Determination of Metals in Walnut Oils by Means of an Optimized and Validated ICP-AES Method in Conventional and Organic Farming Type Samples

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Abstract: Agricultural products are indispensable for equilibrated diets since they discharge minerals and several bioactive constituents. Considering the increasing demand for organic products, research has been conducted over recent years to investigate whether organically grown food products are chemically different compared to those produced with conventional farming. In this work, a novel inductively coupled plasma atomic emission spectrometric method was developed and validated for the determination of nutrient and toxic elements in walnut oils produced with conventional and organic farming. The method presented good linearity ($r^2 > 0.9990$) for each element at the selected emission line. The limits of detection and limits of quantification ranged between 0.09 μg g^{-1} to 2.43 μg g^{-1} and 0.28 μg g^{-1} to 8.1 μg g^{-1}, respectively. Method accuracy and was assessed by analyzing the certified reference materials BCR 278-R and spiked walnut oil samples. The determined metals were quantified, and the results were analyzed by Student's t-test to investigate the differences in the elemental profile of the walnut oils according to type of farming (conventional or organic).

Keywords: ICP-AES; metals; elemental profile; walnut oils; organic; farming type

1. Introduction

Walnuts (Juglans nigra) are an integral part of the Mediterranean diet as they are an important source of nutrients, including bioactive phytochemicals [1] and essential minerals [2]. Their consumption has been associated with several health benefits, including the reduction of coronary heart disease and cholesterol, acting against oxidative stress, diabetes, Alzheimer's disease, and diabetes, among others [3].

Walnut oil is a high-valued product extracted from the walnut. Walnut oils are a rich source of nutrients and bioactive components, i.e., fatty acids, tocopherols, sterols, and phenolic compounds [4] Walnut oils are widely used as a natural raw material in the food industry [5–7], in cosmetics industry [8], while they are used for the production of natural food supplements [9], as well. In recent years, with the increasing emphasis on health care, walnut oil has become popular among consumers as a functional food exhibiting antioxidant, anti-viral, and anti-inflammatory properties [5–8]. According to the literature, dietary walnut oil can inhibit hepatic lipid accumulation [10], its consumption minimizes the risk of atherosclerosis [10], it may serve as a herbal medicine for hyperlipidemic patients with type 2 diabetes [11], and it may also affect the growth of tumors and prevent metastasis [12]. The critical factors that affect the bioactive profile, and in extend the nutritional value and health properties of edible oils, have been related to the species [10], the geographical origin [13], and the farming type (conventional or organic), as well [14]. Organic farming systems avoid the use of pesticides, synthetic fertilizers and growth regulators, and it has been shown that organic foods exhibit lower content of nitrates [15].

In recent years, consumers' demand for organic edible oils has increased remarkably in global scale, and this has necessitated the development of analytical methodologies

for the determination of nutrients and contaminants in attempt to prevent fraudulent actions, guarantee the quality and safety of the products and protect consumers as well. Regulation (EU) 2018/848 of the European Parliament and of the Council, (Regulation (EU), 2018/848) [16], makes requirements about the production and labelling of organic food within the European Union, and refers to controls for the surveillance of products marked with the organic EU-logo respects the rules for organic agriculture. Several works have been published investigating the authentication of agricultural products grown under conventional and organic farming systems, as has been recently reviewed by de Lima et al. [17]. Except for the detection of pesticides and antibiotics which are prohibited in organic agriculture, the detection of different food constituents including bioactive compounds, volatiles, and minerals [18] have been proposed for the discrimination between organic and conventional food products. The content of trace elements has gained importance in recent years due to the fact that the quality of the walnut oils has been related to the elemental concentration [19].

Metals constitute an important group of elements with specific interest due to their essential or toxic nature [2,20]. Nutrient metals include calcium, chromium, copper, iron, magnesium, magnesium, nickel, manganese, and zinc, while lead, mercury, and cadmium are considered to be toxic. Barium and aluminum are non-essential elements. Walnuts, and in extension walnut oils, contain minerals that are essential for human health. The metals that are present in the walnut oils are mainly absorbed by the soil where they are grown. Metals can be also incorporated because of environmental pollution. Considering that toxic elements might be also present in walnut oils due to contamination, and that through consumption trace metals enter the human body, it becomes evident that their determination is critical.

Multi-elemental analysis currently constitutes a promising tool for the authentication of organic products [21]. Atomic absorption spectroscopy (AAS), inductively coupled plasma-optical emission spectroscopy (ICP-AES) and ICP-mass spectrometry (ICP-MS) are the most commonly used techniques [17,22–24]. The most critical step in analysis is the complete dissolution of the samples. Efficient sample preparation strategies for digestion are essential. Different assays have been proposed including dry ashing, wet ashing, microwave digestion and autoclave digestion [20,25,26].

