

Spectroscopic Determination of Metals in Palm Oils from Different Stages of the Technological Process

Aleksandra Szydłowska-Czeriak,^{*,†} Konrad Trokowski,[†] György Karlovits,[§] and Edward Szlyk[†]

[†]Faculty of Chemistry, Nicolaus Copernicus University, ul. Gagarina 7, 87-100 Toruń, Poland

[§]Bunge Europe Research and Development Center, ul. Niepodległości 42, 88-150 Kruszwica, Poland

ABSTRACT: Magnesium, calcium, copper, iron, and lead in palm oils (*Elaeis guineensis*) at various stages of the refining process were determined by inductively coupled plasma mass spectrometry (ICP-MS) after microwave digestion. The mean concentrations of Mg, Ca, Cu, Fe, and Pb in the studied palm oils varied from 20.7 to 7090.1 $\mu\text{g kg}^{-1}$, from 193.9 to 8077.9 $\mu\text{g kg}^{-1}$, from 29.7 to 463.0 $\mu\text{g kg}^{-1}$, from 115.2 to 415.9 $\mu\text{g kg}^{-1}$, and from 1.7 to 16.0 $\mu\text{g kg}^{-1}$, respectively, which are below the Polish legal requirements. The comparable precisions for the proposed ICP-MS (RSD = 0.81–5.99%) and standard GFAAS (RSD = 1.18–5.26%) methods demonstrate the benefit of the ICP-MS method in the routine analysis of metal ions in palm oils. There are significant, positive correlations between Ca and Mg, between Ca and Cu, between Fe and Pb, between Cu and Fe, between Cu and Mg, and between Cu and Pb in palm oils determined by two analytical methods ($r = 0.8798\text{--}0.9817$, $p < 0.05$). Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were used for discrimination of the quality of the analyzed palm oils based on main and trace metal contents determined by the proposed ICP-MS and the standard GFAAS methods. Two main groups were identified by HCA, whereas the classification and characterization of the studied palm oils within each of groups on the basis of metal ions amounts were obtained from PCA. The chemometric analyses demonstrated that crude palm oil had the highest level of the determined metals concentrations. Also, the analyzed metals in palm oils from different steps of the refining process were grouped using HCA to assess the effectiveness of technological processes for their removal.

KEYWORDS: palm oils, refining process, metal ions content, ICP-MS, chemometrics

INTRODUCTION

Palm oil from fruits of palm trees (*Elaeis guineensis*) is one of the major fat sources in the world, widely used in food production and direct consumption purposes.¹ It is an oxidatively and thermally stable vegetable oil due to a fatty acid composition with significant amounts of saturated fatty acids at the sn2-position of the triglycerides, a low level of polyunsaturated fatty acids, and high contents of antioxidant such as tocopherols, tocotrienols, carotenoids, phytosterols, and polyphenolics. However, the refining processes reduce antioxidant compounds content in crude palm oils.² The large variation of antioxidants reduction in different stages of technological process can be explained by differences in plant conditions and design, which would affect the tocols and other antioxidants content in palm oils.³ Moreover, palm oil has a lot of practical applications because of its high productivity and availability and low price, as well as physical characteristics such as plasticity at room temperature and accessibility to fractionation. Therefore, palm oil and its fractions have often been applied in trans fat free products as an alternative source of hard stock in margarines and shortenings, ice creams, and dairy products.⁴

However, the dramatic increase in palm oil cultivation and production has resulted in considerable environmental damage by removal of ecological systems displaced by palm oil tree monocultures in tropical areas such as Malaysia and Indonesia.⁵

On the other hand, metals content in edible oils and their products is one of the most important quality criteria. Lipid oxidation is retarded by antioxidant compounds and accelerated

by prooxidants such as trace metals. Metal ions catalyze the decomposition of hydroperoxides to free radicals and secondary oxidation products such as aldehydes, ketones, acids, and epoxides, which can increase the carcinogenic effect by reacting with other food components.^{3,6} It is noteworthy that the lipid oxidation process is related to several oil physicochemical parameters and sensorial oil properties.

