, while other elements such as Cd, Pb, As, and Hg are subject to legal restrictions due to their toxicity (1,2). Na, K, Ca, Mg, Mn, Fe, Zn,Cu, and P are found at different concentrations in the mesocorp (pulp) of olives. The metallic content of oils and the different forms in which metals are present in edible oils depend on several factors: the presence of metals in the ground itself, their introduction during the production process, or by contamination of the processing material used and/or storage.

The determination of metals in organic samples has always been a challenge in analytical chemistry. There is much literature available on the analysis of lubricating oils but considerably less concerning the analysis of edible oils. Analysis of edible oils for different elements at sub-ppm levels has been performed using different techniques, most of which require sample pretreatment to destroy organic material. These techniques are time-consuming and subject to contamination problems due to the low concentration of metals in olive oil, especially in the best quality virgin olive oil.

A simple and advantageous alternative method used by our research group (3,4) for determining metals in olive oil by inductively coupled plasma mass spectrometry (ICP-MS) (given the low metallic content of this type of oil) and by other authors (5) using inductively coupled plasma optical emission spectrometry (ICP-OES) in spiked sunflower oil is the use of oil-in-water emulsions with an emulsifying agent. One of the main advantages of emulsions is that aqueous solutions can be used for the preparation of calibration standards. If, however, on-line emulsion formation by flow injection analysis (FIA) is used (4), more concentrated emulsions can be introduced and the detection limits are thereby improved. Owing to the transitory nature of FIA, the sample volume introduced is much smaller than in the continuous mode and more concentrated emulsions can therefore be introduced without reducing sensitivity. The analyst hardly takes part in the emulsion preparation and thus the risk of contamination is avoided. The analysis time is significantly reduced (each sample takes about 4 min), resulting in increased sample throughput and improved reproducibility.

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In spite of the numerous factors affecting the chemical composition of the oils and their organoleptic properties, studies were performed with the view of classifying oils according to their geographical origin. Up to now, classifications have always been based on the measurement of the different organoleptic characteristics or the analysis of different organic compounds. Rodríguez-Méndez et al. (6) succeeded in differentiating oils with different organoleptic characteristics from different areas in Spain using the sensorial data provided by an electronic nose. Moreno and co-workers (7) used methods based on an electronic nose and pattern recognition treatments to classify vegetable oils and possible adulterants. The analysis of triglycerides by high performance liquid chromatography (HPLC) has made it possible to differentiate oils geographically from two different types of olives at different stages of maturity (8). The analysis of triacylglycerol by reverse-phase liquid chromatography and chemometric techniques has also been used to detect adulterants in oil (9). Alberghina et al. (10) classified oils from three areas of Sicily using the results obtained from the analysis of fatty acids and sterols by gas chromatography. Lee et al. (11) characterized different types of vegetable oils, including primary evaluation of category similarity, detection of adulterants, and quality control. Measurement of hydrocarbon concentration and composition by gas chromatography (12) and isotopic analysis of ^{13}C, ^{18}O, and D of the organic material (13) with the application of statistical procedures have also permitted the characterization and authentication of olive oil samples.

Generally speaking, the research works found in the literature on the characterization of olive oils by origin and/or the variety of olives showed that chemometrics was used to obtain the analytical results from the determination of organic compounds in oils by HPLC or gas chromatography (in many cases after liquid-liquid extraction or solid phase extraction). This process is time-consuming and involves considerable reagent consumption.

In this paper, we propose the possibility of characterizing olive oils from different Spanish origin denominations by performing multielement determination by ICP-MS with an automated FIA system that permits on-line preparation of oil-in-water emulsions (4). The automated FIA system was coupled to a flame atomic absorption spectrometer for the determination of Na, K, and Mg because these elements are more difficult to determine by ICP-MS due to numerous spectroscopic interferences. There are a limited number of references available that report on the possibility of classifying foodstuffs (different types and from different areas) according to their mineral content [e.g., potatoes (14), honey (15), wine (16), and vinegar (17, 18)].

In our study and in order to differentiate between the different virgin olive oils, Principal Components Analysis (PCA) was applied to the analytical results obtained from the multielement analysis of the virgin olive oil samples from different origin denominations and different parts of Spain with differing climates. In addition, an attempt was made to classify the metals according to the area of their origin, identifying those coming from the soil, those depending on the climatic conditions or type of olive cultivated, and those due to contamination or the type of process involved.

EXPERIMENTAL

Instrumentation

A PerkinElmer SCIEX ELAN® 6000 ICP Mass Spectrometer (PerkinElmer SCIEX, Concord, Ontario, Canada) was used for the multielement determination of Al, Ba, Bi, Cu, Mn, Pb, and Sn. Nickel cones were used.

A PerkinElmer® Model 2380 Flame Atomic Absorption Spectrometer (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA) was used for the determination of Na, Ca, and Mg. Na was determined by emission and Ca and Mg by absorption. Ca and Mg hollow cathode lamps (PerkinElmer) were used.

The different olive oil samples were introduced into the ICP-MS or FAAS in the form of oil-in-water emulsions previously formed on-line using a FIA system with merging zones.