The objective of this work was to analyze Greek walnut oils of the same botanical variety with ICP-AES and elucidate if the elemental profile of organic walnut oils significantly differs from walnut oils produced by walnuts grown with conventional agriculture. For this purpose, 20 samples were analyzed and processed with digestion in Teflon autoclaves to succeed complete dissolution. The metals were determined, and the quantification results were analyzed with Student's t-test to estimate the variance of metal concentrations among the walnut oils grown with different type of farming.

2. Materials and Methods

2.1. Chemical and Reagents

Analytical grade concentrated nitric acid (HNO_3) 65% was supplied by Merck (Darmstadt, Germany). Ultra-pure water prepared by a Milli-Q system (Merck, Darmstadt, Germany) was used throughout the study. During the whole method development and validation procedure, negligible blank values were obtained for the concentrated nitric acid and Milli-Q water. In all steps of the experimental procedure, high purity double distilled water was used. Single stock standard solutions (1000 mg L^{-1}) of Ag, Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb and Zn were supplied by Merck (Darmstadt, Germany). A multi-element stock standard solution was prepared from single ones, while the working standard solutions were daily prepared by appropriate serial dilution of multi-element solution. For this purpose, an intermediate standard solution at a concentration of 100 mg L^{-1} was prepared in 1.5 M nitric acid. Subsequently, working standard solutions were prepared by diluting appropriate aliquots of the intermediate standard solution in Milli-Q water. The concentrations of the working standard solutions ranged between 0.1 mg L^{-1} and

10 mg L^{-1} (i.e., 0.1 mg L^{-1}, 0.2 mg L^{-1}, 0.5 mg L^{-1}, 1.0 mg L^{-1}, 2.0 mg L^{-1}, 5.0 mg L^{-1}, 10.0 mg L^{-1}). Finally, the certified reference material (CRM) BCR 278-R (Community Bureau of Reference Brussels, Belgium) trace elements in mussel tissue was analyzed for the evaluation of the accuracy of the developed method.

2.2. Instrumentation

A Perkin–Elmer Optima 3100XL axial viewing ICP-AES instrument (Perkin Elmer, Waltham, MA, USA) was used for the determination of the nutrient and toxic elements in the walnut oils. The ICP-AES instrument was furnished with a Fassel type torch with a quartz glass 2.0 mm injector and a Scott double-pass spray chamber with a crossflow nebulizer. The main instrumental parameters/conditions of the ICP-AES instrument are summarized in Table 1. All target elements were studied in two wavelengths, those with higher sensitivity according to the manufacturer, while the optimum emission lines were selected for quantification. The analytical wavelengths were set at the first and second sensitivity order spectral atomic or ionic lines of the studied analytes. The recorded wavelengths were: 328.068 nm and 338.289 nm for Ag, 237.313 nm and 308.215 nm for Al, 249.772 nm and 208.957 nm for B, 233.527 nm and 230.425 for Ba, 396.847 nm and 317.933 nm for Ca, 226.502 nm and 214.40 nm for Cd, 238.892 nm and 228.616 nm for Co, 357.869 nm and 238.563 nm for Cr, 324.752 nm and 224.700 nm for Cu, 238.204 nm and 239.562 nm for Fe, 280.271 nm and 279.077 nm for Mg, 257.610 nm and 259.372 nm for Mn, 232.003 nm and 221.648 nm for Ni, 217.000 nm and 202.353 for Pb and 213.857 nm and 202.548 nm for Zn.

Table 1. Instrumental conditions and description of the ICP-AES system.

RF generator	40 MHz, free-running
RF incident power	1350 W (optimized)
Auxiliary Argon gas flow rate	0.6 L min^{-1}
Plasma Argon gas flow rate	15.0 L min^{-1}
Nebulizer Argon gas flow rate	0.85 L min^{-1} (optimized)
Nebulizer uptake flow rate	1.50 mL min^{-1} (optimized)

The sample/standard solutions were introduced into the ICP atomization system with a peristaltic pump equipped with Tygon-type PVC peristaltic pump tubes (i.d. 0.030 in). An Orion EA940 pH-meter was used for pH measurements.

Sample digestion was performed in Teflon$^{®}$ (DuPont, DE, USA) vessels which were placed in a heated six-position aluminum block (Berghof, BTR,941, Eningen, Germany). All glassware and sample preparation apparatus were overnight soaked with nitric acid (10% *v/v*) and extensively washed with double de-ionized water prior to their use to avoid contamination.

2.3. Sample Collection and Preparation

Walnut samples of the Chandler variety produced with conventional (10 samples coded as CWO1-CWO10) and organic farming (10 samples coded as OWO1-OWO10) were acquired from Thessaly in Greece, during November 2020. The organic walnuts were produced using biological and ecologically friendly pesticides and fertilizers. The oil from the walnut kernels was obtained through mechanical extraction, according to Ezzahra Ennoukh et al. [27]. The walnut oils samples were stored in the refrigerator (+4 °C) in sealed dark glass bottles, and each sample was opened prior to analysis.

