Article

Determination of the Toxic and Nutrient Element Content of Almonds, Walnuts, Hazelnuts and Pistachios by ICP-AES

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Abstract: The trace element content of thirty-two nuts including almonds, walnuts, hazelnuts and pistachios available in a Greek market was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES). Wet acid digestion using nitric acid (65%) took place in Teflon autoclaves. The limits of detection (LODs) and limits of quantification (LOQs) ranged between 0.01 (Mg)–2.52 (Cu) $\mu\text{g g}^{-1}$ and 0.02 (Mg)–8.40 (Cu) $\mu\text{g g}^{-1}$, respectively. Good method linearity ($r^2 > 0.9990$) was observed for each element at the selected emission lines. The metals were quantified and one-way analysis of variance (ANOVA) was used to examine whether or not there were any statistically significant differences among the metal concentrations inside the different nut species.

Keywords: trace elements; almonds; pistachios; hazelnuts; walnuts; nuts; digestion; ICP-AES

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1. Introduction

Nuts are considered to be one of the most economically and nutritionally available food sources and they are therefore widely consumed all over the world. Nuts contain a large amount of antioxidants, vitamins [1], minerals and beneficial nutrients such as proteins, amino acids and unsaturated fatty acids [2]. They are considered to be cholesterol-free foods and they are preferred among consumers because of their unique taste, high nutritional value and health benefits [3,4]. A few of the reported health benefits derived from nut consumption include the control of blood pressure and body weight and a reduction of blood cholesterol levels, coronary heart disease risk and triacylglycerols [5,6].

The large-scale consumption of nuts worldwide has emerged the development of analytical methodologies for the determination of contaminants and pollutants in bulk foods such as nuts and guarantee the quality and safety of the nut product in an attempt to protect consumers' health and wellbeing [7].

Metals are a significant group of elements that exist in foods and they are important for human health due to their essential or toxic nature [8,9]. Nutrient metals including calcium, chromium, copper, iron, magnesium, nickel, manganese and zinc are essential for human health and they play an important role in human metabolism [10]. On the other hand, metals including lead, mercury and cadmium that exist in food matrices mainly due to environmental contamination are considered to be toxic elements. Finally, metals such as barium and aluminum are considered to be non-essential elements and their biological function for living organisms is not known [8]. Through the consumption of food in the human diet, trace metals that are present in food are directly taken into the body [9]. Therefore, their determination in foodstuffs is critical.

Nuts contain multiple minerals that are essential for human health. However, some toxic elements that can have potential health effects in the human body such as lead and cadmium might also be present in nuts due to contamination during food processing and packaging [4]. There are various factors that influence the mineral composition of nuts, as also in the case of other food items, including the climate and soil characteristics (e.g., the organic matter content and its pH value) of the cultivation location. These factors

vary significantly among different regions and therefore the elemental composition of nuts depends strongly on their cultivation site [8,11]. Thus, the inorganic components of nuts have been proposed as potential markers for the geographical origin [12]. Several works have been published in the literature reporting the elemental profile of different nuts species available in the Brazilian [13], Italian and Turkish market [12,14], as well as nuts from Iran and the United States [15]. Therefore, there is an emerging need to monitor the concentrations of toxic metals in commercially available nuts and evaluate the macro-nutrient and micro-nutrient levels with analytical methodologies.

Several digestion procedures have been evaluated for the complete dissolution of plant samples. Typical examples of these procedures include dry ashing, wet ashing with different chemicals and conventional heating procedures and autoclave digestion [8,16,17]. The dry ashing method of mineralization has been employed for the digestion of walnuts, almonds, hazelnuts, Brazil nuts, cashews, pistachios and peanuts [17]. Autoclave digestion with nitric acid, sulfuric acid and hydrogen peroxide has been employed for the digestion of peanuts, almonds, hazelnuts and walnuts [8]. Microwave digestion in closed vessels has been also successfully employed for the digestion of nuts in order to reduce the digestion time [3]. Moreover, a plethora of analytical techniques including flame atomic absorption spectrometry (FAAS) [18], electrothermal atomic absorption spectrometry (ETAAS) [19], inductively coupled plasma atomic emission spectrometry (ICP-AES) [10] and inductively coupled plasma mass spectrometry (ICP-MS) [20] have been employed for the determination of metals in foodstuffs. ICP-AES exhibits multiple advantages such as a high sensitivity, ease of operation, rapid measurements and ease in overcoming interferences [21].