The instrumental setup, described in greater detail in a previous paper (4), can be seen in Figure 1. The setup consisted of two peristaltic pumps (P1 and P2, Gilson Minipuls™-3, Villiers le Bel, France), three 6-way automatic injection valves (V1, V2 and V3, from Eurosas, Zaragoza, Spain) with V1 and V2 being used as the sample injection valve and emulsifier injection valve (with and without standards), respectively, and V3 as the by-pass valve of the emulsion formed to the ICP-MS. The injection loops, consisting of a PTFE tube with an internal diameter (i.d.) of 0.8 mm, had an injection volume of 0.4 mL for V1 and V2. The two valves were connected to Y (Omnifit) by means of two PTFE tubes (C1 and C2). Connected to Y was the emulsion formation reactor R consisting of a PTFE tube (1 m length, i.d. 1.6 mm). The reactor and Y were both submerged in an ultrasonic bath (J.P. Selecta,

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TABLE I A
Instrumental Operating Conditions and Measurement Parameters for Quantitative FIA-ICP-MS Analysis

Forward Power	1150 W
Sample Uptake Rrate	3 mL min^{-1}
Coolant Argon Flow	14 L min^{-1}
Sweeps/Reading	1
Readings/Replicate	280
Replicates	1
Dwell Time	50 ms
Scan Mode	Peak Hopping

Carretera Nacional II, Km 585.1, Barcelona, Spain), 250 W power and 40 KHz frequency. A PTFE tube with an internal diameter of 0.8 mm, connectors (Omnifit) and different peristaltic pump tubes consisting of Teflon® tubes with an internal diameter of 1.42 mm (to propel the V1 carrier solution) and 2.79 mm (for the V2 carrier) were also used. The whole system was computer-controlled.

Details of the instrumental operating conditions and measurement parameters for the quantitative analysis by ICP-MS and FAAS are given in Tables IA and IB. Prior to all experiments, the ICP-MS instrument was optimized for routine multielement analysis following the manufacturer's instructions. Nebulizer gas flow rate, oxides, lens voltage, and the daily performance of the instrument were optimized by aspirating a solution containing Rh, Mg, Pb, Ba, and Ce (10 µg L^{-1} of each); the autolens calibration was optimized by aspirating a solution of Be, Co, In, and U (10 µg L^{-1} of each).

The computer program UNSCRAMBLER (Version 7.5, CAMO ASA, Oslo, Norway) was used for the statistical treatment of the data obtained.

TABLE I B
Instrumental Parameters for Quantitative FIA-FAAS Analysis

	Na	Ca	Mg
Wavelength (nm)	589	422.7	289
Bandwith (nm)	0.2	0.7	0.7
Observation Height (cm)	8	8	8
Air Flow Rate (L min^{-1})	12.5	12.5	12.5
Acetylene Flow Rate (L min^{-1})	2	2	2

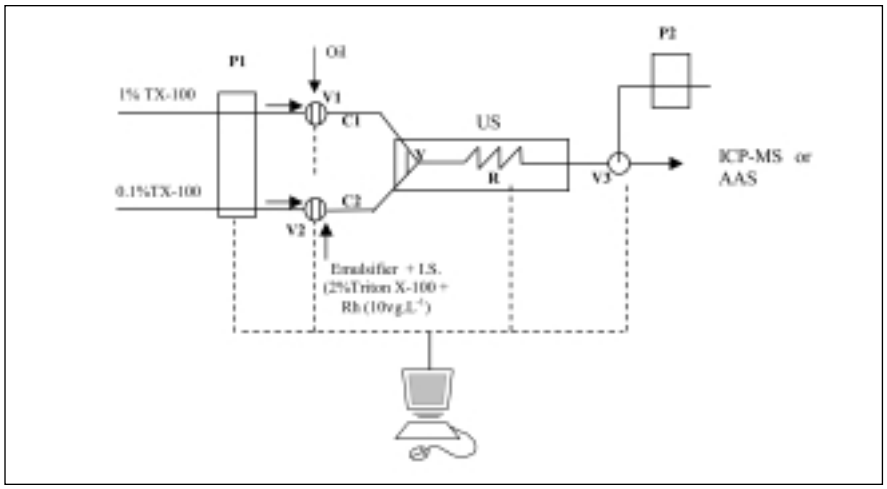

Fig. 1. Schematic diagram of the FIA system for on-line emulsion formation: P1 and P2: Peristaltic pumps; V1: oil valve (injection volume: 0.4 mL); V2: emulsifier valve (injection valve: 0.4 mL), the emulsifier solution contains the internal standard and the calibration standards or spikes for the recovery studies; C1: Connection between V1 and Y (Length=1 m, i.d.=0.8 mm); C2: Connection between V2 and Y (Length=0.5 m, i.d.=2.4 mm); US: ultrasonic bath; R: Emulsion formation reactor (Length= 1 m, i.d.=1.6 mm); V3: By-pass valve. Emulsion formation flow rate: 3 mL.min^{-1}.

Reagents, Standards, and Sample Preparation

Nitric acid (Baker Instra-analyzed, J.T. Baker B.V., Deventer, Holland) and Triton® X-100 (pro analysis, Merck, Darmstadt, Germany) were used. A 5% (v/v) HNO_3 solution was prepared and introduced continuously into the ICP-MS when the emulsion was not being introduced. 0.1%, 1%, and 2% (m/v) solutions of Triton X-100 were prepared by dilution of a more concentrated 10% (m/v) solution of Triton X-100.

Two different aqueous stock solutions with different concentrations of different metals were used:

Standard 1 (500 mg L^{-1} Al; 100 mg L^{-1} As, Be, Cr, Co, Cu, Fe, Pb, Mn, Ni, and Zn; 250 mg L^{-1} V; 25 mg L^{-1} Cd; and 5 mg L^{-1} Hg) (Baker Instra-analyzed, J.T. Baker, Phillipsburg, NJ, USA);

Standard 2 (500 mg L^{-1} Ba, Ca, Mo, and Na; 100 mg L^{-1} Mg; 100 mg L^{-1} K) (Baker Instra-analyzed, J.T. Baker, Phillipsburg, NJ, USA).

A monoelement solution of Sn (1000 mg L^{-1}, Atomic Absorption Standard, Fluka AG, Buchs, Switzerland) was also used. 1 mg L^{-1} intermediate solutions were prepared from the Sn standard and Standards 1 and 2.

The different multielement solutions used in the study were prepared with these intermediate solutions. A multielement solution prepared by dilution of the 1-mg L^{-1} intermediate solution containing 10 µg L^{-1} of Ba, Bi, Cu, Mn, Pb, Sn and 50 µg L^{-1} of Al in 2% Triton X-100 was used to optimize the FIA system employed for the on-line formation of emulsions and Rh (10 µg L^{-1}) as the internal standard.