Digestion of the walnut oils was performed by weighting 100 mg of the sample in Teflon$^{®}$ vessels. Subsequently, 7 mL of concentrated nitric acid was added, and the vessels were placed in a heated six-position aluminum block. The aluminum block was heated at 120 °C for 60 min to achieve complete dissolution of the walnut oils. After the completion of the dissolution, the Teflon vessels were left to cool down to room temperature and they were opened in a fume hood. The digested sample was transferred into a 25 mL volumetric

flask and the volume was made up to the mark with Milli-Q water. Finally, the samples were placed into test tubes and analyzed by ICP-AES.

2.4. Statistical Analysis

Statistical differences between the walnut oils produced by walnuts grown with conventional and organic type of farming were estimated employing t-test at the 5% level ($p < 0.05$) of significance. Statistical analyses were performed using the Data Analysis tool of Microsoft Excel (Microsoft, Redmond, WA, USA).

3. Results and Discussion

3.1. Dissolution Procedure

To find the optimum dissolution protocol for the walnut oil samples various procedures were investigated. Nitric acid was employed as dissolution agent due to its widespread use for the digestion of foodstuffs. Initially, aliquots of 100 mg of the oil samples were placed in a beaker and different quantities of concentrated nitric acid was added (i.e., 3 mL, 5 mL, and 7 mL). No sample dissolution was observed after 24 h, indicating that a heating step was required. Since no dissolution of the walnut oils was observed, the addition of hydrogen peroxide was subsequently evaluated. For this purpose, different amounts and ratios of nitric acid and hydrogen peroxide was examined (i.e., 2 mL of nitric acid and 1 mL of hydrogen peroxide, 3 mL of nitric acid and 1 mL of hydrogen peroxide, 3 mL of nitric acid and 2 mL of hydrogen peroxide, 5 mL of nitric acid and 2 mL of hydrogen peroxide). However, also in this case no sample dissolution was observed without heating (for a time span of 24 h). Therefore, open-vessel digestion was examined as a dissolution approach. For this purpose, aliquots of 100 mg of the oil samples were placed in a beaker and 7 mL of concentrated nitric acid was added, followed by sample heating at 120 °C. After 20 min of open-vessel digestion, no complete sample dissolution was observed. Finally, pressure-assisted autoclave digestion was examined by adding aliquots of 100 mg of the oil in Teflon vessels together with 7 mL of concentrated nitric acid was added. The Teflon vessels were placed in a steel autoclave and the mixture was heated at 120 °C for 60 min. Subsequently, the Teflon vessels were left to cool down to room temperature and opened in a fume hood. Complete sample dissolution was obtained with the simultaneous use of heat and pressure and thus this protocol was chosen to achieve dissolution of the walnut oil samples.

3.2. Optimization of ICP-AES Conditions

Among the recorded emission spectral lines for each element, these with higher sensitivity, better linearity and reproducibility as well as absence of interferences were considered to be optimum and used for further study. These emission lines are Ba; 233.527 nm, Cd; 226.502 nm, Cu; 324.752 nm, Fe; 238.204, Pb; 217.00 nm, Mn; 257.610 nm, Ni; 232.003 nm, Zn; 213.857 nm, Al; 237.313 nm, Cr; 357.869 nm, Co; 238.892 nm, Ag; 328.068 nm, Ca; 396.847 nm, Mg; 280.271 nm, B; 249.772 nm.

Because the signal emission intensities of each analyte depend on sample matrix, the optimization procedure was proceeded using a mixed walnut oil digest solution spiked with 0.5 mg L^{-1} of the multi-element standard solution. Regarding the ICP parameters, which significant affect the atomization and excitation performance, the radio frequency (RF) incident power, the nebulizer gas flow rate, and the nebulizer uptake flow rate were studied.

In general, RF incident power effects positively the plasma temperature resulting in higher emission intensities [28]. The RF incident power was studied in the range 1200–1400 W for each analyte. The obtained results shown that the recorded intensity increased by increasing the RF power up to 1350 W, remaining almost stable for higher RF values, for all analytes. For RF power values below 1200 W extinction of the plasma was observed. Thus, an RF incident power of 1350 W was adopted as optimum for further study.