Moreover, heavy metals are very dangerous for human health. Lead is considered to be one of the most dangerous elements for human life, because it can cause blood and brain disorders and damage the human nervous system. Pb and Cd, in particular, are cumulative poisons.

However, antioxidants of palm oil offer some protection against Pb and Cd toxicity by reducing their accumulation in animal tissues.^{7,8} Also, copper and iron have a biological action at low doses, but can be toxic in larger amounts. Both metals are necessary trace elements found in nearly all living organisms. Many enzymes and proteins containing Cu or Fe participate in biological oxidations and transport. The determination of these trace metals is very important, because they can catalyze oxidation processes of fatty acid chains, exerting a deleterious influence on shelf life and nutritional value.⁹ Calcium and magnesium are common cellular ionic messengers with many functions and are found as structural

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elements in bones. Both metals are complexing agents and can inactivate some important compounds such as phenolics.

The content of heavy metals in palm oils depends on several factors. These metals are naturally present in fruits of palm trees (*E. guineensis*), because they may be absorbed from the soil and fertilizers or environmental pollutants. Moreover, the production processes, materials of packaging, and storage of palm oils and their products may be sources of heavy metals contamination.^{3,10,11}

It has been known that palm kernel is a good source of mineral components such as Ca, Mg, K, and P.^{12,13} Therefore, it is highly probable that crude palm oils contain Ca, Mg, and other cations, which may form oil-soluble soaps and non-hydratable gums consisting mainly of the calcium and magnesium salts of phospholipids. In addition, the oil may be contaminated with these cations during the washing process with hard water or from adsorbents used in cleaning processes. On the other hand, mono- and divalent cations (Na^+ and Ca^{2+}) were indicated to efficiently reduce acrylamide formation. It was postulated that these ions could interact with asparagine so that the Schiff base formation was again prevented.¹⁴

Metal cations in palm oils may be present as (1) complexes surrounded by proteins, phospholipids, lipids, or nonlipid carriers and (2) suspended solid impurities in oil. These metal complexes can have a deleterious effect on oil quality and should be removed in refining steps. Therefore, it is important to determine the concentration of the trace heavy metals and minerals in palm oils at various stages of technological processes. Analysis of metal ions in palm oils and other fats requires a sample preparation step to release elements from the fat matrix.

Different procedures of sample preparation, for example, wet digestion,^{12,13} microwave digestion,¹⁵ ultrasound-assisted extraction,¹⁰ dissolving oil in hexane and then eluting with hexane through a column of aluminum oxide,¹¹ dry ashing,^{16–19} and homogenization of freeze-dried samples,²⁰ were applied in analytical techniques for analysis of metals in palm kernel, fruit, leaf, frond, husk, shell, seeds, and oils.

Among others, atomic absorption spectrometry (AAS),^{10–12,15,16,18} inductively coupled plasma (ICP) spectrophotometry,^{13,15,17,19} flame photometry,¹² anodic stripping chronopotentiometry,¹⁰ and capillary electrophoresis¹⁸ were used for trace metal analysis in palm and its products.

It has been known that trace quantities of Cu, Fe, Mn, and Ni substantially reduce the oxidative stability of fats and oils, whereas Ca, Mg, and Na reduce the efficiency of the refining, degumming, bleaching, and hydrogenation systems. Therefore, it seems worthy to consider the determination of trace metals of nutritional and toxicological interest in a wide range of concentrations in palm oils at different stages of conventional technological operations.

To the best of our knowledge, there has been no reference on the determination of Mg, Ca, Cu, Fe, and Pb levels in palm oils (*E. guineensis*) during various steps of the refining process using the inductively coupled plasma mass spectrometry (ICP-MS) method after microwave digestion. Only Rossi et al.¹¹ used a graphite furnace atomic absorption spectrometry (GFAAS) method to study the effect of concentration and type of bleaching clays on heavy metals contents such as Cu, Fe, and Pb in the crude, degummed, bleached, and refined palm oils.