In this study, almonds, pistachios, walnuts and hazelnuts available in a Greek market were analyzed by ICP-AES for the determination of toxic and nutrient elements (Ba, Pd, Al, Cd, Cr, Ca, Cu, Fe, Mn, Ni, Zn and Mg) in an attempt to assess the elemental profile of these species available in Greece. For this purpose, thirty-two samples were acquired from local markets and producers and processed with digestion in Teflon autoclaves to achieve complete dissolution. The metals were quantified and further analyzed with analysis of variance (ANOVA) to estimate the variance of metal concentrations among the different nut species.

2. Materials and Methods

2.1. Materials and Reagents

For the acidic digestion, supra pure nitric acid 65% was supplied by Merck (Darmstadt, Germany). Milli-Q water was employed for the preparation of the solutions and for washing the sample preparation apparatus. For all elements included in the study stock standard solutions (1000 mg L^{-1}) from Merck (Darmstadt, Germany) were used and working standard solutions were prepared by appropriate serial dilutions of the stock solutions in 1.5 M supra pure nitric acid.

2.2. Instrumentation

The determination of the trace elements in nuts was achieved with the use of a Perkin-Elmer Optima 3100 XL axial viewing ICP-AES instrument (Perkin-Elmer, Waltham, MA, USA). The ICP-AES system was equipped with a cyclonic spray chamber, a Fassel-type fully demountable torch and a GemTip cross-flow nebulizer for the efficient selection of small size particles into the plasma. The internal diameter of the torch alumina injector was 2.0 mm. The radiofrequency incident power and the radiofrequency generator were 1350 W and 40 MHz (free running), respectively. The nebulizer argon gas flow rate, the auxiliary argon gas flow rate and the plasma argon gas flow rate were 0.8, 0.5 and 15 L min^{-1} , respectively. A segmented-array charge-coupled detector (SCD) with 235 sub-arrays was used. All elements were recorded in two wavelengths and the optimum emission lines were selected for quantification. As a signal measurement mode, peak areas were used and for each peak seven points were used. The sample solution was introduced into the

ICP-AES system with a three-channel peristaltic pump through Tygon-type PVC peristaltic pump tubes. The flow rate of the peristaltic pump was set at 1.5 mL min^{-1} .

All sample preparation apparatus (glassware, storage bottles and Teflon vessels) were soaked in 10% (*v/v*) supra pure nitric acid for at least 24 h and finally extensively washed with Milli-Q prior to their use.

2.3. Sample Pretreatment

Thirty-two nut samples including almonds ($n = 6$), pistachios ($n = 15$), walnuts ($n = 6$) and hazelnuts ($n = 5$) were purchased from producers and local markets in Greece during 2019. Each sample weighed about 500 g and was homogenized in a mortar. In a further step, about 500 mg of each sample was weighed prior to digestion.

For the digestion of the nut samples, wet digestion with nitric acid was employed. For this purpose, the weighed homogenized samples (500 mg) were placed in Teflon vessels followed by the addition of 5 mL of nitric acid (65%). Subsequently, the vessels were placed inside a six-position aluminum block (Berghof, BTR, 941, Eningen, Germany) and the block was heated at 130°C for 90 min to achieve complete dissolution. When the digestion procedure ended, the block was left to cool down to room temperature. The obtained clear solutions were transferred into 25 mL conical flasks and their volume was made up to the mark with Milli-Q water.

2.4. Statistical Analysis

Statistical analysis was performed using analysis of variance (ANOVA) from the data analysis tool of Microsoft Excel (Microsoft, Redmond, WA, USA). ANOVA is used to examine if there are significant statistical differences among the variances of independent groups of data. In this work, ANOVA was used for the comparison of the average metal concentration levels between the different nut species (almonds, pistachios, walnuts and hazelnuts). The quantification results were tested for statistically significant differences by one-way ANOVA. Furthermore, to evaluate the results, a *p*-value was used for a confidence level of 95%. If the *p*-value was higher than 0.05, there was no significant statistical difference. On the contrary, if the *p*-value was lower than 0.05, there were significant statistical differences among the samples.