The effect of flow rate of nebulizer gas on the sensitivity considering the emission intensity was investigated in the range 0.6 to 1.0 L min^{-1}. Maximum intensity values were recorded at nebulizer gas flow rate of 0.85 L min^{-1} for all analytes, as is shown representative for Cd, Co, Fe, Ni, Pb, and Zn in Figure 1. Therefore, a nebulizer gas flow rate of 0.85 L min^{-1} was chosen as optimum.
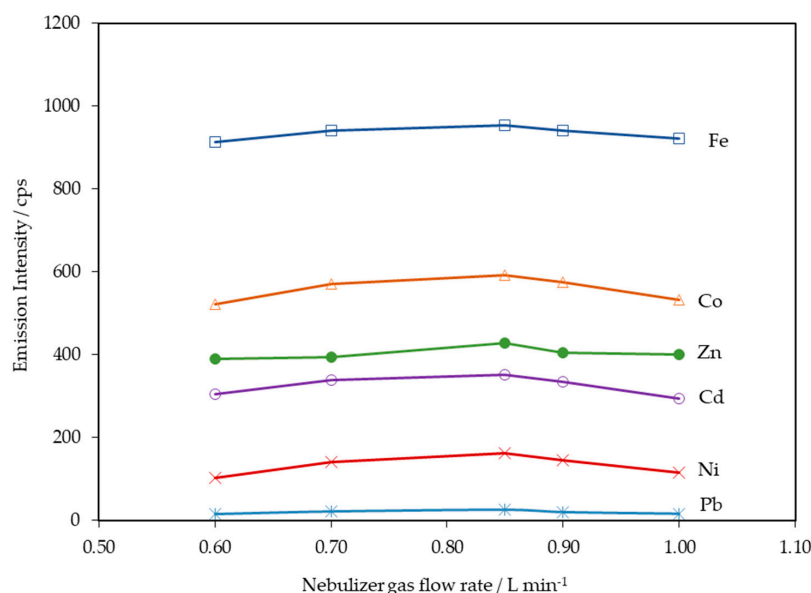
Figure 1. Effect of nebulizer gas flow rate on the emission intensity of 0.5 mg L^{-1} Fe, Co, Zn, Cd, Ni, and Pb. All other parameters are presented in the text.

Since the nebulizer uptake flow rate affects the amount of sample that is inserted into plasma and thus the sensitivity of the developed method, this flow rate was investigated in the range 1.0–3.0 mL min^{-1}. The obtained results presented an optimum nebulizer uptake flow rate at 1.5 mL min^{-1} for all studied elements and representative demonstrated for Fe, Co, Ba, Zn, Cd, and Ni in Figure 2. Thus, this value was used as optimum throughout the study.
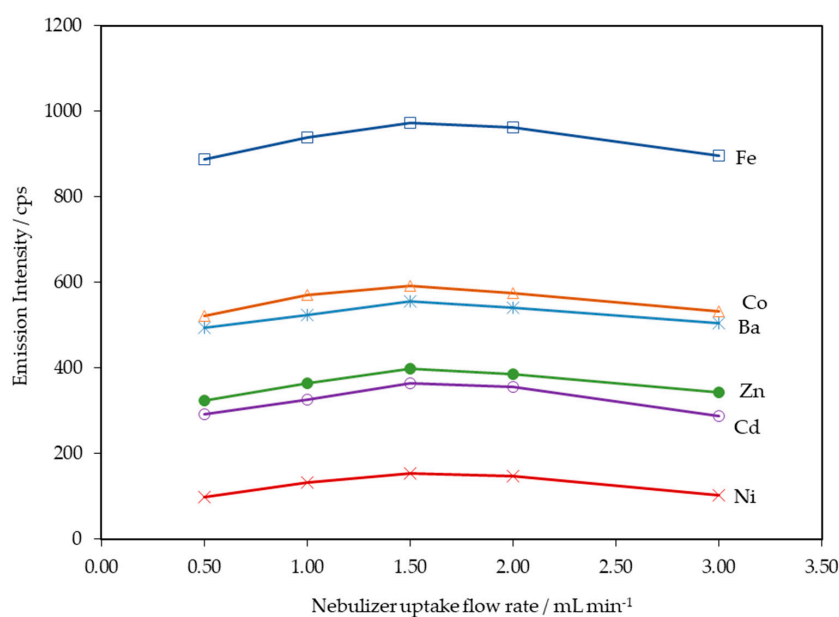
Figure 2. Effect of nebulizer gas flow rate on the emission intensity of 0.5 mg L^{-1} Fe, Co, Ba, Zn, Cd, and Ni. All other parameters are presented in the text.

3.3. Figures of Merit

The herein optimized developed ICP-AES method was validated in terms of linearity, limits of detection (LODs), limits of quantification (LOQs), accuracy and precision for each target analyte. To assess method performance, least square linear regression analysis was employed.

The slope (±standard deviation) and the r^2 values for each metal is presented in Table 2. The LODs and LOQs for the examined analytes were calculated according to the guidelines of the International Union of Pure and Applied Chemistry (IUPAC) [29]. As such, ten separate blank solutions were prepared and the LODs were equal to the three times the standard deviation of the measurements for the blank solutions divided by the slope of the calibration curves for each element, while the LOQs were equal to ten times the standard deviation of the measurements for the blank solutions divided by the slope of the calibration curves for each element. The LODs and LOQs together with the upper limit of the linear range of the ICP-AES method are also given in Table 2.

Table 2. Analytical performance characteristics of the developed ICP-AES method.