Therefore, in the present paper, an inductively coupled plasma mass spectrometric (ICP-MS) method was used for the quantitative analysis of Mg, Ca, Cu, Fe, and Pb in crude, rinsed,

degummed, bleached, and deodorized palm oils after microwave digestion and compared to the official standard GFAAS method for accuracy and precision. Because the GFAAS procedure does not demonstrate a benefit in the routine analysis of metal ions in fat samples due to its time-consuming and monoelement analysis characteristics, ICP-MS was proposed for simultaneous determination of Mg, Ca, Cu, Fe, and Pb in palm oils. Besides, correlations between the metals contents determined by ICP-MS and GFAAS methods were examined and discussed. Also, chemometric methods such as principal component analysis (PCA) and hierarchical cluster analysis (HCA) were used for grouping of the determined metal ions and the studied palm oils, their characterization, and detection of differences.

MATERIALS AND METHODS

Reagents and Materials. All reagents were of analytical ultrapure grade. Concentrated nitric acid (65%) of ultrapure grade (maximum $0.01 \mu\text{g mL}^{-1}$ of Cu and Pb, $0.1 \mu\text{g mL}^{-1}$ of Fe, Mg, and Ca, respectively) was supplied by POCh (Gliwice, Poland). Hydrogen peroxide ($\geq 30\%$, TraceSELECT Ultra, for ultra trace analysis) and palladium(II) nitrate in 15% nitric acid solution ($10.0 \pm 0.3 \text{ mg mL}^{-1}$ Pd, for GFAAS) were purchased from Sigma-Aldrich (Poznań, Poland). Standard solutions for each element ($10 \mu\text{g}$ of Cu mL^{-1} , $10 \mu\text{g}$ of Fe mL^{-1} , $10 \mu\text{g}$ of Pb mL^{-1} , $50 \mu\text{g}$ of Ca mL^{-1} , and $50 \mu\text{g}$ of Mg mL^{-1}) were obtained by diluting unipure commercial standards ($1000 \mu\text{g mL}^{-1}$, Spectrosol, United Kingdom) in the deionized water. Deionized water (DW, specific conductivity = $0.05 \mu\text{S cm}^{-1}$) was obtained from a Hydrolab system (Hydrolab, Poland).

All solutions and samples were prepared in plastic volumetric flasks using deionized water. To eliminate possible contamination from detergents or materials, all glassware and polyethylene containers were soaked in 6 M HNO_3 solution (for at least 24 h) and rinsed several times with deionized water. Finally, dried material was appropriately kept until use. Polyethylene tips were cleaned according to the same procedure and then used for micropipetting.

Samples. Five palm oils at various stages of physical refining processes, crude palm oil (CPO), palm oil after rinsing (POAR), degummed palm oil (DPO), palm oil after bleaching and filtration (POABF), and palm oil after deodorization (POAD), were kindly provided by a local vegetable oil factory. All palm oils in the original packing (polypropylene (PP) containers) were stored at 4°C in the dark until the analysis.

Sample Preparation for Determination of Metal Ions. Palm oil samples were heated to 40°C in a water bath for 15 min to obtain a representative portion of homogeneous materials. The liquid oil samples were digested as follows: samples were accurately weighed on an analytical balance in the range $0.3\text{--}0.4 \text{ g}$ (± 0.0001) and transferred into the reaction vessel (Teflon Hostafen TFM, Berghof); 10 mL of nitric acid and 3 mL of hydrogen peroxide were added and digested in a closed microwave system (Ertec, Wrocław Poland). The program of mineralization included three stages: (I) 5 min at 180 W ($T = 190\text{--}200^\circ\text{C}$, pressure = $16\text{--}19 \text{ MPa}$), (II) 10 min at 240 W ($T = 295\text{--}300^\circ\text{C}$, pressure = $27\text{--}30 \text{ MPa}$), and (III) 10 min at 300 W ($T = 295\text{--}300^\circ\text{C}$, pressure = $30\text{--}40 \text{ MPa}$). After cooling, obtained clear solutions were transferred quantitatively into 50 mL plastic volumetric flasks and made up to the mark with deionized water. The digestion procedure was done in triplicate for each oil sample. Blank digestions were carried out in the same way.