A monoelement solution of Rh (1000 mg L^{-1}) was prepared from $(NH_4)_3RhCl_6$ (Puriss, Fluka AG, Buchs, Switzerland) and used as the internal standard. A 1-mg L^{-1} intermediate solution was prepared from this standard solution of Rh.

Several standard solutions were prepared for the quantitative analysis with increasing concentrations of Al, Ba, Bi, Cu, Mn, Pb, and Sn in 2% Triton X-100. Rh (10 µg L^{-1}) was added as the internal standard for the calibration.

Several types of virgin olive oil from different regions of Spain were analyzed. These oils are listed in Table II together with their geographical origins (see "Code" column in Table II and Figure 2), origin denominations (O.D), and the type of olive used for the oil. Most of the oils analyzed were not processed from a single type of olive, but the main variety in each oil was around 80%.

The different areas from which the oils studied chemometrically came from are given in Figure 2 (A, B, C, D, and E refer to the geographical origin of oils in Table II). It should be noted that areas A, B, and C are in close proximity, D and E come from areas with very similar climates, and A and B come from the same type of olive (Empeltre).

TABLE II
Different Virgin Olive Oil Samples

Area of Origin	Product Name	Main Type of Olive Used	Bottling Material	Code[b]
Campo de Borja	Oliambel	Empeltre	Glass	1A
	Sigmun Naturalis		Glass	2A
Bajo Aragón[a]	Arboleda[a]	Empeltre	Glass	3B
	Arboleda [a]		PVC	4B
	Reales Almazaras[a]		Glass	5B
	Vadueña[a]		Glass	6B
	Alcober[a]		Glass	7B
Cataluña	Sensat	Arbequina	Glass	8C
	Oleastrunverd[a]		Glass	9C
Baena[a]	Germán Baena[a]	Picudo	Glass	10D
	Nª Sra. De Guadalupe[a]		Glass	11D
	Valle de las Flores[a]		Glass	12D
Plasencia	La Chinata	Manzanilla	Tin Plate	13E

[a]With origin denomination.
[b]Code: Letters refer to area of origin (see Figure 2).

Fig. 2. Map of Spain showing the different areas of origin of the virgin olive oil samples.

Recovery studies were carried out by adding spikes to the oils containing 10 µg L^{-1} of Ba, Bi, Cu, Mn, Pb, and Sn; and 50 µg L^{-1} of Al. Rh (10 µg L^{-1}) was used as the internal standard for the analysis of the different spiked and unspiked samples.

The salts used for determining Na, Ca and Mg by FAAS were as follows: LiCl (Pro Analysis, Merck, Darmstadt, Germany), NaCl, $MgCl_2$,

TABLE III
FIA Program for On-line Emulsion Formation and ICP-MS or FAAS Determination

Step	Time (s)	Vo	Ve	Vp	Ub	P	Flow Rate (mL min^{-1})	Trg	Comments
1	0–8	1	1	1	0	1	28.2	0	Reactor cleaning
2	8–20	0	0	1	0	1	6[a], 22.2[b]	0	Oil and emuls. injection
3	20–210	0	0	0	1	1	3	1	ICP-MS or FAAS measurement
4	210–220	1	1	1	0	1	28.2	0	Cleaning reactor
5	220–221	1	1	1	0	0	28.2	0	Stand-by

Vo, Ve = Injection Valves for Oil and Emulsifier: 1= Loading, 0= Injection.
Vp= By-pass Valve, 1= Waste, 0= ICP-MS.
Ub= Ultrasonic Bath, 1= ON, 0= OFF.
P= Peristaltic Pump, 1= ON, 0= OFF.
Trg= Trigger sends measuring order to ICP-MS.

[a]Oil Flow Rate; [b]Emulsifier Flow Rate.

and $CaCl_2$ (Suprapur®, Merck, Darmstadt, Germany). Individual solutions containing 1000 mg L^{-1} of Na, Ca, and Mg, and 2000 mg L^{-1} of Li were prepared. Intermediate solutions of 50 mg L^{-1} were prepared for each metal from the solutions of 1000 mg L^{-1} of Na, Ca, and Mg. Several standard solutions were prepared with increasing concentrations of Na, Ca, and Mg in 2% Triton X-100 (0.1-5 mg L^{-1}) for the quantitative analysis. Li (400 mg L^{-1}) was added as the spectral buffer to the blank, standard solutions, and spiked and unspiked samples for the determination of Na by atomic emission spectrometry. Recovery studies were also carried out by adding spikes to the oils containing 0.5 mg L^{-1} of Na, 1 mg L^{-1} of Ca, and 0.5 mg L^{-1} of Mg.

Aqueous solutions were prepared using ultrapure water, with a resistivity of 18.2 MΩ obtained from a Milli-Q™ water purification system (Millipore, Saint Quentis Yvelines, France). All polyethylene material was decontaminated with nitric acid (10% v/v) for at least 72 h, rinsed with ultrapure water, and dried.

Procedure

The on-line emulsion formation process by FIA-merging zones is completely computer–controlled and has been described previously (4). The program used is listed in Table III. The emulsion formation process was as follows: a quantity of the olive oil sample (0.38 g) was introduced through valve V1 using a 1% Triton X-100 solution as the carrier. The emulsifier solution, a 2% Triton X-100 solution needed for emulsion formation, was introduced by V2. This solution contains the internal standard (Rh, 10 µg L^{-1}) for the analysis of the olive oil samples or internal standard and standards for the calibration or recovery studies. A 0.1% Triton X-100 solution was used as the carrier in V2. In step 2 of the program, injection from the two valves was simultaneous so that mixing of the olive oil sample (or blank solution) and 2% Triton X-100 emulsifier (with or without standards) was synchronized in Y. Formation of the emulsion in the reactor R takes place during step 4 in which the ultrasonic agitator is connected automatically. The emulsion formed is then introduced into the ICP-MS or FAAS through V3 for multi-element determination. The final step of the program is cleaning of the reactor.