3. Results and Discussion

3.1. Selection of the Optimum Emission Line

In order to find the optimum emission line for each metal, two different wavelengths were recorded. The analytical wavelengths were 233.527 nm and 230.425 nm for barium, 226.502 nm and 214.440 nm for cadmium, 324.752 nm and 224.700 nm for copper, 238.204 nm and 239.562 nm for iron, 217.000 nm and 220.353 nm for lead, 257.610 nm and 259.372 nm for manganese, 232.003 nm and 221.648 nm for nickel, 213.857 nm and 202.548 nm for zinc, 308.215 nm and 237.313 nm for aluminum, 283.563 nm and 357.869 nm for chromium, 279.077 nm and 280.271 nm for magnesium and 317.933 nm and 315.887 nm for calcium.

The selection of the optimum emission line was based on the intensity and the sensitivity of their calibration curves in combination with the absence of spectral interferences. As a result, the following emission lines were chosen: Ba II 230.425 nm, Cd II 226.502 nm, Cu I 324.752 nm, Fe II 238.204 nm, Pb I 217.000 nm, Mn I 257.610 nm, Ni II 232.003 nm, Zn I 213.857 nm, Al II 308.215 nm, Cr I 357.869 nm, Mg II 280.271 nm and Ca II 317.933 nm. I indicates that a spectral atomic emission line was used, while II indicates that a spectral ionic emission line was used.

3.2. Figures of Merit

For the evaluation of the linearity, calibration curves for each element were constructed by plotting the peak area of the optimum emission line to the concentration of the standard solution. Subsequently, a least squares linear regression analysis was employed to determine the slope and the intercept of the calibration curve and to evaluate the coefficients of

determination [22]. In order to construct the calibration curves, multi-element solutions at a concentration of 10, 25, 50, 250 and 1000 and 2500 $\mu\text{g g}^{-1}$ were added to 1.5 M nitric acid. Table 1 summarizes the calibration curves and the coefficients of determination of the ICP-AES method. As can be observed, the coefficients of determination for all metals in the entire working range were good ($r^2 > 0.9970$).

Table 1. Regression analysis, limits of detection (LOD) and limits of quantification (LOQs) of the inductively coupled plasma atomic emission spectrometry (ICP-AES) method.

Element	Emission Line (nm)	Slope	Intercept	r^2	LOD ¹ ($\mu\text{g g}^{-1}$)	LOQ ² ($\mu\text{g g}^{-1}$)
Ba	230.425	64.34	+936.22	0.9998	0.50	1.66
Cd	226.502	40.52	+607.75	0.9999	0.33	1.11
Cu	324.752	2375.00	+74,996.00	0.9999	2.52	8.40
Fe	238.204	105.91	+1036.10	0.9970	0.89	2.96
Pb	217.000	3.95	−61.61	0.9999	1.17	3.90
Ni	232.003	20.41	−240,197	0.9999	0.95	3.16
Mn	257.61	857.99	+7192.31	0.9999	0.23	0.78
Zn	213.857	32.33	+32.33	0.9999	0.42	1.40
Al	308.215	233.08	+2654.20	0.9998	2.40	7.99
Cr	357.869	1480.30	−18,850.00	0.9999	0.16	0.54
Mg	280.271	1820.00	+76,096.00	0.9990	0.01	0.02
Ca	317.933	104.66	+5696.90	0.9993	0.83	2.76

¹ Limits of detection; ² Limits of quantification.

For the calculation of the limits of detection (LODs) and the limits of quantification (LOQs) of the proposed ICP-AES method, blank solutions were used based on the guidelines of the International Union of Pure and Applied Chemistry (IUPAC). For this purpose, ten separate blank solutions were prepared and analyzed. The LOD values of the ICP-AES technique equal to three times the standard deviation of the measurements for the blank solutions was divided by the slope of the calibration curves for each element. Similarly, the LOQ value of the ICP-AES technique equal to ten times the standard deviation of the measurements for the blank solutions was divided by the slope of the calibration curves for each element. The LODs and LOQs of the ICP-AES method are also shown in Table 1. As it can be observed, the LODs and the LOQs ranged between 0.01–2.52 $\mu\text{g g}^{-1}$ and 0.02–8.40 $\mu\text{g g}^{-1}$, respectively.