Instruments. Inductively Coupled Plasma Mass Spectrophotometric Analysis. Total Mg, Ca, Cu, Fe, and Pb were quantified using an inductively coupled plasma mass spectrometer (ICP-MS 7500 CX, Agilent Technologies, Japan). The apparatus was equipped with radial and axial configurations of operation and software. The samples were introduced into the ICP-MS via a cross-flow nebulizer, spray chamber, and autosampler. The instrument was calibrated with aqueous reference standards, and precautions were taken against spectral

interferences. Operating conditions of the ICP-MS quadrupole spectrometer are listed in Table 1.

Table 1. Operating Conditions of the ICP-MS Spectrometer

parameter	data
generator RF frequency (MHz)	27.12
RF power (W)	500–1600
carrier gas	Ar X50S 5.0
mass analyzer	quadrupole
mass range	2–260
gas in collision chamber	He
resolution m/z	238 amu
background	<5 cps (9 amu)
isotope ratio precision CeO/Ce	<3%
short-term stability	<3% RSD
long-term stability	<4% RSD/2 h
sensitivity (cps/ppm)	80×10^6 ^{89}Y
isotopes measured	^{24}Mg , ^{43}Ca , ^{63}Cu , ^{57}Fe , ^{208}Pb
detection limit ($\mu\text{g mL}^{-1}$)	
Mg	9.69×10^{-4}
Ca	7.46×10^{-4}
Cu	3.11×10^{-5}
Fe	5.83×10^{-5}
Pb	4.15×10^{-6}

Three multielement calibration solutions were prepared at different concentration levels (0.25–5 $\mu\text{g mL}^{-1}$) from 10 $\mu\text{g mL}^{-1}$ single-element unipure grade standards using ultrapurity nitric acid matched to the samples matrix. A calibration curve, at 10 different concentrations (0.05–1.0 $\mu\text{g mL}^{-1}$), was made using these multielement standards ($R^2 = 0.9997$). A further three-point calibration curve at higher concentration levels (5, 10, and 25 $\mu\text{g mL}^{-1}$) was required to perform the analysis of Mg and Ca due to the high concentration level of these elements in the samples.

The detection limits (DL) were calculated as the concentrations corresponding to signals equal to 3 times the standard deviation of 10 reagent blank solutions (Table 1). To check for instrumental drift, one of the multielement standards was analyzed for every 10 samples.

Atomic Absorption Spectroscopic Analyses. A Varian 20 ABQ GFAAS with deuterium lamp background corrector was used. Metals amounts were determined according to the GFAAS method adding, for each injection, 5 μL of 10 mg mL^{-1} palladium(II) nitrate in 15% nitric acid solution as a matrix modifier. Analyses were carried out at the most sensitive analytical spectral lines of the metals (Mg, 285.2 nm; Ca, 422.7 nm; Cu, 324.8 nm; Fe, 248.3 nm; Pb, 283.3 nm). The within-day precision (repeatability) was found by regression analysis of curves: $y = (0.0461 \pm 0.0014)x - (0.0005 \pm 0.0026)$, $y = (0.0493 \pm 0.0011)x - (0.0001 \pm 0.0007)$, $y = (0.0434 \pm 0.0027)x - (0.0062 \pm 0.0017)$, $y = (0.0129 \pm 0.0005)x - (0.0009 \pm 0.0065)$, and $y = (0.0047 \pm 0.0009)x - (0.0020 \pm 0.0010)$ for Mg, Ca, Cu, Fe, and Pb, respectively, and expressed as the relative standard deviation (RSD% < 5, $n = 5$) of the slopes. The calibration plots were linear ($R^2 = 0.9952$,

Table 2. Determination of Mg, Ca, Cu, Fe, and Pb Ions in Palm Oils from Different Stages of the Refining Process^a