Element	Spectral Line (nm)	Slope ± Standard Deviation (cps mg^{-1} L)	r^2	LOD [1] (µg g^{-1})	LOQ [2] (µg g^{-1})	Upper Limit of Linear Range (µg g^{-1})
Ag	328.068	20,800 ± 171	0.9997	0.58	1.94	2500
Al	237.313	352 ± 0.8	0.9999	0.49	1.64	2500
B	249.772	3645 ± 50.6	0.9992	0.48	1.59	2500
Ba	233.527	1125 ± 7.1	0.9998	0.66	2.22	2500
Ca	396.847	1,715,367 ± 6567	0.9999	0.65	2.18	2500
Cd	226.502	756 ± 4.4	0.9999	1.53	5.09	2500
Co	238.892	1177 ± 1.4	0.9998	0.76	2.53	2500
Cr	357.869	43,901 ± 170	0.9998	0.29	0.95	2500
Cu	324.752	65,948 ± 440	0.9998	0.45	1.49	2500
Fe	238.204	1962 ± 12.4	0.9998	1.20	4.01	2500
Mg	280.271	50,276 ± 540	0.9995	0.14	0.47	2500
Mn	257.610	23,317 ± 242	0.9996	0.09	0.28	2500
Ni	232.003	315 ± 0.6	0.9999	1.65	5.49	2500
Pb	217.000	47.1 ± 0.6	0.9993	2.43	8.11	2500
Zn	213.857	813 ± 4.3	0.9999	0.72	2.40	2500

[1] LOD: Limit of detection. Equivalent to wet digestion method. The LOD values correspond to 3.3 standard deviation of the signals of the blank for each element related to a sample mass of 0.1 g finally diluted in 25 mL. [2] LOQ: Limit of quantification. Equivalent to wet digestion method. The LOQ values correspond to 10 standard deviations of the signals of the blank for each element related to a sample mass of 0.1 g finally diluted in 25 mL.

The LODs ranged between 0.09–2.43 µg/g, and were comparable with other works that used microwave digestion for the dissolution of the edible oils [30–33]. Considering this fact, the proposed method could be used as an alternative to microwave assisted digestion. For the evaluation of method accuracy and precision spiked samples of conventional and organic walnut oil were prepared at three different concentration levels (i.e., 100 µg g^{-1}, 500 µg g^{-1}, 2500 µg g^{-1}). Accuracy was expressed in terms of relative recovery (RR%) by comparing the experimentally measured concentration and the nominal concentration of the spiked sample. Precision was expressed in terms of relative standard deviation (RSD%) that was derived from the replicate analysis (*n* = 5) of the spiked samples [34]. Samples CWO1 and OWO1 were used to prepare spiked walnut oil samples of conventional and organic walnut oil, respectively. As shown in Table 3, the RR% ranged between 85.0–110.2%, indicating the herein developed method shows satisfactory accuracy. Moreover, the RSD% values were lower than 8.4%, showing satisfactory method precision.

Table 3. Accuracy and precision results of the ICP-AES method (mean value $\pm$ standard deviation of five measurements).