3.3. Real Samples Analysis

All nut samples were analyzed in triplicate ($n = 3$) with relative standard deviation (RSD) $< 8\%$ for all metals. The concentration ranges of the determined calcium, magnesium, iron, manganese, copper, zinc, aluminum and chromium are presented in Table 2. The concentration ranges for calcium, magnesium, iron, manganese, copper, zinc and aluminum were similar with those of other studies about the elemental composition of nuts [3,8,23,24]. The concentrations for chromium ranged between 0.03 to 3.64 $\mu\text{g g}^{-1}$ and were in accordance with those reported in a previous study [8] for the majority of samples. Cadmium and lead were below the LOQs, which was also the case in other studies [8,17] as well. However, in this study barium and nickel were below the LOQs while their concentrations in nuts were previously reported to be from not detected to 0.11 $\mu\text{g g}^{-1}$ for barium [8] and 0.10–0.51 $\mu\text{g g}^{-1}$ for nickel [24].

Table 2. Metal concentration ranges in nut samples of a Greek market.

Element ($\mu\text{g g}^{-1}$)	Almond	Pistachio	Walnut	Hazelnut
Ca	817–1439	86.9–787	328–932	738–1265
Mg	1150–1681	112–718	573–901	688–883
Fe	29.8–68.2	4.25–26.6	19.3–56.8	27.0–64.0
Zn	49.7–56.4	8.51–35.1	31.1–52.9	35.2–70.1
Al	1.17–3.82	0.85–3.00	1.50–2.98	1.28–3.04
Mn	9.21–31.5	6.98–19.4	5.86–23.1	0.57–33.2
Cu	7.24–15.0	1.77–9.85	0.19–5.92	6.61–10.8
Cr	0.85–3.64	0.90–3.29	0.79–2.52	0.03–3.64

The quantification results of the determined metals were further analyzed with ANOVA to evaluate if there was a significant statistical difference in the concentrations of calcium, magnesium, iron, zinc, aluminum, manganese and copper.

The highest average concentration for calcium was determined in almonds ($1105 \mu\text{g g}^{-1}$) followed by $650 \mu\text{g g}^{-1}$ in pistachios, $571 \mu\text{g g}^{-1}$ in walnuts and $950 \mu\text{g g}^{-1}$ in hazelnuts. The Box and Whisker plot for the concentrations of calcium in different nut species is presented in Figure 1. After comparing the variances of calcium content in almonds, pistachios, walnuts and hazelnuts, it was concluded that there was a statistical difference among the nut species because the ANOVA showed a p -value < 0.05 ($p = 3.34 \times 10^{-7}$).

Figure 1. Box and Whisker plot for the concentrations of calcium among different nut species.

The average concentration for magnesium was $1313 \mu\text{g g}^{-1}$ in almonds followed by $805 \mu\text{g g}^{-1}$ in hazelnuts and $713 \mu\text{g g}^{-1}$ in walnuts while the lowest concentration was observed in pistachios ($310 \mu\text{g g}^{-1}$) as shown in Figure 2. The ANOVA demonstrated a p -value of 3.68×10^{-7} and showed a significant statistical difference among the nut samples.

Figure 2. Box and Whisker plot for the concentrations of magnesium among different nut species.

In the case of iron, Figure 3 indicates that the highest average concentration was determined in almonds ($48 \mu\text{g g}^{-1}$) followed by hazelnuts ($37.5 \mu\text{g g}^{-1}$), walnuts ($34.4 \mu\text{g g}^{-1}$) and pistachios ($12.8 \mu\text{g g}^{-1}$). Concerning the concentration of iron, the ANOVA showed that there was a significant statistical difference for the analyzed almonds, pistachios, walnuts and hazelnuts ($p = 8 \times 10^{-6}$).

Figure 3. Box and Whisker plot for the concentrations of iron among different nut species.

Figure 4 graphically illustrates that the highest average concentration of zinc was determined in almonds ($52.2 \mu\text{g g}^{-1}$). Walnuts and hazelnuts exhibited close average concentrations of 43.5 and $46.4 \mu\text{g g}^{-1}$, respectively. The lowest was determined in pistachios ($24.0 \mu\text{g g}^{-1}$). The ANOVA analysis showed that there was a significant statistical difference in zinc concentration between almonds, pistachios, walnuts and hazelnuts

($p = 6.83 \times 10^{-5}$). In addition, there was a significant statistical difference in the concentration of aluminum among all nut species ($p = 0.02$).

