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Optimized pretreatment for accurate determination of elemental composition of extra virgin olive oil (EVOO) using ICP-OES and ICP-MS: A comprehensive study

Paolo Inaudi [a,*], Ilenia Certomà [b], Mery Malandrino [c], Laura Favilli [a], Riccardo Cecire [c], Stefano Bertinetti [c], Ornella Abollino [a], Agnese Giacomino [a]

[a] Department of Drug Science and Technology, University of Torino, Torino 10125, Italy
[b] Regione Piemonte, Environment, Energy and Territory Directorate, Torino, Italy
[c] Department of Chemistry, University of Torino, Torino 10125, Italy

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ABSTRACT

Precisely identifying trace elements within vegetable edible oils remains a significant analytical problem due to their low concentrations and the complex composition of the matrixes. The objective of this research was to develop an analytical approach for quantifying Al, Be, Co, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Sb, Se, V, Zn, and REEs using inductively coupled plasma optical emission spectroscopy (ICP-OES) and high resolution ICP mass spectrometry (HR-ICP-MS), according to their concentration, in 13 Italian extra virgin olive oils (EVOOs). To determine the most efficient sample preparation technique, different type of pretreatment were tested, and validated by testing a certified reference material (SRM NIST 1573a). The sample manipulation involved the following steps: i. agitation for 120 min; ii. sonication for 10 min; iii. mineralization with 4 mL of HNO_3 and 2 mL of H_2O_2 for 240 min at room temperature. Principal component analysis (PCA) was conducted to assess whether oils from different regions would separate based on their inorganic content.

1. Introduction

The elemental composition of foods holds significance in both nutritional and toxicological contexts, serving as a vital quality parameter (Abbatangelo et al., 2019; Acar, 2012; Inaudi et al., 2020). This is particularly true for extra-virgin olive oil (EVOO), where trace element concentrations play a crucial role in assessing its quality, storable period, and freshness (Wali et al., 2021). Derived from the fruits of the olive tree (*Olea Europea. L*), olive oil stands as a powerful source of vitamins and nutrients, making it a cornerstone of the Mediterranean diet, renowned not only for its distinct flavour but also for its potential health benefits (Bajoub et al., 2017; Bakkali et al., 2012).

In the context of olive oil quality assurance, the International Olive Council (IOC) has set forth stringent criteria, including maximum residue levels (MRL) for As, Cu, Pb (0.1 mg kg^{-1}), and Fe (3 mg kg^{-1}) in both olive oils and olive-pomace oils (International Olive Council IOC, 2019, n.d.). Additionally, regulatory standards, such as Codex Stan 33–1981, have established MRLs for Cu and Fe in various vegetable oils, ranging from 0.1 to 5 mg kg^{-1} (Codex Stan 33-1981 2021, n.d.). This emphasis on quality control aligns with the recent surge in interest regarding element determination in EVOO samples, particularly for purposes of geographical traceability and authentication (Beltrán et al., 2015; Cecchi and Alfei, 2013; Giacomino et al., 2022). Against the backdrop of a global olive oil industry which saw production approaching 3 million tons between 2021 and 2022, with Europe contributing nearly 2 million tons (t) (including Spain, 1400 t; Italy, 329 t; Greece, 232 t; and Portugal, 206 t) and non-European countries contributing approximately 1 million tons (such as Tunisia, 240 t; Turkey, 235 t; Morocco, 200 t; Algeria, 91 t; Egypt, 20 t; and Argentina, 3 t), the need for robust quality assessment measures becomes ever more pressing (Chiaudani et al., 2023; International Olive Oil Council, n.d.).

Despite being celebrated for its positive health impacts, olive oil, like other vegetable oils, can harbour pollutants, including toxic elements (Cabrera-Vique et al., 2012). These contaminants may stem from soil composition, environmental pollution, or contamination during production and storage processes. Thus, understanding and monitoring trace element levels in olive oil is imperative for ensuring its safety and nutritional integrity. On the other hand, among the factors influencing

* Corresponding author.
E-mail address: paolo.inaudi@unito.it (P. Inaudi).

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its quality and nutritional profile, the elemental composition and presence of rare earth elements (REEs) play significant roles, influencing both flavour and health attributes (Chiaudani et al., 2023; Gu et al., 2018).

In recent years, advancements in analytical techniques have enabled precise characterization of the elemental composition of EVOO (Jiang et al., 2015; Ma et al., 2025; Maléchaux et al., 2020; Rotondo et al., 2024).

Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) have emerged as powerful tools for elemental analysis due to their sensitivity and ability to detect trace elements (Angioni et al., 2006; Astolfi et al., 2021; Chiaudani et al., 2023; Cindric et al., 2007; Gaggero et al., 2020; Giacomino et al., 2022; Llorent-Martínez et al., 2011; Zeiner et al., 2005). These techniques offer insights into the concentration of essential minerals and potentially harmful elements, thereby informing quality control measures and ensuring consumer safety. Moreover, the direct connection between the soil composition and the elemental content in EVOO suggests the use of the element's concentrations for the origin recognition of the products, useful in the fight against food frauds.

However, the accurate determination of elemental content in EVOO is contingent upon effective sample pretreatment procedures. Various factors such as matrix interference and sample complexity necessitate meticulous optimization of pretreatment conditions to minimize analytical errors and maximize sensitivity. In this study, we tested different procedure to identify the optimal pretreatment conditions for the analysis of EVOO samples.