Element	Added (μg g^{-1})	Conventional Walnut Oil			Organic Walnut Oil		
		Found (μg g^{-1})	RR%	RSD%	Found (μg g^{-1})	RR%	RSD%
Ag	0	<LOD	-	-	<LOD	-	-
	100	90.0 $\pm$ 4.4	90.0	4.9	93.1 $\pm$ 2.6	93.1	2.8
	500	447 $\pm$ 11.6	89.4	2.6	482 $\pm$ 26.2	96.5	5.4
	2500	2263 $\pm$ 11.3	90.5	0.5	2263 $\pm$ 9.1	90.5	0.4
Al	0	<LOD	-	-	<LOD	-	-
	100	110 $\pm$ 5.1	110.2	4.6	111 $\pm$ 1.2	110.8	1.1
	500	487 $\pm$ 8.0	97.4	1.6	510 $\pm$ 13.0	102.0	5.9
	2500	2331 $\pm$ 23.0	93.3	1.0	2215 $\pm$ 2.3	88.6	0.1
B	0	<LOD	-	-	<LOD	-	-
	100	90.8 $\pm$ 7.6	90.8	8.4	92.1 $\pm$ 0.9	92.1	1.0
	500	475 $\pm$ 19.2	94.9	4.2	442 $\pm$ 22.9	88.3	5.2
	2500	2231 $\pm$ 88.9	89.3	4.0	2234 $\pm$ 75.9	89.3	3.4
Ba	0	<LOD	-	-	<LOD	-	-
	100	96.8 $\pm$ 7.2	96.8	7.4	98.8 $\pm$ 1.6	98.8	1.6
	500	407 $\pm$ 1.0	81.4	0.2	401 $\pm$ 19.6	80.2	4.9
	2500	2253 $\pm$ 37.4	90.1	1.7	2266 $\pm$ 18.8	90.6	0.8
Ca	0	19.8 $\pm$ 0.3	-	1.5	61.7 $\pm$ 2.9	-	4.6
	100	116 $\pm$ 10.4	96.6	8.9	167 $\pm$ 0.7	105.2	0.4
	500	541 $\pm$ 7.1	104.3	1.3	568 $\pm$ 11.6	101.2	2.0
	2500	2150 $\pm$ 34.5	85.0	1.6	2390 $\pm$ 91.3	93.1	3.8
Cd	0	<LOD	-	-	<LOD	-	-
	100	97.6 $\pm$ 4.4	97.6	4.5	99.5 $\pm$ 2.6	99.5	2.6
	500	453 $\pm$ 2.7	90.5	0.6	467 $\pm$ 23.8	93.4	5.1
	2500	2277 $\pm$ 10.2	91.1	0.5	2280 $\pm$ 4.8	91.2	0.2
Co	0	<LOD	-	-	<LOD	-	-
	100	105 $\pm$ 7.6	104.6	7.3	97.5 $\pm$ 2.6	97.5	2.1
	500	434 $\pm$ 14.8	86.8	3.4	459 $\pm$ 22.4	91.7	4.9
	2500	2310 $\pm$ 12.9	92.4	0.6	2312 $\pm$ 3.0	92.5	0.1
Cr	0	<LOD	-	-	<LOD	-	-
	100	90.1 $\pm$ 4.3	90.1	4.8	101 $\pm$ 1.3	100.6	1.3
	500	475 $\pm$ 7.3	95.1	1.5	494 $\pm$ 31.5	98.9	6.4
	2500	2387 $\pm$ 12.9	95.5	0.5	2403 $\pm$ 22.3	96.1	0.9
Cu	0	<LOD	-	-	<LOD	-	-
	100	91.0 $\pm$ 4.9	91.0	5.4	101 $\pm$ 2.0	101.1	2.0
	500	520 $\pm$ 8.8	104.1	1.7	494 $\pm$ 33.4	98.8	6.8
	2500	2358 $\pm$ 92.8	94.3	3.9	2617 $\pm$ 20.9	104.7	0.8
Fe		13.8 $\pm$ 1.2	-	8.7	21.8 $\pm$ 1.0	-	4.6
	100	114 $\pm$ 6.6	100.3	5.8	119 $\pm$ 0.9	97.5	0.8
	500	507 $\pm$ 8.8	101.3	1.7	541 $\pm$ 33.4	103.8	6.2
	2500	2427 $\pm$ 20.1	96.5	0.8	2441 $\pm$ 19.3	96.8	0.8
Mg		1.5 $\pm$ 0.1	-	4.5	11.7 $\pm$ 1.1	-	9.6
	100	100 $\pm$ 6.9	98.9	6.9	110 $\pm$ 1.6	98.1	1.5
	500	424 $\pm$ 2.1	94.0	0.5	439 $\pm$ 23.1	85.5	5.3
	2500	2329 $\pm$ 7.2	93.1	0.3	2331 $\pm$ 11.4	92.8	0.5
Mn	0	<LOD	-	-	<LOD	-	-
	100	104 $\pm$ 6.3	104.9	6.0	102 $\pm$ 1.2	101.8	1.2
	500	432 $\pm$ 1.8	86.4	0.4	435 $\pm$ 27.3	87.0	6.3
	2500	2385 $\pm$ 21.5	95.4	0.9	2384 $\pm$ 50.1	95.4	2.1

Table 3. *Cont.*

Element	Added (µg g^{-1})	Conventional Walnut Oil			Organic Walnut Oil		
		Found (µg g^{-1})	RR%	RSD%	Found (µg g^{-1})	RR%	RSD%
Ni	0	<LOD	-	-	<LOD	-	-
	100	102 ± 6.4	101.9	6.5	95.3 ± 5.3	95.3	5.6
	500	453 ± 1.2	90.7	0.3	459 ± 16.7	91.8	3.6
	2500	2372 ± 32.8	94.9	1.4	2369 ± 5.0	94.8	0.2
Pb	0	<LOD	-	-	<LOD	-	-
	100	105 ± 8.5	105.3	8.1	86.4 ± 8.5	86.4	3.3
	500	488 ± 20.4	97.5	4.2	446 ± 30.8	89.2	6.9
	2500	2314 ± 104.1	92.6	4.5	2276 ± 53.5	91.0	2.4
Zn	0	8.1 ± 0.5	-	6.4	15.6 ± 0.8	-	-
	100	105 ± 10.3	97.0	9.8	111 ± 1.2	95.2	1.1
	500	498 ± 2.8	97.9	0.6	526 ± 13.0	102.0	2.5
	2500	2303 ± 10.4	91.8	0.5	2320 ± 13.2	92.2	0.6

The inter-day precision ($n = 3 \times 5$) for a quality control sample (100 µg g^{-1}) ranged between 1.3 and 9.2%, while for the LOQ of each metal the intra-day precision ranged between 2.4 and 9.6% and the inter-day precision ranged between 3.2 and 9.8%. The accuracy (intra-day and inter-day) for the LOQ of each metal, as well as the inter-day accuracy of the quality control sample was in the range of 83.0–115.0% for all the examined analytes. The analytical results of the analysis of the certified reference materials BCR 278-R are presented in Table 4. As it can be observed, the proposed method shows satisfactory accuracy.