Figure 4. Box and Whisker plot for the concentrations of zinc among different nut species.

According to Figure 5, the highest aluminum concentration was determined in almonds ($2.50 \mu\text{g g}^{-1}$) followed by $2.09 \mu\text{g g}^{-1}$ in walnuts and $1.89 \mu\text{g g}^{-1}$ in hazelnuts while the lowest average concentration was determined in pistachios ($1.36 \mu\text{g g}^{-1}$). The ANOVA showed a statistically significant difference among the samples ($p = 0.02$).

Figure 5. Box and Whisker plot for the concentrations of aluminum among different nut species.

Concerning the ANOVA analyses of copper and manganese, the average concentrations did not show a statistically significant difference for both elements because the calculated p -values were equal to $p = 0.06$ and $p = 0.43$, respectively. Figure 6 demonstrates that the highest average concentration of copper was determined in almonds ($10.9 \mu\text{g g}^{-1}$). The second highest average concentration of copper was determined in

hazelnuts ($8.32 \mu\text{g g}^{-1}$); $5.15 \mu\text{g g}^{-1}$ were determined in pistachios and $2.62 \mu\text{g g}^{-1}$ were determined in walnuts.

Figure 6. Box and Whisker plot for the concentrations of copper among different nut species.

As far as manganese concentration was concerned, the average concentration in almonds was $17.5 \mu\text{g g}^{-1}$ followed by $14.1 \mu\text{g g}^{-1}$ in walnuts, $13.1 \mu\text{g g}^{-1}$ in hazelnuts and $11.1 \mu\text{g g}^{-1}$ in pistachios (Figure 7). The ANOVA test showed a significant statistical difference in manganese concentrations among the nut species ($p = 0.004$).

Figure 7. Box and Whisker plot for the concentrations of manganese among different nut species.

The average concentrations of chromium in pistachios, walnuts and hazelnuts were very close (1.00 , 1.78 and $1.03 \mu\text{g g}^{-1}$, respectively) as shown in Figure 8. The average concentration in almonds was higher ($3.56 \mu\text{g g}^{-1}$). The ANOVA test exhibited a significant statistical difference in chromium concentration among all of the nut species with $p = 0.005$. However, there was no statistically significant difference between pistachios, walnuts and hazelnuts ($p = 0.22$).

Figure 8. Box and Whisker plot for the concentrations of chromium among different nut species.

Finally, the average concentrations of calcium and magnesium among the different nut species are shown in Figures 9 and 10, which graphically illustrate the average concentrations of the rest of the metals (Mn, Cu, Fe, Zn, Cr, Al) determined at lower concentrations. As it can be observed, almonds exhibited the highest mineral content based on the metals determined followed by pistachios, walnuts and hazelnuts.

Figure 9. Average content of Ca and Mg among almonds, pistachios, walnuts and hazelnuts available in a Greek market.

Figure 10. Average content of Mn, Cu, Fe, Zn, Cr and Al among almonds, pistachios, walnuts and hazelnuts available in a Greek market.

This study gives considerable evidence that the nuts available in Greek markets contained a wide range of trace elements in significant amounts. The concentration levels of the determined metals varied depending on the nut species (almonds, pistachios, walnuts or hazelnuts). Overall, the findings support that nuts are rich in minerals that have already been related to health benefits and can contribute to the prevention of nutritional insufficiencies [25], amplifying that they constitute a valuable nutritional source.

4. Conclusions

This study contributes to the existing knowledge concerning the nutritional value and mineral profile of different nut species including almonds, pistachios, walnuts and hazelnuts available in a Greek market. The reported results are in accordance with the dietary guidelines recommended of each element for the daily intake. The ICP-AES method exhibited a wide linearity range and good coefficients of determination as well as low LODs ($0.1\text{--}2.52\text{ }\mu\text{g g}^{-1}$) and LOQs ($0.02\text{--}8.40\text{ }\mu\text{g g}^{-1}$). Calcium and magnesium were the most abundant elements in all samples followed by zinc and iron. Nickel, lead, cadmium and barium were below the LOQs, assuring the safety of the analyzed samples. Overall, the highest average elemental concentrations were observed in almonds followed by pistachios, walnuts and hazelnuts. The results highlight the elemental micro-nutrient profile of nuts, recognizing their high nutritional value and potential contribution to health promotion.

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