Furthermore, the vast datasets generated by ICP-OES and ICP-MS demand sophisticated data analysis techniques to extract meaningful insights. Chemometric methods offer a robust framework for multivariate analysis, facilitating the interpretation of complex data matrices and uncovering hidden patterns within the elemental profiles of EVOO samples. By employing chemometric processing, we aim to unravel the intricate relationships between elemental composition, geographical origin, and processing methods, shedding light on the factors influencing the quality and authenticity of Italian EVOO.

In this paper, we present the results of our investigation, encompassing the optimization of pretreatment conditions for elemental analysis of EVOO using ICP-OES and ICP-MS, followed by chemometric treatment of the acquired data. Our findings contribute to the comprehensive understanding of the elemental composition and REE content of Italian EVOO, laying the groundwork for future studies aimed at enhancing its quality, nutritional value, and authenticity.

2. Material and methods

2.1. Instruments and reagents

Dissolution of samples took place in vessels made of polytetrafluoroethylene (PTFE) using the Milestone MLS-1200 Mega microwave laboratory unit (Milestone, Sorisole, Italy). Sample analyses were conducted using an Optima 7000 ICP-OES (Perkin Elmer, Norwalk, CT, USA) and a sector field ICP-MS Element 2 (HR-ICP-MS, Thermo Fisher Scientific, Waltham, Massachusetts, MA, USA). High purity water (HPW) from a Milli-Q apparatus (Millipore, Burlington, MA, USA) was utilized for sample preparation and standard solutions. Analytical grade reagents were employed consistently throughout the experiments, and standard metal solutions were prepared from 1000 mg L^{-1} single element Merck Titrisol stock solutions (Merck, Darmstadt, Germany).

2.2. Samples

2.2.1. Standard reference material

To validate the experimental methodology and evaluate the impact of vegetal matrices on elemental quantification, in absence of recognized standard reference material (SRM) for the elemental quantification in olive oils, we used the SRM 1573a provided by the National Institute of Standards and Technology (NIST), specifically Tomato Leaves, for quality control analysis. The determined elements were Al (598 ± 12 mg kg^{-1}), Ca (50,500 ± 900 mg kg^{-1}), Cd (1.52 ± 0.04 mg kg^{-1}), Co (0.57 ± 0.02 mg kg^{-1}), Cr (1.99 ± 0.06 mg kg^{-1}), Cu (0.57 ± 0.02 mg kg^{-1}), Fe (368 ± 7 mg kg^{-1}), K (27,000 ± 500 mg kg^{-1}), Mn (246 ± 8 mg kg^{-1}), Na (136 ± 4 mg kg^{-1}), Ni (1.59 ± 0.07 mg kg^{-1}), P (2160 ± 40 mg kg^{-1}), and Zn (30.9 ± 0.7 mg kg^{-1}).

2.2.2. EVOO test sample

A commercial extra virgin olive oil was used as test sample (T-EVOO) for the development of the method of analysis of the inorganic component. This EVOO is produced in Imperia (Liguria, North Italy) on local farm which integrates and coordinates all the stages of EVOO production. As such, this oil is unfiltered and cold extracted.

2.2.3. EVOO samples

The EVOO samples analyzed in this work are 13 and come from different Italian regions. The choice to analyze only Italian samples stems from the desire to concur in the efforts to valorise the high quality of the Italian EVOOs. Table 1 shows the codes of the analyzed samples, the type of packaging, and their respective origins. The low number of samples is certainly a limitation for data interpretation; however, this study was designed as an intensive test of sample-pretreatment optimization and, as such, did not permit the evaluation of a large sample set.

2.3. Procedures

2.3.1. T-EVOO sample pretreatment

T-EVOO aliquots of 0.5 g were weighed inside the PTFE vessels (Benincasa et al., 2007). Then, the properly mixture of 65 % w/w nitric acid and 30 % w/w hydrogen peroxide was added. The amount of the two reagents in the extraction solution was tested in two different ratios, as reported in Section 2.3.2. After closing the vessels, they were placed in the microwave mineralizer and subjected to a stepwise heating program: i. 250 W; 1 min, ii. 0 W; 1 min, iii. 250 W; 5 min, iv. 400 W, 5 min, v. 650 W 5 min, vi. Ventilation (0 W), 20 min.

At the end of this program, the vessels were left under a hood to cool for approximately 2 h. Subsequently, they were opened, and the contents were filtered through Whatman 5 filters and transferred into falcon 50 mL test tubes. Finally, each sample was diluted to a volume of 30 mL with HPW. Each extraction conditions were tested in triplicate.

2.3.2. Acid mixture

EVOO has a very high content of organic matter that needs to be mineralized with the appropriate acid mixture for the complete solubilization of the inorganic component of interest. A mixture composed of nitric acid and hydrogen peroxide is commonly used, which, when combined, enhance the oxidizing properties of the two reagents

Table 1
EVOOs samples.

Sample	Packaging	Region of origin
EVOO1	Tin container	Liguria (North West Italy)
EVOO2	Glass bottle container	Liguria (North West Italy)
EVOO3	Glass bottle container	Liguria (North West Italy)
EVOO4	Glass bottle container	Liguria (North West Italy)
EVOO5	Glass bottle container	Veneto (North East Italy)
EVOO6	Glass bottle container	Veneto (North East Italy)
EVOO7	Glass bottle container	Toscana (Center Italy)
EVOO8	Glass bottle container	Sardegna (South Italy)
EVOO9	Glass bottle container	Sardegna (South Italy)
EVOO10	Tin container	Sardegna (South Italy)
EVOO11	Glass bottle container	Puglia (South Italy)
EVOO12	Glass bottle container	Calabria (South Italy)
EVOO13	Glass bottle container	Calabria (South Italy)

(Benincasa et al., 2007). In this work, the HNO_3/H_2O_2 mixture was studied in the following ratios: a. 4 mL of HNO_3/2 mL of H_2O_2; b. 6 mL of HNO_3/1 mL of H_2O_2.