statistical parameter	CPO		POAR		DPO		POABF		POAD	
	ICP ^b	AAS ^c	ICP ^b	AAS ^c	ICP ^b	AAS ^c	ICP ^b	AAS ^c	ICP ^b	AAS ^c
Mg ($\mu\text{g kg}^{-1}$)	5561.2 c,y	5578.3 c,y	7090.1 c,z	7050.3 c,z	753.5 d,x	746.3 d,x	27.1 b,w	25.7 b,w	20.7 b,w	19.7 b,w
SD ($\mu\text{g kg}^{-1}$)	263.2	108.1	194.9	177.4	45.1	12.1	1.6	1.3	0.3	0.5
RSD (%)	4.73	1.94	2.75	2.52	5.99	1.62	5.90	5.06	1.45	2.54
F_{calcd}		5.93		1.21		13.97		1.50		2.45
t_{calcd}		0.13		0.30		0.33		1.33		3.42
Ca ($\mu\text{g kg}^{-1}$)	8077.9 d,x	8012.2 d,x	8006.2 d,x	7916.0 d,x	193.9 c,w	188.0 c,w	218.2 d,w	208.3 d,w	402.4 e,w	381.9 e,w
SD ($\mu\text{g kg}^{-1}$)	127.4	150.1	441.7	201.0	11.5	8.8	4.9	2.7	12.7	15.2
RSD (%)	1.58	1.87	5.52	2.54	5.93	4.68	2.25	1.30	3.16	3.98
F_{calcd}		1.39		4.83		1.72		3.11		1.44
t_{calcd}		0.63		0.39		0.81		3.68		1.97
Cu ($\mu\text{g kg}^{-1}$)	463.0 b,z	453.1 b,z	322.8 b,y	331.3 b,y	67.9 b,x	67.6 b,x	29.9 b,w	28.7 b,w	29.7 c,w	32.5 c,w
SD ($\mu\text{g kg}^{-1}$)	19.1	21.8	10.2	13.7	3.3	0.8	1.2	1.0	1.0	1.7
RSD (%)	4.13	4.81	3.16	4.14	4.86	1.18	4.01	3.48	3.37	5.23
F_{calcd}		1.30		1.83		17.12		1.50		3.04
t_{calcd}		0.65		0.93		0.19		1.58		2.60
Fe ($\mu\text{g kg}^{-1}$)	415.9 b,z	411.4 b,z	234.2 b,y	228.3 b,y	190.2 c,x	190.1 c,x	118.0 c,w	115.6 c,w	115.2 d,w	112.7 d,w
SD ($\mu\text{g kg}^{-1}$)	22.0	15.1	1.9	8.0	11.1	8.2	4.7	2.1	5.8	4.5
RSD (%)	5.29	3.67	0.81	3.50	5.84	4.31	3.98	1.82	5.03	3.99
F_{calcd}		2.12		16.86		1.86		4.88		1.64
t_{calcd}		0.34		1.25		0.02		0.96		0.70
Pb ($\mu\text{g kg}^{-1}$)	16.0 a,z	16.5 a,z	8.4 a,y	8.3 a,y	6.8 a,x	6.7 a,x	1.7 a,w	1.9 a,w	6.0 a,x	6.2 a,x
SD ($\mu\text{g kg}^{-1}$)	0.7	0.8	0.4	0.1	0.4	0.2	0.05	0.1	0.3	0.2
RSD (%)	4.38	4.85	4.76	1.20	5.88	2.99	2.94	5.26	5.00	3.23
F_{calcd}		1.04		15.2		5.00		4.44		4.42
t_{calcd}		0.86		0.44		0.32		2.72		1.62

^aSD, standard deviation; RSD, relative standard deviation; $F_{\text{theor}} = 19.25$ and $t_{\text{theor}} = 2.78$ for $p = 0.05$. Different letters (a–e) within the same column indicate significant differences between metal ions contents determined in each palm oil (one-way ANOVA and Duncan test, $p < 0.05$). Different letters (w–z) within the same row for each metal ion level in palm oils from different stages of the refining process analyzed by ICP-MS and GFAAS methods, respectively, differ significantly ($p < 0.05$). ^b $n = 5$. ^c $n = 3$.

0.9990, 0.9951, 0.9946, and 0.9932) in the concentration ranges between 1.02 and 16.30 $\mu\text{g mL}^{-1}$, between 1.13 and 17.02 $\mu\text{g mL}^{-1}$, between 0.20 and 5.33 $\mu\text{g mL}^{-1}$, between 0.61 and 1.84 $\mu\text{g mL}^{-1}$, and between 0.01 and 0.80 $\mu\text{g mL}^{-1}$ for Mg, Ca, Cu, Fe, and Pb, respectively. The calculated detection limits were 0.33, 0.19, 0.064, 0.097, and 0.0032 $\mu\text{g mL}^{-1}$ for Mg, Ca, Cu, Fe, and Pb, respectively.