Table 4. Analytical results of in CRM BCR 278-R by the developed ICP-AES method.

BCR 278-R	Certified Value (µg g^{-1})	Found [1]	$t_{exp.}$	Recovery (%)
Cd	0.348 ± 0.007	0.331 ± 0.016	1.840	95.1
Cr[2]	0.78 ± 0.06 [2]	0.74 ± 0.04	1.732	94.9
Cu	9.45 ± 0.13	9.31 ± 0.6	0.404	98.5
Mn	7.69 ± 0.23	7.56 ± 0.28	0.804	98.3
Pb	2.00 ± 0.04	1.89 ± 3.5	1.270	94.5
Zn	83.1 ± 1.7	80.0 ± 1.7	1.534	96.3

[1] Mean value ± standard deviation based on three replicates ($n = 3$). $t_{crit.} = 4.30$ at 95% probability level. [2] For chromium, a sample quantity of 0.2 g was employed since its concentration was lower than the LOQ of the herein developed ICP-AES method.

3.4. Real Samples Analysis

All walnut oils were analyzed in triplicate ($n = 3$) with relative standard deviation (RSD) <8% for the determined metals. The results are presented in Table 5. As can be observed, the toxic metals lead, mercury, and cadmium were not determined in any of the analyzed samples, reassuring in this way their quality and safety. The non-essential elements, barium and aluminum, as well as silver, chromium, copper, nickel, and manganese were not determined, either. The higher concentrations were calculated for iron, zinc, calcium, and manganese. Iron ranged between <LOD and 13.84 µg g^{-1} in conventional walnut oils and between <LOD and 46.65 µg g^{-1} in organic walnut oils. Zinc ranged between 8.10 and 14.51 µg g^{-1} in conventional walnut oils, and between 6.42 and 15.58 µg g^{-1} in those produced with organic farming. As for calcium, it ranged between 5.10 and 45.26 µg g^{-1} in conventional walnut oils, and between 1.63 and 67.44 µg g^{-1} in organic walnut oils. Magnesium ranged between <LOQ and 2.72 µg g^{-1} in conventional walnut oils, while higher concentrations were observed for those grown with organic farming ranging between <LOQ and 11.68 µg g^{-1}. Regarding the metals that were not detected in the examined samples, an increase in the sample mass could be performed to

evaluate whether these analytes do not exist in the walnut oil samples, or their detection was not possible due to the sample amount. Therefore, if bigger digestion vessels are available, sample mass could be potentially increased. The concentration ranges are in accordance with those previously reported in the literature [33,35].

The statistical analysis with Student's *t*-test showed that there was no significant statistical difference for iron and zinc ($p > 0.05$) among walnut oils grown with different type of farming. Significant statistical differences were observed, however, in the case of calcium and magnesium ($p < 0.05$).

The Box and Whisker plots of calcium and magnesium which differed statistically among the analyzed samples, are presented in Figure 3.
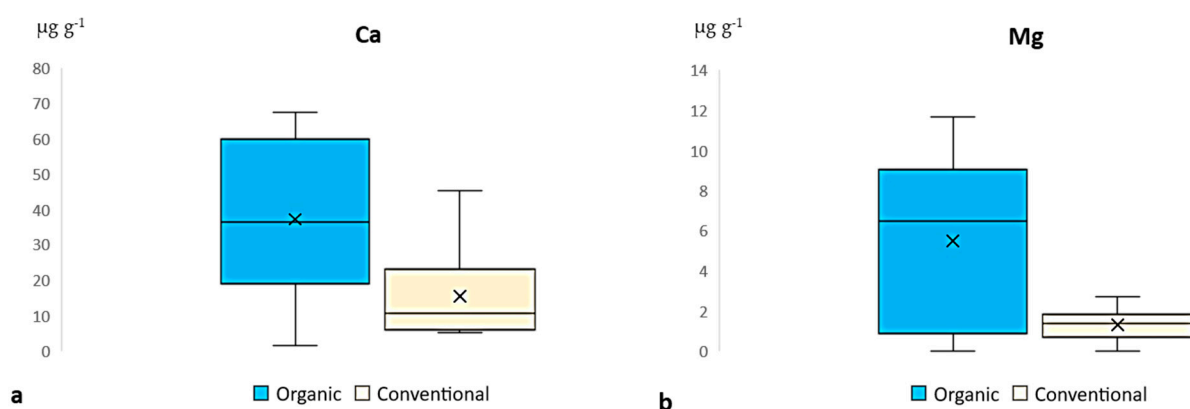
Figure 3. (**a**) Box and Whisker plot for the concentrations of calcium among organic (n = 10) and conventional (n = 10) walnut oils; (**b**) Box and Whisker plot for the concentrations of magnesium among organic (n = 10) and conventional (n = 10) walnut oils.