RESULTS AND DISCUSSION

The previously described procedure with on-line emulsion formation by flow injection was used for the multielement determination in different oil samples. As stated previously, Al, Ba, Bi, Cu, Mn, Pb, and Sn were determined simultaneously using an ICP-MS. Na was determined by FAES (Flame Atomic Emission Spectrometry) and Ca and Mg by FAAS, although these two elements could not be determined in some samples because their concentration was below the detection limits. Three different analyses were carried out for all samples on different days with four replicates of each sample per day. As can be seen in Table II, there were 13 samples, and the results obtained for the determination of the different metals in the samples are gathered in Table IV. The detection limits (DL) (based on three times the standard deviation of the blank signals, n=10) and the recovery values for the different olive oils from a single analysis are also given in Table IV. It can be observed that the recoveries were quite satisfactory, in most cases around 100%. Moreover, the concentrations found for most of the elements are in agreement with those in the literature for olive oil samples (19–21).

TABLE IV
Mean Metal Concentration of Triplicate Analyses (ng g^{-1}) in Olive Oil Samples, Recoveries (%), and Detection Limits (ng g^{-1}), Together With Descriptive Statistics: Mean, Standard Deviation (SD), Minimum and Maximum Value

	Al	Mn	Cu	Pb	Sn	Bi	Ba	Na[a]	Ca[a]	Mg[a]
1A	21.08 ± 1.70	14.39 ± 0.17	1.47 ± 0.61	1.22 ± 0.03	0.57± 0.07	0.25 ± 0.10	1.76 ± 0.02			
[b]	81.6	121	100.3	125	80.1	98	107.1			
2A	19.09 ± 1.43	8.10 ± 0.04	5.48 ± 0.40	9.35 ± 0.23	< DL	< DL	1.44 ± 0.47			
[b]	90.5	97.7	101.9	119.1	108	141	98.8			
3B	25.21 ± 1.02	3.77 ± 0.10	2.31 ± 0.11	0.90 ± 0.22	0.53 ± 0.20	0.16 ± 0.08	0.50 ± 0.09			
[b]	99.3	113.4	108.3	120.2	108.7	104.6	100.4			
4B	30.37 ± 1.66	6.40 ± 0.42	2.50 ± 0.27	1.79 ± 0.26	< DL	0.14 ± 0.07	0.43 ± 0.19			
[b]	93	98.7	108	119.2	98.1	102.7	94.6			
5B	21.23 ± 0.86	3.54 ± 0.62	1.39 ± 0.27	1.03 ± 0.46	0.13 ± 0.07	< DL	0.37 ± 0.12			
[b]	114	120	127	109	92.5	125	82.8			
6B	23.91 ± 2.85	3.33 ± 0.50	2.26 ± 0.29	2.12 ± 0.32	0.20 ± 0.10	0.39 ± 0.15	0.57 ± 0.004			
[b]	95.7	82.4	106.8	113.1	90.3	113	93.8			
7B	18.10 ± 1.71	3.96 ± 1.12	2.45 ± 1.33	0.23 ± 0.036	< DL	< DL	0.28 ± 0.07			
[b]	114	120	127	109	92.5	125	82.8			
8C	22.38 ± 1.25	19.98 ± 0.27	1.80 ± 0.10	1.71 ± 0.12	0.67 ± 0.03	< DL	5.82 ± 0.14	910 ± 32	560 ± 8	120 ± 16
[b]	72.5	70.9	89.1	116.3	71.3	89	107	62.5	91.3	104.5
9C	9.75 ± 0.70	1.97 ± 0.07	1.79 ± 0.02	0.64 ± 0.02	0.53 ± 0.03	0.45 ± 0.20	0.61 ± 0.16			
[b]	88.7	101.4	105.0	105.1	76.8	86.3	102			
10D	39.56 ± 2.46	50.25 ± 1.36	5.59 ± 0.12	6.22 ± 0.25	0.85 ± 0.06	0.48 ± 0.20	27.16 ± 0.49	1750 ± 99	2970 ± 276	350 ± 23
[b]	107.3	116	108.2	118	97.7	139	157	88.4	89	91.6
11D	43.61 ± 4.73	16.24 ± 1.13	1.83 ± 0.15	0.83 ± 0.13	0.34 ± 0.038	0.45 ± 0.14	5.55 ± 0.13			
[b]	117	106	118	98	74.3	130	87			
12D	54.34 ± 0.21	25.93 ± 0.01	3.28 ± 0.26	1.64 ± 0.062	< DL	1.48 ± 0.50	9.85 ± 0.24	470 ± 31	1500 ± 71	295 ± 23
[b]	124	129	114	72.1	85	119	83.1	97.5	83.3	73.5
13E	47.40 ± 3.19	38.63 ± 1.41	4.66 ± 1.50	0.66 ± 0.21	4.66 ± 0.22	< DL	4.99 ± 0.41		841 ± 79	173 ± 9
[b]	108	161	122	99.1	80.8	127	83.8		117	90.3
DL	5.31	0.98	1.09	0.33	0.44	0.02	0.15			
Median	29.05	16.73	2.83	2.16	0.71	0.3	4.56	299	573	78
S.D.	13.14	19.65	1.47	2.64	1.19	0.4	7.42	501	822	120
Minimum Value	9.75	1.97	1.39	0.23	0.22	0.01	0.28	75	175	9.5
Maximum Value	54.34	71.25	5.59	9.35	4.6	1.48	27.16	1750	2972	349

[a]If there is no value for Na, Ca, and Mg, it is because the metal content in the sample is below the detection limit.
[b]Recoveries (%) obtained for each virgin olive sample.
DL: Detection limits (ng g^{-1}).