Statistical Analysis. Statistical analyses of data were performed using Statistica (Windows software package, version 8.0). The results of metals contents were presented as mean ($n = 5$ for ICP-MS method and $n = 3$ for GFAAS method) with their standard deviation (SD). The within-day precision of analytical methods expressed as the relative standard deviation (RSD%) was assessed. One-way analysis of variance (ANOVA), followed by the Duncan test, was performed to analyze the significant differences between data ($p < 0.05$). The proposed ICP-MS and the official standard GFAAS methods were compared for within-day (repeatability) precision using the F test. Student's t test was also employed to identify significant differences ($p < 0.05$) between mean concentrations of analytes determined by the two analytical methods. Moreover, the Pearson correlation test was used to determine the correlations between metals contents in palm oils from different stages of the refining process determined by the two analytical methods. Moreover, data were submitted to chemometric analyses such as PCA and HCA. PCA was employed to study clustering and differentiation of five palm oils from different stages of technological processes on the basis of Mg, Ca, Cu, Fe, and Pb results. The scores and loadings of the data analyzed by PCA were displayed as biplots. HCA with Ward's method using Euclidean distances was also applied to identify palm oils on the basis of the degree of similarity among their metals concentrations. Besides, HCA was used for grouping different metals to recognize their amounts in palm oils at various stages of the refining process and assess the effectiveness of the technological processes. The palm oil samples and metals similarities were represented on two-dimensional diagrams (dendrograms).

RESULTS AND DISCUSSION

Metal Ions Contents in Palm Oils at Different Stages of the Refining Process. The mean concentrations of Mg, Ca, Cu, Fe, and Pb in the analyzed palm oils at various stages of the refining process determined by the proposed ICP-MS method were compared with these obtained by the GFAAS method (Table 2).

The conventional physical refining process of crude palm oil (CPO) involves degumming with 50% citric acid and subsequent adsorptive cleaning with bleaching earth to remove undesirable impurities such as trace metals, moisture, insolubles, pigments, and phospholipids and reduce the oxidation products. The bleached palm oil is deodorized by steam distillation at elevated temperature under reduced pressure to remove volatile compounds. Among the analyzed samples, CPO revealed the highest amounts of metal ions, whereas the final product of the refining process (POAD) contained the lowest levels of Mg, Cu, and Fe only (Table 2). The results collected in Table 2 (t values and Duncan test) indicate that there are no significant differences between the mean contents of the analyzed metal ions in all palm oil samples assayed by both analytical methods. However, it is evident that the mean levels of metals in palm oils from different steps of technological processes are significantly different from one another (Duncan test, Table 2). The Duncan test indicated that POABF and POAD did not differ significantly in mean amounts of Mg, Ca, Cu, and Fe, whereas, unexpectedly, the Pb level in POAD was >3 higher than in POABF. This can be explained by the fact that Pb was probably introduced to the final product during the deodorization process from the used equipment (deodorizer), which can contain metal parts. Also, insignificant differences for mean Ca