2.3.3. Standard reference material

The selected HNO_3/H_2O_2 mixture was then tested with the SRM NIST 1573a to evaluate the recovery capability of the presented method. 0.5 g of SRM was subjected to the same operational procedure also used for T-EVOO sample.

2.3.4. Evaluation of effectiveness of agitation, sonication and precontact time on the reproducibility of the extraction

To improve the reproducibility of the extraction by MW procedure, the incidence of three additional phases in the oil pretreatment has been studied: agitation, sonication, and a sample reaction time with reagents before microwave treatment. Initially, agitation and sonication were studied separately; subsequently, their combined effect at different times was evaluated through different tests. The initial homogenization trials of the sample were conducted to assess whether the chosen treatments could indeed yield satisfactory results for the intended purpose. Two aliquots of 25 mL of T-EVOO were transferred into two 50 mL polypropylene tubes with screw caps, which were sealed with protective parafilm. One sample (S1) was designated for agitation treatment (2 h), while the second (S2) was placed inside the sonicator (2 h). Afterwards, 0.5 g of each sample were left to react for 12 hours with 3 mL of HNO_3 and 2 mL of H_2O_2. The remaining 1 mL of H_2O_2 was used to transfer the sample solution into the PTFE vessels. Following this procedure, a sample loss was observed, resulting in incomplete transfer into the vessels. Therefore, in subsequent optimization tests, the pre-mineralization reaction was always conducted within the vessels themselves.

2.4. Multivariate statistical analysis

All statistics were executed in Python 3.12 using NumPy 1.26, pandas 2.2, SciPy 1.12, scikit-learn 1.5, factor_analyzer 0.5., and Matplotlib 3.9. The dataset was organised as a rectangular matrix in which each row corresponds to one EVOO sample (n = 13) and each column to a single elemental variable (p = 22) after removing non-numerical identifiers. Values below the limit of quantification (LOQ) were imputed as LOQ / $\sqrt{2}$ (Hornung and Reed, 1990) and all variables were auto-scaled. Sampling suitability was checked with the Kaiser–Meyer–Olkin (KMO) index (Kaiser, 1974) and Bartlett's test of sphericity (Bartlett, 1954); variables with KMO < 0.50 or > 50 % censoring were flagged for sensitivity analysis. Dimensionality reduction was explored by principal component analysis (PCA) retaining ten components; 10 000 non-parametric bootstrap resamples provided 95 % confidence intervals for the variance captured by each PC. All multivariate procedures were repeated after removing low-KMO variables and highly missing values (>50 %) variables to gauge robustness of the conclusions.

3. Results and discussion

The determination of the inorganic component in samples of EVOO was carried out using two instrumental techniques: ICP-OES and ICP-MS. Specifically, the first analytical technique enabled us to determined Al, Be, Co, K, Li, Mg, Na, Sb, Se, V; while Fe, Zn, Ni, Cu, Mn, and rare earth elements were determined with the second one. The limit of detection (LOD=3σblank) and limit of quantification (LOQ=10σblank) for all the analyzed elements are reported in Table S1, while in Table S2 are reported the wavelength and the *m/z* of the isotopes used for the quantification.

3.1. Preliminary test

The sample used for T-EVOO testing was mineralized in triplicate, and the results of element quantification by ICP-OES are illustrated in Table S3.

The results obtained show comparable behaviour of both mixtures (paired *t*-test for mean element concentration and RSD% p-value > 0.05). As observed, the RSD% values obtained are very high; for instance, even for major elements such as Ca, K, and Na, they exceed 15 %. This high variability, that the denotes a significant inhomogeneity of the concentration in the T-EVOO, masked any difference in the performance of the different extraction mixtures. Therefore, the 4:2 HNO_3/H_2O_2 mixture was chosen, as it is also reported in the literature and requires smaller volumes of reagents. In addition, the use of the 4:2 mixture brought satisfactory recovery when applied to the SRM NIST 1573a (Table S4). Finally, the use of the 4:2 mixture allows for clearer final solutions and reduced residual organic material compared to the 6:1 mixture. In addition to the elements listed, the contents of B, Cd, Cr, Mn, Ni, Si, and Sn were also evaluated, but these elements were found to be present in concentrations lower than their respective instrumental detection limits.

As shown in Table S5, the RSD% for most of the elements improved compared to previous cases where the oil was analyzed without pretreatment, indicating the importance of the performed it. Comparing the two types of pretreatments, it can be noted that in some cases, such as for Ca, K, Mg, Na, and P, agitation seems to yield better results than sonication both in terms of RSD% and detected concentrations. Conversely, opposite results regarding RSD% values are obtained for some minor elements such as Al, Ni, and Zn.

Despite the significant number of metals detected, the RSD% values remain notably high, even concerning the major elements. For this reason, to optimize the pretreatment procedure, different sonication, agitation and reaction times were tested.