The results for all the metals vary considerably, indicating significant differences in the mineral composition of the different oil samples. Al and Mn and, in particular, Na, Ca, and Mg have much higher standard deviations than the other metals. Preprocessing (autoscaling) of the original matrix data was carried out for the subsequent statistical treatment so that all the analytes would have the same variance.

Correlation Study

The correlation matrix (see Table V) shows the correlation between pairs of variables (metals). The elements of the matrix are the Statistic named as Correlation Pearson coefficient (22,23). The matrix elements, which are close to the unit, show a correlation between pairs of variables. The correlated variables are due to the same cause or source of variance, that is, the same fundamental variable. Variables that display similarities (as their Pearson coefficients are close to 1) are shown in bold type. A close relationship can be seen between the alkaline earth metals (Ba, Ca, and Mg) and those with Mn. Na (alkaline) differs significantly from the other metals.

The mean values for Mn and Ba for olive oils from the Campo de Borja, Bajo Aragón, Catalonia, and Baena areas are plotted in Figure 3 in order to determine whether these elements have a differentiating capacity. It should be noted that the greater the differentiating capacity, the farther apart the points representing each type of oil should be. The oils of the Baena origin denomination clearly differ from the rest. The Mn and Ba variables are therefore suitable for differentiating oils from the north vs. those from the south, the differences being basically the climatic conditions, the soil, and the different types of olive used.

Principal Components Analysis

Principal Component Analysis (PCA) (24) was used to find groupings of variables, that is, to find out which manifest variables (metals) are interrelated and related to a specific principal component (latent variable) which is associated with a fundamental variable (for instance, climate, type of soil, etc.). Each principal component provides information about fundamental variables, which causes the buildup of one metal or another. Another objective was to determine whether the metallic content can be used to establish differences between the oils studied.

PCA (24) transforms the original data matrix $X_{n \times m}$ (Table IV) into a product of two matrices, one of which contains information about the samples (Scores matrix $S_{n \times m}$), and the other about manifest variables (metals) (Loadings matrix $L_{m \times m}$); "n" is the number of samples and "m" is the number of variables.

TABLE V
Correlation Matrix (Pearson Coefficients)

	Al	Mn	Cu	Pb	Sn	Bi	Ba	Na	Ca	Mg
Al	1.00									
Mn	0.64	1.00								
Cu	0.46	0.67	1.00							
Pb	0.04	0.36	0.76	1.00						
Sn	0.42	0.46	0.41	-0.13	1.00					
Bi	0.59	0.29	0.16	0.02	-0.17	1.00				
Ba	0.55	**0.94**	0.60	0.42	0.15	0.41	1.00			
Na	0.31	**0.83**	0.47	0.41	0.02	0.26	**0.92**	1.00		
Ca	0.58	**0.93**	0.63	0.39	0.22	0.47	**0.97**	**0.89**	1.00	
Mg	0.70	**0.89**	0.61	0.27	0.33	0.58	**0.88**	**0.80**	**0.95**	1.00

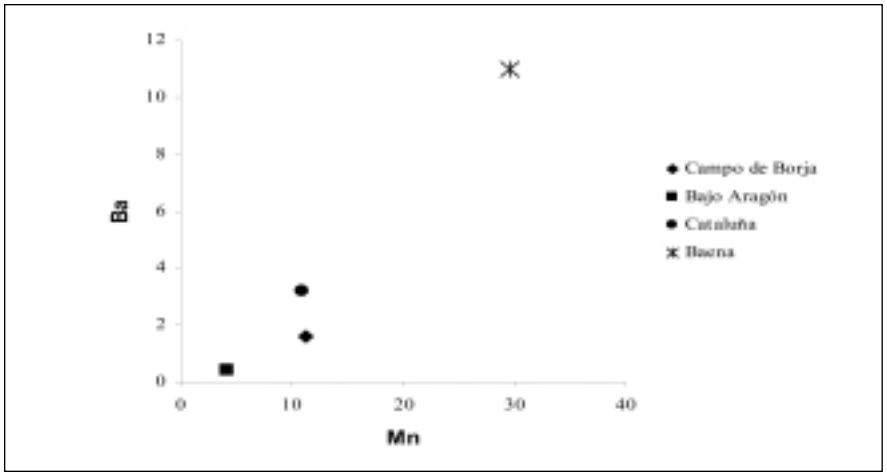

Fig. 3. Correlation plot between Mn and Ba.

The PCA results are graphically displayed using two plots. The Score plot shows the relationships between the samples (olive oil samples). Each point whose coordinates are the scores on the principal components (axes on the plot) corresponds to a sample. Groups or clusters of similar samples can be detected visually. The Loading plot shows the similarities (correlations) between the variables. Each point, whose coordinates are the loadings on the principal components, corresponds to a variable (metal). The variables with small factor loadings on a specific principal component have little influence. The most important variables on a principal component have high factor loadings. Two variables are strongly correlated when their factor loadings are similar to each other, that is, when there is a small distance between them on the Loading plot. The score and loading plots are complementary and give the most valuable information about both the samples and the variables when studied together. When a sample has a position in the score plot that is corresponding to the position of the variable in the loading plot, it means that the sample has a high value for the variable.

The maximum amount of variance contained in the data is concentrated in the first component (PC1). The second principal component, orthogonal to the first, contains the next largest possible variation (PC2).

As previously mentioned, the matrix data required preprocessing by hole filling for unknown data when the concentrations were below the detection limits. It is assumed that the values of the holes are half the detection limit (22). Scaling of the original data was also carried out; this will make the result independent on the scale of the original variables. PCA was subsequently applied to the autoscaled data using the UNSCRAMBLER program.

The first component accounts for 58% of the total variability and the second one represents 16% of the total variance. PC1 and PC2 explain the largest possible variation in the data and therefore PC1 and PC2 account for most of the information.