Table 5. Elemental analysis of organic and conventional walnut oils sample (concentrations expressed in $\mu g\ g^{-1}$).

Sample	Ag	Al	B	Ba	Ca	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Zn
CWO1	nd [1]	nd	nd	nd	19.7 ± 0.3	nd	nd	nd	nd	13.8 ± 1.2	1.5 ± 0.07	nd	nd	nd	8.1 ± 0.5
CWO2	nd	nd	nd	nd	11.23 ± 0.3	nd	nd	nd	nd	5.5 ± 0.3	1.9 ± 0.08	nd	nd	nd	8.9 ± 0.8
CWO3	nd	nd	nd	nd	11.2 ± 1.1	nd	nd	nd	nd	6.7 ± 0.4	1.6 ± 0.13	nd	nd	nd	11.8 ± 1.1
CWO4	nd	nd	nd	nd	5.8 ± 0.4	nd	nd	nd	nd	nd	1.8 ± 0.09	nd	nd	nd	9.3 ± 0.2
CWO5	nd	nd	nd	nd	45.3 ± 3.5	nd	nd	nd	nd	4.01 ± 0.3	2.7 ± 0.22	nd	nd	nd	10.4 ± 0.9
CWO6	nd	nd	nd	nd	6.5 ± 0.6	nd	nd	nd	nd	nd	1.2 ± 0.11	nd	nd	nd	10.0 ± 0.6
CWO7	nd	nd	nd	nd	6.1 ± 0.5	nd	nd	nd	nd	nd	1.1 ± 0.10	nd	nd	nd	9.4 ± 0.8
CWO8	nd	nd	nd	nd	5.1 ± 0.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	14.7 ± 1.3
CWO9	nd	nd	nd	nd	10.0 ± 0.9	nd	nd	nd	nd	nd	0.75 ± 0.06	nd	nd	nd	11.4 ± 1.1
CWO10	nd	nd	nd	nd	33.5 ± 2.5	nd	nd	nd	nd	nd	0.45 ± 0.03	nd	nd	nd	14.5 ± 1.2
OWO1	nd	nd	nd	nd	61.7 ± 2.8	nd	nd	nd	nd	21.7 ± 1.0	11.7 ± 1.1	nd	nd	nd	15.6 ± 0.8
OWO2	nd	nd	nd	nd	59.5 ± 7.3	nd	nd	nd	nd	4.6 ± 0.6	10.7 ± 0.91	nd	nd	nd	10.8 ± 1.0
OWO3	nd	nd	nd	nd	25.5 ± 2.1	nd	nd	nd	nd	12.4 ± 1.6	5.5 ± 0.4	nd	nd	nd	6.4 ± 0.6
OWO4	nd	nd	nd	nd	67.4 ± 3.3	nd	nd	nd	nd	33.8 ± 2.3	8.5 ± 0.6	nd	nd	nd	11.2 ± 1.0
OWO5	nd	nd	nd	nd	52.3 ± 4.5	nd	nd	nd	nd	45.6 ± 4.1	8.2 ± 0.2	nd	nd	nd	9.8 ± 0.8
OWO6	nd	nd	nd	nd	47.4 ± 6.3	nd	nd	nd	nd	41.1 ± 3.9	7.4 ± 0.5	nd	nd	nd	10.5 ± 1.0
OWO7	nd	nd	nd	nd	1.6 ± 0.1	nd	nd	nd	nd	nd	nd	nd	nd	nd	10.4 ± 1.0
OWO8	nd	nd	nd	nd	20.3 ± 1.9	nd	nd	nd	nd	nd	1.23 ± 0.1	nd	nd	nd	7.9 ± 0.6
OWO9	nd	nd	nd	nd	19.5 ± 1.2	nd	nd	nd	nd	nd	0.91 ± 0.08	nd	nd	nd	11.8 ± 0.8
OWO10	nd	nd	nd	nd	17.3 ± 1.3	nd	nd	nd	nd	nd	0.73 ± 0.04	nd	nd	nd	11.3 ± 0.7

[1] nd: not detected.

4. Conclusions

The work presented describes the elemental profile of Greek walnut oils produced in 2020 with conventional and organic type of farming. An ICP-AES method was developed and validated for the determination of metals in walnut oils. Under optimum conditions, the herein developed method showed good linearity, low LODs and LOQs, as well as satisfactory accuracy and precision. Calcium and magnesium were the most abundant elements determined in the analyzed samples, followed by iron and zinc. The statistical analysis with Student's *t*-test showed that there was no significant statistical difference for iron and zinc among walnut oils grown with different type of farming, while significant statistical differences were observed for calcium and magnesium.

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