Observing the bottle of EVOO, a slight residue was noticed at the bottom. The deposit may be due to plant materials from the olives, which do not necessarily compromise the quality of the oil, or to low storage temperatures. In some cases, the presence of plant residues may increase the amount of polyphenols, but also the availability of enzymes capable of triggering fat oxidation processes. However, such processes are generally hindered at storage temperatures around 14–15 °C. From another point of view, a slight deposit at the bottom of the bottle can be evidence of the genuineness of the product and the absence of industrial manipulations or cuts. It was therefore thought that a different quantity of the sought-after inorganic component could remain in that residue and at the bottom of the bottle compared to the overlying liquid phase. For this reason, two sample aliquots was taken from the surface layer and from the bottom zone respectively. The bottle of EVOO was initially kept in a vertical position and then inverted overnight. The comparison between the obtained data is shown in Table 2.

It is possible to observe that for Ca, K, Mg, Na, and P, the

Table 2
Element concentrations and RSD values for EVOO sampled from the surface and the bottom of the bottle.

	Surface aliquot			Bottom aliquot	
	mg kg^{-1}±σ	RSD %		mg kg^{-1}	RSD %
Al	0.76 ± 0.075	9.93	**Al**	0.85 ± 0.10	11.9
Ba	0.12 ± 0.0063	5.11	**Ba**	0.17 ± 0.035	20.1
Ca	8.00 ± 2.20	27.5	**Ca**	22.2 ± 16.1	33.4
Fe	0.44 ± 0.26	57.6	**Fe**	0.51 ± 0.086	17.00
K	1.74 ± 1.10	63.6	**K**	3.62 ± 0.99	27.3
Li	n.d.	-	**Li**	n.d.	-
Mg	1.04 ± 0.42	40.6	**Mg**	3.89 ± 1.59	40.8
Mo	7.77 ± 0.17	2.25	**Mo**	7.92 ± 0.24	3.01
Na	0.58 ± 0.71	77.6	**Na**	4.56 ± 1.02	22.4
P	1.68 ± 0.33	19.4	**P**	2.95 ± 0.17	5.65
Sb	0.081 ± 0.095	116	**Sb**	0.37 ± 0.12	32.0
Se	1.10 ± 0.33	29.7	**Se**	1.73 ± 0.56	32.6
Sr	0.011 ± 0.027	18.9	**Sr**	0.088 ± 0.047	53.3
V	0.0062 ± 0.075	430	**V**	0.0075 ± 0.017	223

concentration is higher in the bottom aliquot than from the surface one. Performing a paired *t*-test on the mean values of the elements, a p-value slightly less than 0.05 was observed, confirming the inhomogeneity of the EVOO product. Additionally, as can be observed, the RSD% values are still relatively high for both aliquots. These observations confirm two issues: i). the necessity to homogenize the sample before the sampling; ii) the importance of reducing variability among results obtained for replicates of the same sample, thus decreasing the RSD% to enhance data repeatability. The optimization of the sample treatment procedure, preliminary to acid digestion with microwave, will be illustrated to reduce variability by optimally homogenizing the sample.

The factors chosen for this optimization work on the sample pretreatment method are: agitation time (10–120–720 min), sonication time (10–120–720 min), and cold reaction time in the vessels (90–240–720 min).

The response is the RSD%, and the goal is to find the best condition that allow to reduce it to better recognize even small differences in the inorganic composition of the EVOOs from different productor regions. All trial experiments obtained from the tests (Table S6) were conducted in the laboratory using the same T-EVOO. The trials were performed randomly. The concentration of elements was determined using the ICP-OES.

In T-EVOO, 27 elements were determined, most of them present in very low concentrations (< 8 mg kg^{-1}). Since it was not feasible to consider the RSD% of all elements as a response, a system had to be developed to provide a unique value. The RSD% values are the result of averaging the percentage standard deviations relative to the concentration of five elements (Be, Ca, K, Mg, Na, Se) for each individual test. The choice was driven by the low concentrations found in the sample: by considering only the elements present in concentrations higher than 0.5 mg kg^{-1}, the influence of possible instrumental fluctuations was avoided, and the results obtained from different experiments could be better compared. In particular, test N9 (agitation:sonication:reaction=120:10:240) has the lowest RSD% of all others. For this reason, and due to the greater number of analytes determined with this procedure, its application to the samples was chosen.

The optimal conditions for the pretreatment of EVOO samples were identified. The procedure performed on the samples thus involved the following steps: i. agitation for 120 min; ii. sonication for 10 min; iii. cold reaction in vessels with 4 mL of HNO_3 and 2 mL of H_2O_2 for 240 min.

3.2. ICP-OES

Table 3 reports the results obtained for the samples of Italian EVOO analyzed using ICP-OES are reported, and represented in Fig. 1.

For all samples, in addition to the elements listed in the table, the determination of As, B, Ba, Cd, Cr, Cu, Mn, Ni, P, Pb, Si, Sn, Sr was also attempted, but their content was not quantified since they were present in concentrations below the detection limit. One essential and one probably essential micronutrient, recorded in low concentrations in almost all samples of EVOO, are Co and V, which probably derive from the natural soil composition. Regarding geogenic elements, Al is also likely derived from the natural soil composition; however, it is a toxic metal for plants. As seen in Table 3, concentrations vary within a narrow range of values. Among the essential and most important elements from a biochemical point of view, K stands out with concentrations above 1 mg kg^{-1} in almost all samples except for EVOO8 and EVOO9, followed by Na with a minimum concentration (0.68 mg kg^{-1}) in EVOO4 and a maximum (1.76 mg kg^{-1}) in EVOO12, and Mg with a maximum concentration recorded as 0.71 mg kg^{-1} in EVOO7. Also, Li and Se, as shown, fall among the elements with concentrations greater than or equal to 1 mg kg^{-1}. Lithium, a non-essential element, is found in traces in numerous plants that absorb it from the environment. Selenium, on the other hand, is a micronutrient naturally present in both animal and plant species. The marked Se enrichment in Sardinian oils aligns with

Table 3
Concentrations of 10 elements in Italian EVOOs obtained by ICP- OES. The results are expressed in mg kg^{-1}.