When outliers are found in a PC plot, one should first identify and then eliminate the outliers and carry out the PCA again. It was assumed that there were no anomalous samples as the validity of the method has been previously shown (4), the recovery values were quite satisfactory, and anomalous metallic concentration was due to randomized sources of contamination. The criterion used was selection of the minimum number of variables needed for a correct classification. The variables that do not provide sufficient information (they have small factor loadings in any principal component) were excluded from the chemometric study.

The factor loadings for all the variables in both components are given in Table VI. Ba, Mn, Ca, Mg,

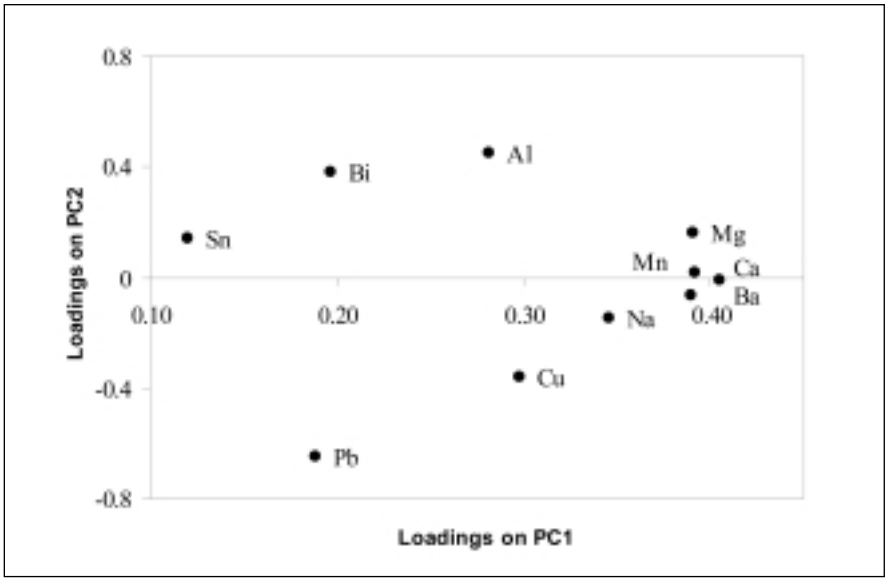

Fig. 4. Loadings plot (10 variables).

TABLE VI
Factor Loadings of Principal Components

Principal Component	Variable									
	Al	Mn	Cu	Pb	Sn	Bi	Ba	Na	Ca	Mg
1	0.282	0.392	0.298	0.189	0.120	0.197	0.39	0.346	0.405	0.391
2	0.449	0.017	–0.363	–0.647	0.139	0.381	–0.068	–0.147	–0.008	0.164

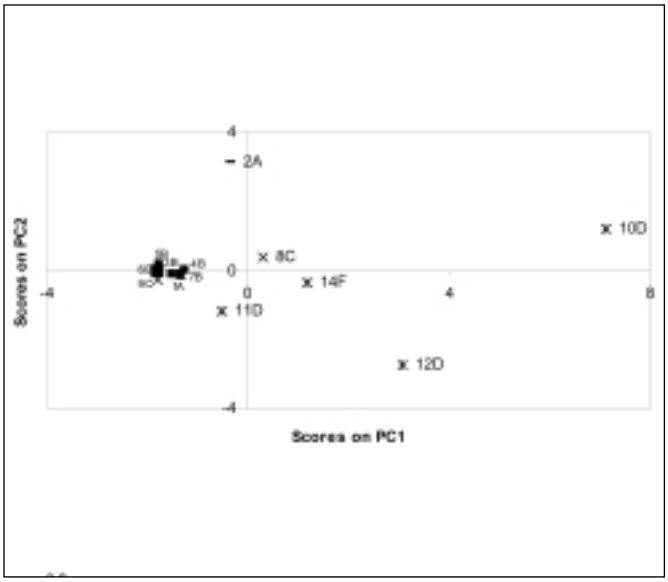

Fig. 5a. Scores plot (9 variables).

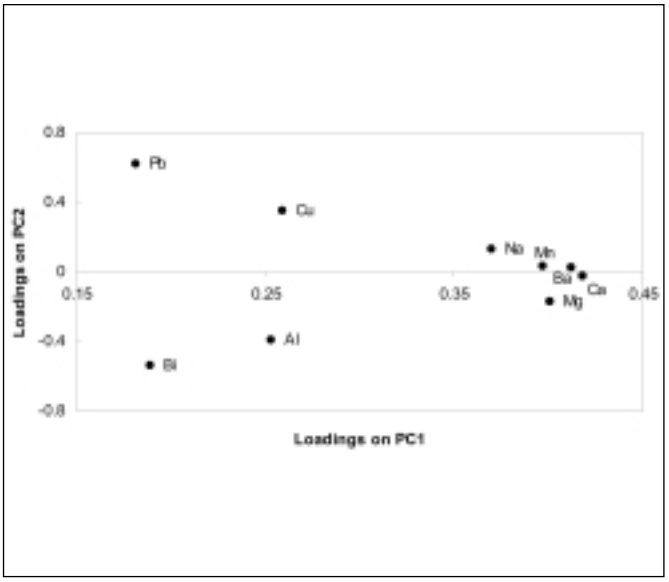

Fig. 5b. Loadings plot (9 variables).

and Na are the predominant elements in the first component as they have the highest factor loadings. Moreover, they are correlated to each other because they have similar factor loadings. Sn does not seem to be important in any principal component as this element is the closest to the origin in the loading plot (see Figure 4) and it has no influence on any other component. It was therefore excluded on account of its poor differentiating capacity with all the variables. When the scores and loadings were plotted for the remaining variables (Figures 5a and 5b), no clearly defined clusters were observed in the scores plot. When comparing the two plots (Figures 5a and 5b), it can be seen that Pb (high loading value in the second component, small value in the first) coincides with sample 2A (high score value in the second component, small value in the first). This sample (2A) is Sigmun Naturalis, and it can be seen in Table IV that it has an abnormally high Pb concentration which could be due to atmospheric contamination, fertilizers, or herbicides used. The origin of Pb is randomized and hence avoids the clusters between samples.