	Al	Be	Co	K	Li	Mg	Na	Sb	Se	V
EVOO1	0.31 ± 0.15	0.29 ± 0.0032	0.053 ± 0.0082	3.47 ± 0.34	1.11 ± 0.038	0.035 ± 0.0098	0.85 ± 0.14	n.d.	1.66 ± 0.25	0.37 ± 0.039
EVOO2	0.19 ± 0.031	0.28 ± 0.0061	0.079 ± 0.023	2.04 ± 0.51	1.10 ± 0.018	n.d.	0.82 ± 0.025	n.d.	2.78 ± 0.13	0.34 ± 0.011
EVOO3	0.17 ± 0.0049	0.24 ± 0.0015	0.053 ± 0.0047	0.88 ± 0.48	1.07 ± 0.022	0.19 ± 0.22	1.20 ± 0.047	0.32 ± 0.095	1.18 ± 0.12	0.15 ± 0.018
EVOO4	0.15 ± 0.055	0.19 ± 0.011	0.020 ± 0.0021	1.09 ± 0.20	1.05 ± 0.015	n.d.	0.68 ± 0.12	n.d.	1.67 ± 1.28	n.d.
EVOO5	0.18 ± 0.0063	0.15 ± 0.018	0.053 ± 0.021	2.42 ± 0.29	1.07 ± 0.018	0.46 ± 0.058	1.59 ± 0.15	n.d.	0.99 ± 0.13	0.024 ± 0.0026
EVOO6	0.49 ± 0.42	0.25 ± 0.013	0.057 ± 0.0076	2.49 ± 0.17	1.07 ± 0.021	0.36 ± 0.18	1.46 ± 0.098	n.d.	2.73 ± 0.34	0.33 ± 0.022
EVOO7	0.13 ± 0.034	0.17 ± 0.018	0.023 ± 0.00066	1.55 ± 0.10	1.07 ± 0.030	0.71 ± 0.47	1.41 ± 0.15	0.95 ± 0.25	2.93 ± 0.68	0.20 ± 0.14
EVOO8	0.11 ± 0.020	0.19 ± 0.0038	0.041 ± 0.0077	0.54 ± 0.14	1.07 ± 0.014	0.57 ± 0.22	0.93 ± 0.03	0.70 ± 0.01	2.19 ± 0.78	0.18 ± 0.03
EVOO9	0.23 ± 0.021	0.22 ± 0.0061	0.045 ± 0.017	0.26 ± 0.16	1.05 ± 0.036	n.d.	0.86 ± 0.12	0.58 ± 0.10	3.24 ± 0.18	0.21 ± 0.016
EVOO10	0.23 ± 0.013	0.22 ± 0.017	0.054 ± 0.0016	1.04 ± 0.17	1.09 ± 0.015	n.d.	0.83 ± 0.19	0.46 ± 0.0076	2.96 ± 0.81	0.37 ± 0.12
EVOO11	0.097 ± 0.0029	0.24 ± 0.0053	0.065 ± 0.003	1.48 ± 0.51	1.07 ± 0.024	n.d.	1.20 ± 0.036	0.25 ± 0.097	1.48 ± 0.46	0.28 ± 0.072
EVOO12	n.d.	0.24 ± 0.020	0.031 ± 0.011	4.39 ± 0.058	1.06 ± 0.012	n.d.	2.19 ± 0.53	0.15 ± 0.0049	2.18 ± 0.92	0.22 ± 0.0041
EVOO13	0.15 ± 0.025	0.26 ± 0.012	0.063 ± 0.0011	1.85 ± 0.15	1.05 ± 0.019	0.46 ± 0.077	1.41 ± 0.070	0.44 ± 0.0010	2.05 ± 0.035	0.35 ± 0.015
MEAN	0.20	0.23	0.049	1.81	1.07	0.40	1.19	0.48	2.16	0.25
MEDIAN	0.175	0.24	0.053	1.55	1.07	0.46	1.2	0.45	2.18	0.25
RANGE	0.097–0.49	0.15–0.29	0.02–0.079	0.26–4.39	1.05–1.11	0.035–0.71	0.68–2.19	0.15–0.95	0.99–3.24	0.024–0.37

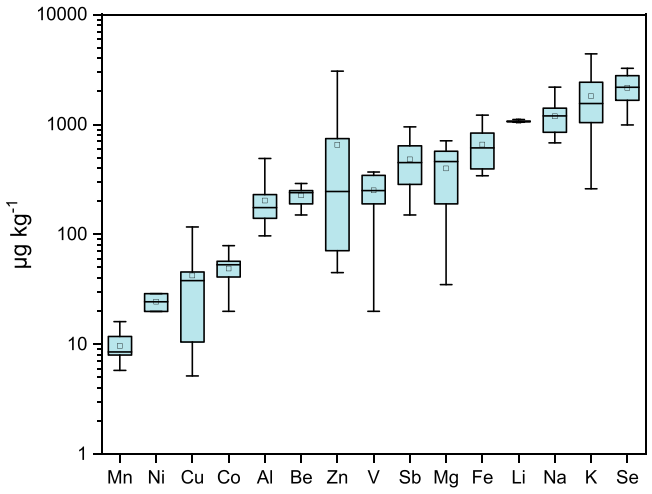

Fig. 1. Distributions of major and minor element concentrations in the EVOO samples.

the island's basaltic–volcanic soils, while the Li- and Al-rich Ligurian samples reflect granite-derived coastal substrates. Finally, the presence of antimony, although in low concentrations in all samples of EVOO, cannot be neglected: antimony is a potentially toxic element with no biochemical relevance that can be found in traces in soils.