A comparison of the scores and loadings plots after repetition of the PCA without Pb shows that Bi coincides with sample 12D (Valle de las Flores). As occurred with Pb, the buildup of Bi in the sample is abnormal (see Table IV) and has an adverse effect on cluster formation. Bi was therefore excluded as a variable. The scores and loadings plots after exclusion of Sn, Pb, and Bi can be seen in Figures 6a and 6b, respectively.

It can be observed in the loadings plot that Al is the principal element in component 2. PC2 depends on Al as this element has a high loading value in this principal component, while Mg, Mn, Ca, and Ba are correlated because the distance between them is very small. Moreover, these elements predominate in component 1 as the loading values are very high. Cu does not contribute in either compound since its factor loadings in PC1 and PC2 are the smallest. Na, however, has a significant contribution in both.

Judging from the initial data (Table IV), Na, Mg, and Ca are present at very high concentrations in the oils from the south but are below detection limits in the others. These elements were therefore excluded as they provide very little information about the oils from the other areas studied. When the study was repeated with the remaining variables (Al, Mn, Ba, and Cu), two clusters or groupings disappeared. It was observed that Cu coincides with sample 2A (*Sigmun Naturalis*) which has a much higher Cu concentration than the other samples from similar geographical origins. Elements were therefore excluded not only because of the type of soil, type of olive or climate, but above all on sources of contamination which possibly caused the abnormal accumulation of these elements in certain samples.

The scores and loadings graphs (Figures 7a and 7b) were plotted with three variables, after exclusion of the variables that provided little information. Three clearly defined clusters can be observed.

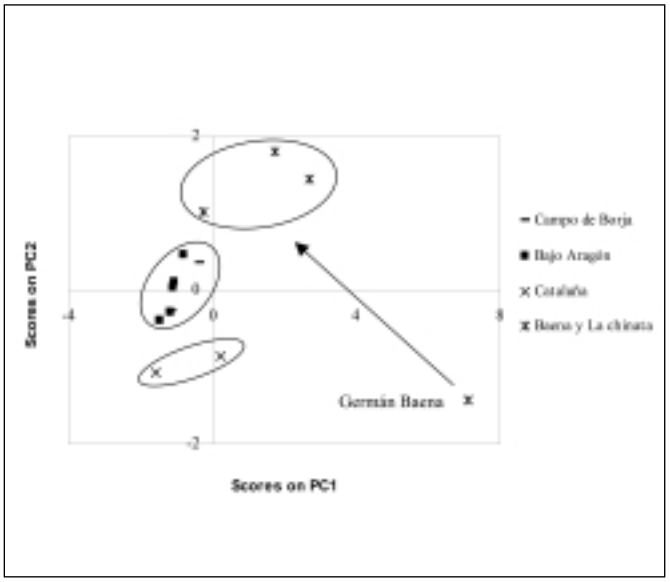

Fig. 6a. Scores plot (7 variables).

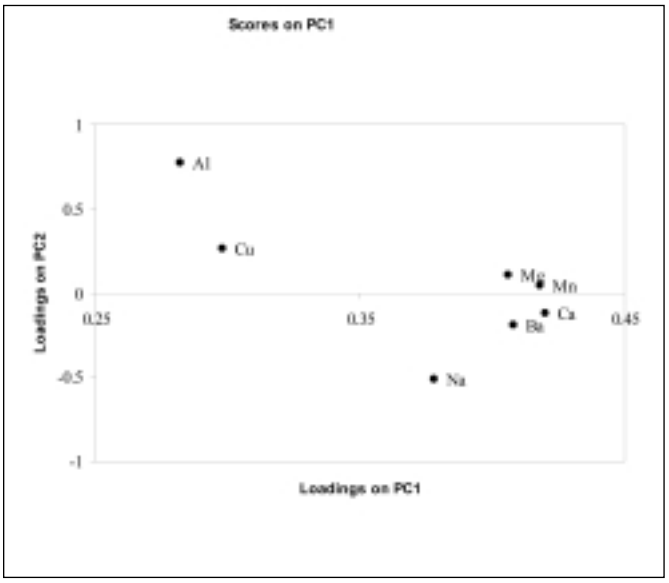

Fig. 6b. Loadings plot (7 variables).

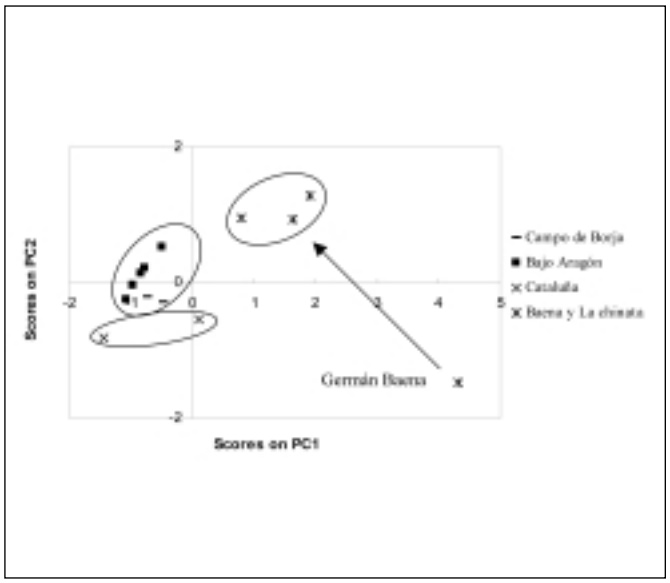

Fig. 7a. Scores plot (3 variables).