3.3. ICP-MS

ICP-MS was employed to determine several metals including Cu, Fe, Mn, Ni, Zn. Attention was focused onto these five trace metals because they have an influence on the flavour and oxidative stability of the oil due to their catalytic effect on the auto-oxidation mechanism. Moreover, the rare earth elements (Ce, Dy, Er, Eu, Gd, Ho, La, Lu, Nd, Pr, Sc, Sm, Tb, Tm, Y, Yb) have been detected due to their correlation with geogenic source. Analyses were conducted at both low and medium resolution. The results of minor elements (µg kg^{-1}) are reported in Table 4 and represent in Fig. 1. Rare Earth element's concentrations are reported in Table S5 for metals (ng kg^{-1}).

Table 4
Concentrations of 5 elements in Italian EVOOs obtained by ICP- MS. The results are expressed in µg kg^{-1}.

	Cu	Fe	Mn	Ni	Zn
EVOO1	n.d.	612 ± 34.1	10.4 ± 5.57	n.d.	n.d.
EVOO2	n.d.	342 ± 23.5	5.80 ± 1.55	n.d.	n.d.
EVOO3	n.d.	395 ± 52.0	6.24 ± 4.91	n.d.	n.d.
EVOO4	n.d.	354 ± 50.7	8.01 ± 4.72	n.d.	n.d.
EVOO5	n.d.	837 ± 27.3	11.8 ± 2.13	n.d.	44.9 ± 22.9
EVOO6	n.d.	364 ± 86.0	8.42 ± 0.55	n.d.	618 ± 31.6
EVOO7	36.8 ± 13.6	788 ± 74.7	13.8 ± 3.70	n.d.	350 ± 9.37
EVOO8	10.5 ± 7.57	514 ± 28.1	8.90 ± 3.88	n.d.	n.d.
EVOO9	45.5 ± 2.47	1100 ± 120	8.53 ± 1.12	n.d.	142 ± 25.3
EVOO10	n.d.	419 ± 14.2	8.33 ± 5.67	n.d.	68.9 ± 37.7
EVOO11	5.17 ± 0.16	733 ± 20.3	6.19 ± 0.52	n.d.	73.2 ± 28.8
EVOO12	117 ± 13.6	1217 ± 95.5	16.1 ± 11.0	19.9 ± 8.28	3055 ± 202
EVOO13	39.2 ± 12.8	858 ± 78.2	13.3 ± 2.96	28.9 ± 6.38	871 ± 215
MEAN	42.3	656	9.68	24.4	653
MEDIAN	38	612	8.53	24.4	246
RANGE	5.17–117	342–1217	5.8–16.1	19.9–28.9	44.9–3055

It was not possible to obtain a satisfactory calibration curve for ^{56}Fe at low resolution due to interference from $^{40}Ar^{16}O$; therefore, this element was determined by considering the isotopes 54 and 56 at medium resolution. Additionally, the concentrations of Ni, Cu, and Zn were derived from the averages of their isotopes: 60 and 62 for nickel, 63 and 65 for copper, and 64, 66, and 68 for zinc. For rare earth elements, only concentrations above the instrumental detection limit are reported.

Iron is a micronutrient utilized by plants in chlorophyll formation; copper is also indispensable for plants as it plays a crucial role in photosynthesis, respiration, protein synthesis, and cell wall metabolism. Fe and Cu content in EVOOs can derive from the soil composition, from some fertilizers used in olive cultivation (copper salts), or from contamination during oil processing and storage. As seen in the table, both Cu and Fe exhibit significant variability in concentration values, ranging from approximately 10 to 117 µg kg^{-1} and from 342 to 1217 µg kg^{-1}, respectively. The importance of these two metals for EVOO quality lies in their ability to act as pro-oxidants by catalyzing the autoxidation reactions of the oil itself. As for Zn, noticeable variability in concentrations can be observed; for example, the value of approximately 3055 µg kg^{-1} in EVOO12 stands out compared to those samples where no zinc was detected. Considering that the median value is 246 µg kg^{-1}, a contamination in EVOO12, during analysis, cannot be ruled out. Calabrian EVOO 12-13 show the highest suite of REEs, consistent with weathered granitoid soils common in that area. Zinc is an essential element in plants, which can absorb and accumulate it, as it serves as an activator for some enzymes. Periodic application of Bordeaux-type copper fungicides and Zn/Mn-containing foliar fertilisers plausibly explains the Cu peaks in EVOO7 and the Zn outlier in EVOO12. It is considered a pollutant when present in high concentrations; in EVOOs, its presence is deleterious as it is one of those metals that increase the speed of oil autooxidation reactions. The levels of Mn are lower than 20 µg kg^{-1} in all samples; it can be hypothesized that they derive from soil composition rather than oil processing. Literature reports that its catalytic activity falls between that of Cu and Fe, and a Mn concentration near 0.6 mg kg^{-1}, much higher than the one found in this study, induces a 50 % decrease in oil resistance to oxidative processes (Li et al., 2021). Finally, nickel was detected only in samples of EVOO12 and EVOO13, albeit in low concentration. Veneto orchards are partly irrigated with moderately saline groundwater from the Po-delta alluvium, which can raise Na and Mn in the fruit. Manganese and iron uptake are enhanced during late-season maturation; the oils collected in November (Veneto, Sardinia) therefore show higher Mn and Fe than early-harvest Ligurian oils.