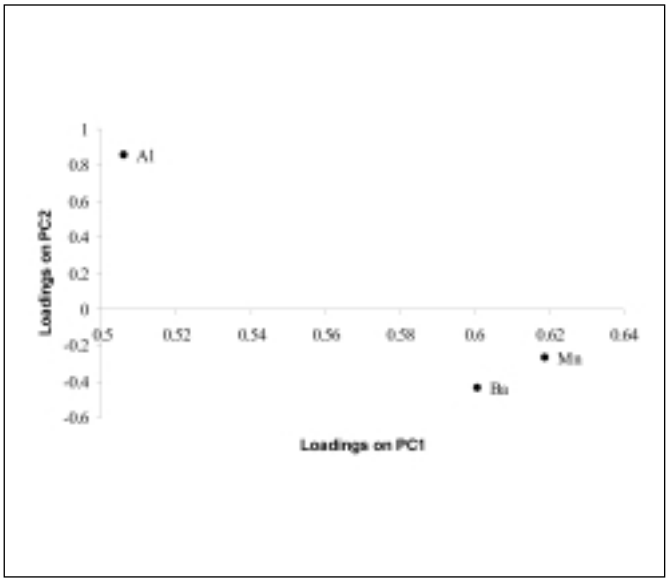

Fig. 7b. Loadings plot (3 variables).

The group including the Baena and La Chinata oils are farther apart from the rest after exclusion of Cu and are located in the positive quadrant. As occurred previously with the oils from Bajo Aragón and Campo de Borja, they cannot be differentiated, which could be due to their geographical proximity and because the olive variety is the same (Empeltre). These oils differ from the oils from Catalonia because, if not geographically far apart, the olive variety cultivated is different. The La Chinata oil from Plasencia (Cáceres) is in the group of oils belonging to the Baena origin denomination and, although the olive used is different, the climate and soil are very similar. The Germán Baena oil, on the other hand, is located at a distance from the other samples. When compared with the other samples, Germán Baena has very high Mn and Ba concentrations. However, it was not considered an anomalous

sample as its exclusion had a negative effect on the appearance of clusters.

The loadings from the PCA showed that Al is the principal element in the second component, while Mn and Ba predominate in the first, although Al is also present. Mn and Ba are interrelated and their presence in the oil is for the same reason. In comparing the scores and loadings plots (Figures 7a and 7b), it can be concluded that the oils from Bajo Aragón and Campo de Borja can only really be differentiated from those from Catalonia on account of Al, which predominates in the second component. The Baena and La Chinata groups differ from most of the oils from the north not only with regard to Al, but Mn and Ba as well. The oils from the north (Bajo Aragón, Campo de Borja, and Catalonia) come from areas in close proximity and it can, therefore, be assumed that the type of soil has little influence. Consequently, it is impossible to differentiate between Bajo Aragón and Campo de Borja on the basis of their geographical proximity, climate, and type of olive (Empeltre in both cases). The Catalonian oils can be differentiated, but given the geographical proximity, this probably has nothing to do with the type of soil. As far as climatic conditions are concerned, temperature and rainfall may have an influence on the mineral composition of the olives. However, the controlling bodies of the origin denominations studied define their respective climates as warm continental, with cold winters and hot summers and a similar annual rainfall. The main difference lies in the type of olive used: Empeltre in Bajo Aragón and Arbequina in Catalonia.

The Baena oils differ from those from the north of Spain on account of the two principal components and, consequently, the Al, Mn, and Ba variables. The olive variety is not the same as those predominantly used in the north, which marks the first principal component (Al) as the difference. Moreover, the Baena oils differ in the second principal component where Mn and Ba predominate. Unlike the oils from the north, the second component in the Baena olive oil is related to the type of soil. In other studies carried out with different foodstuffs, Herrero et al. (14) showed that Mn, Ca, Mg, Rb, and Li are related to the type of soil and make it possible to differentiate between potatoes from Galicia (Spain) and potatoes from other countries. According to M.J. Latorre (15), Mn is a geographical indicator for differentiating between honeys and wines from Galicia and products from other geographical origins. It should be noted that although the Germán Baena sample is different from the samples from the north, it cannot be included in the Baena group as the concentrations of Mn and Ba are significantly higher, possibly because the process leads to an accumulation of Mn and Ba in the sample.

CONCLUSION

The determination of metals in different virgin olive oil samples from different areas and origin denominations was carried out using ICP-MS by on-line emulsion formation with a view to their possible classification. The statistical treatment of the data was performed applying Principal Component Analysis, and the metals with a differentiating capacity (Al, Mn, and Ba) were identified. The fact that some metals (Sn, Pb, Bi, and Cu) do not permit classification or produce anomalous samples could be due to contamination or the type of process involved.

Al, Mn, and Ba permitted differentiation between samples from Bajo Aragón and Catalonia and those from Baena, but were not sufficiently accurate to differentiate them from the Campo de Borja samples owing to the geographical proximity and the fact that the same olive was used. The Al content appears to depend, above all, on the olive variety. This theory, however, has not been proven; there is no mention in the literature about how the genetics of the olive tree influences the Al concentration in the olive. In fact, the Mn and Ba concentrations depend basically on the type of soil and permit differentiation between oils from the north (Campo de Borja, Catalonia, and Bajo Aragón) and oils from the south of Spain (Baena and La Chinata). The relationship between Mn and alkaline earth metals and the soil has been proven and used to differentiate between foodstuffs of different geographical origin. It is suggested that a future study focus on whether genetics has an influence on the Al concentration by analyzing different types of olives grown in the same soil.

Na, Ca, and Mg make it possible to distinguish between oils from the south of Spain (Baena and La Chinata) and oils from the north (Bajo Aragón, Campo de Borja, and Catalonia) but provide no information concerning the other oils. A more sensitive determination technique would be required for Na, Ca, and Mg since most of the samples available for this study were below detection limits when FAAS or FAES was used.

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