In general, the data obtained exhibit significantly high values of RSD % for the rare earth elements. It should be noted that pretreatment tests aimed at optimizing the analysis procedure was conducted by determining metals using ICP-OES, when the response of rare earth elements was not yet known. Furthermore, low concentrations could also be a potential cause of the high RSD% values obtained.

3.4. Multivariate statistical analysis

Multivariate analysis was performed to investigate patterns in the elemental composition of EVOO samples. Prior to imputation, Cu, Ni, Ce, and Gd showed over 50 % missing values and were flagged for sensitivity analysis. The overall KMO value for the full dataset was 0.567, indicating a moderate level of sampling adequacy suitable for exploratory multivariate analysis. However, several variables exhibited individual KMO values below the commonly accepted threshold of 0.50 (Table S8), suggesting they may not share sufficient common variance with other variables to support reliable factor extraction. Specifically, the elements La, Sm, Nd, Ni, V, Se, Al, Li, and Sb all fell below this cut-

off, with KMO values ranging from 0.491 to 0.392. These elements were therefore marked for sensitivity analysis, and their influence on PCA outcomes was examined. Bartlett's test of sphericity returned a highly significant result ($\chi^2 = 1402.6$, $p < 0.001$), decisively rejecting the null hypothesis that the correlation matrix is an identity matrix. This indicates that the dataset contains substantial inter-variable correlations, thereby justifying the application of multivariate techniques such as principal component analysis (PCA) and factor analysis. The result supports the presence of latent structure within the elemental composition data and confirms that dimensionality reduction is appropriate in this context. Given the small sample size and moderate adequacy indicators, the results should be considered preliminary and exploratory, serving to identify patterns and limitations rather than to support definitive classification or prediction.

Bootstrapping was employed to assess the stability of the principal component analysis (PCA) results and to quantify the uncertainty associated with the proportion of variance explained by each component (Table S9). The resulting 95 % confidence intervals (CIs) for the first ten principal components provided a robust, measure of the variability inherent in the eigenvalue estimates. Notably, the CIs confirmed that the first two components consistently captured a substantial portion of the total variance, supporting their retention for interpretation, while the remaining components explained increasingly marginal and statistically unstable variance. These results lead to the conclusion that the observed variance patterns are not artifacts of sampling noise.

As can be seen (Fig. 2), The PCA biplot displays the score projections of the 13 EVOO samples onto the first two principal components (PC1 and PC2), which together explain 54.3 % of the total variance (34.3 % and 20.0 %, respectively). Sample points are coloured by region, revealing partial grouping by geographic origin. Notably, Calabria samples tend to spread along the positive PC1 axis, with one clear outlier, while Liguria oils are more tightly clustered on the negative side of PC1 and slightly above PC2. Sardegna and Puglia samples occupy intermediate positions, overlapping with Toscana and Veneto. The red arrows represent the scaled loadings of each element with the principal components. Elements such as Na, Zn, Fe, and Ce load strongly on PC1, suggesting that variation in these trace elements drives separation along the horizontal axis. In contrast, elements like Be, V, and Nd show stronger associations with PC2. Several rare-earth elements (e.g., Sm, Gd, La, Sb) contribute minimally to both PCs, as indicated by their shorter, centralised arrows. Overall, the plot suggests that geographic trends are partially captured by compositional differences in a subset of trace elements, particularly alkali metals and early transition elements.

Removing the twelve doubtful variables (those with KMO < 0.50 or > 50 % censored values) had only a modest effect on PCA results. After filtering, PC1 and PC2 still explain virtually the same share of total variance (49.0 % and 22.0 %, compared with 46 % and 20 % in the full matrix), confirming that the dominant compositional structure is carried by the well-behaved elements. The corresponding biplot (Fig. 3) mirrors the original one: Liguria samples remain clustered along the negative PC1 axis, Calabria oils still project positively on PC1, and the intermediate positioning of Sardegna, Puglia, Toscana and Veneto is preserved. Likewise, the main drivers of separation (Na, Zn, K, Fe and Y on PC1; Be and Mg on PC2) remain unchanged, whereas the removed low-KMO/poor-precision elements contributed little directional information. Thus, the sensitivity analysis demonstrates that the multivariate patterns reported in the manuscript are robust and not an artefact of variables with weak sampling adequacy or high levels of imputation.

4. Conclusion

The conducted study enabled the development of a procedure for determining the element content within extra virgin olive oils. The homogenization of the samples before their mineralization was found to be of paramount importance to obtain repeatable concentrations. Chemometric treatment revealed the possibility of using them as markers for oil

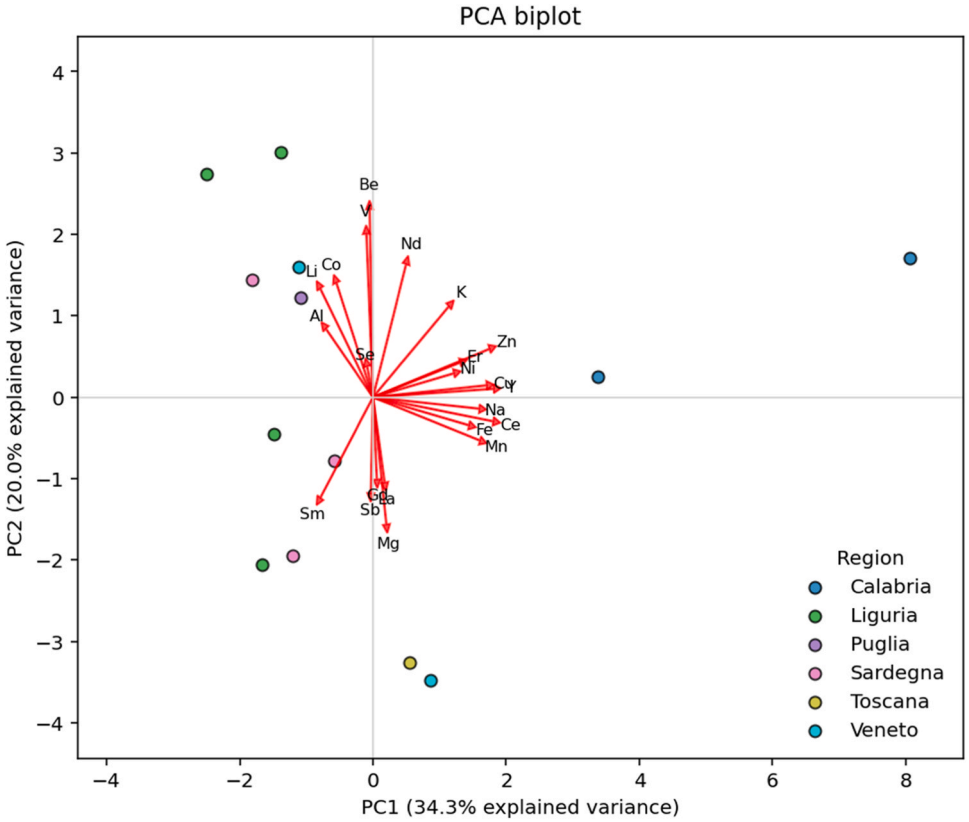

Fig. 2. Biplot of PC1 vs PC2 with region-colored scores considering all dataset.

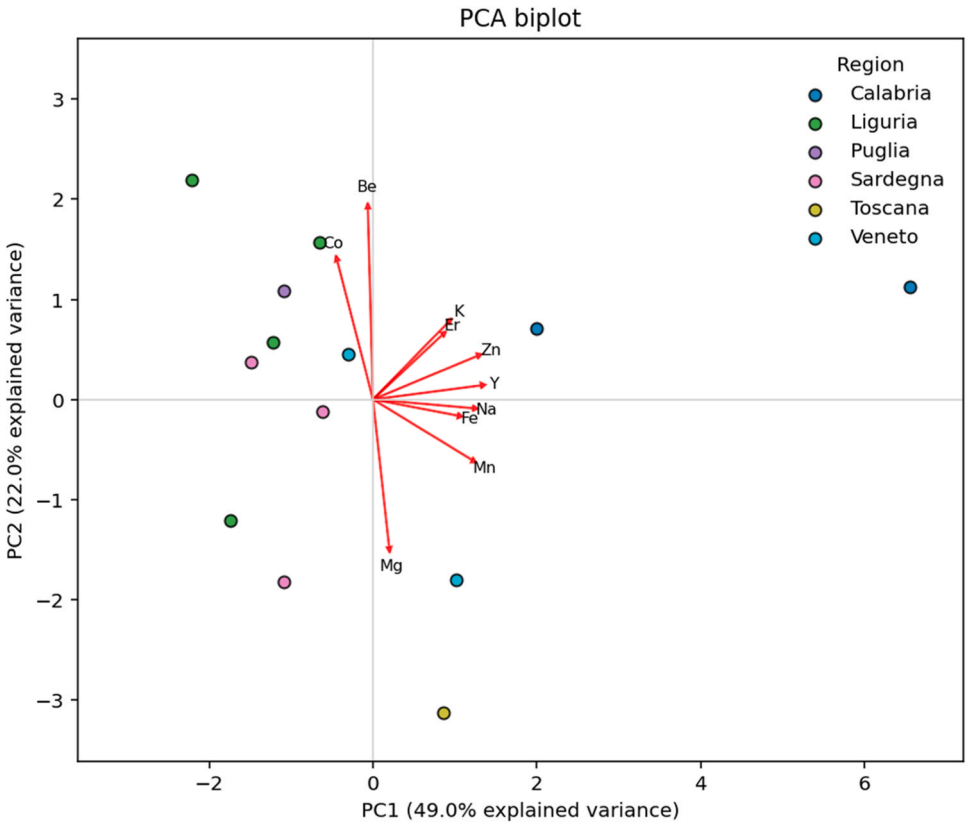

Fig. 3. Biplot of PC1 vs PC2 with region-colored scores considering filtered dataset.

typification according to their origin. In the case of rare earth elements, however, the obtained results showed high variability, likely due to the low concentrations involved. Therefore, future developments will be dedicated to identifying optimal pretreatment conditions that allow for minimized variability in the rare earth element content across different aliquots of the same sample. Specifically, a specific experimental design will be conducted with the aim of enhancing the discriminatory capacity of the inorganic component toward the geographical origin of the sample.

CRediT authorship contribution statement

Ilenia Certomà: Writing – original draft, Investigation, Formal analysis. **Mery Malandrino:** Writing – review & editing. **Paolo Inaudi:** Writing – original draft, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Laura Favilli:** Visualization, Data curation. **Riccardo Cecire:** Visualization. **Stefano Bertinetti:** Writing – review & editing, Data curation. **Ornella Abollino:** Writing – review & editing, Visualization, Supervision. **Agnese Giacomino:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2025.107846.

Data availability

No data was used for the research described in the article.

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