values were observed between CPO and POAR (Table 2). Therefore, Ca was not removed from CPO by hot demineralized water washing, and the addition of water to CPO did not increase the concentration of Ca ions in POAR. On the contrary, the mean content of Mg in POAR was about 27% higher than its level in CPO (Table 2). Probably, CPO was washed with water that contained high amounts of Mg ions. Therefore, the highest Mg concentration was revealed in POAR, whereas the level of this element was lowest for POAD. This indicated that the refining process of CPO ensures a decrease of the Mg content in refined oil. It is noteworthy that the mean concentration of Ca in palm oils obtained by traditional technological processes ranged between 193.9 ± 11.5 and $8077.9 \pm 127.4 \mu\text{g kg}^{-1}$ and between 188.0 ± 8.8 and $8012.2 \pm 150.1 \mu\text{g kg}^{-1}$ for ICP-MS and GFAAS methods, respectively. The results of metal chelating determinations suggested that the studied palm oils are richer sources of Ca than Mg ions. For comparison, in Njoku's work,¹⁶ the contents of Mg and Ca ions in crude palm oils extracted from three species of *E. guineensis* were much lower and varied between 370 and 1130 $\mu\text{g Mg kg}^{-1}$ and between 340 and 460 $\mu\text{g Ca kg}^{-1}$. However, defatted and undefatted palm kernels ($c_{\text{Mg}} = (22.5\text{--}55.4) \times 10^5 \mu\text{g kg}^{-1}$ and $c_{\text{Ca}} = (7.5\text{--}23.5) \times 10^5 \mu\text{g kg}^{-1}$),^{12,13} palm berries ($c_{\text{Ca}} = 26.0 \times 10^5 \mu\text{g kg}^{-1}$),²⁰ fronds, shells, husks, and leaves ($c_{\text{Mg}} = (30.0\text{--}60.0) \times 10^5 \mu\text{g kg}^{-1}$ and $c_{\text{Ca}} = (20.0\text{--}60.0) \times 10^5 \mu\text{g kg}^{-1}$)¹⁸ contain about 3 orders higher amounts of Mg and Ca than the studied CPO. Also, much higher amounts of chelating ions in palm seeds ($c_{\text{Mg}} = (6.1311\text{--}17.5903) \times 10^5 \mu\text{g kg}^{-1}$ and $c_{\text{Ca}} = (0.6451\text{--}5.6666) \times 10^5 \mu\text{g kg}^{-1}$) were determined by Ali-Mohamed and Khamis.¹⁵

Moreover, production processes such as degumming, bleaching, filtration, and deodorization did not significantly affect the Ca amounts in those palm oils (Duncan test, Table 2), although a surprisingly high mean level of Ca in refined palm oil (POAD) was determined.

It is evident that the highest decrease of two nutrient elements, Mg (about 87%) and Ca (about 97%), and prooxidant Cu ions (about 85%) took place during the degumming of the palm oil. However, the highest removal of two heavy metals, prooxidant Fe and toxic Pb (about 45%), from CPO was observed after rinsing of palm oil (Table 2).

Among the determined heavy metal ions, the mean content of Cu was the highest in all palm oil samples and ranged between 28.7 ± 1.0 and $463.0 \pm 19.1 \mu\text{g kg}^{-1}$. However, the mean level of Cu in all of the studied palm oils was lower when compared to results obtained by Cypriano et al. ($380\text{--}2670 \mu\text{g kg}^{-1}$).¹⁰ Unfortunately, the content of Cu in CPO is somewhat higher than the Polish Standard PN-EN ISO 8294:2000²¹ prescribed limit of $400 \mu\text{g Cu kg}^{-1}$ crude oil. Nevertheless, the level of Cu in the final product (POAD) is significantly lower than that recommended by Polish Standard PN-EN ISO 8294:2000²¹ for refined vegetable oils ($100 \mu\text{g kg}^{-1}$). For comparison, in Rossi's work,¹¹ the concentration of Cu in the crude palm oil was about 8 times lower ($60 \mu\text{g kg}^{-1}$), whereas similar levels of this element in the degummed ($60 \mu\text{g kg}^{-1}$) and refined ($30\text{--}40 \mu\text{g kg}^{-1}$) palm oils were reported. Palm kernels ($15900\text{--}27000 \mu\text{g kg}^{-1}$)^{12,13} and seeds ($2710\text{--}13420 \mu\text{g kg}^{-1}$)¹⁵ contain significantly higher amounts of Cu ions.

In addition, the mean concentration of prooxidant Fe ions in all analyzed oil samples ranged between 115.2 ± 5.8 and $415.9 \pm 22.0 \mu\text{g kg}^{-1}$ for ICP-MS and between 112.7 ± 4.5 and $411.4 \pm 15.1 \mu\text{g kg}^{-1}$ for GFAAS methods (Table 2). The obtained Fe results for the studied palm oils from different steps of the

Table 3. Correlation Coefficients (*r*) between Metal Ions Contents in Palm Oils at Various Stages of the Refining Process Determined by Two Analytical Methods^a

	Ca		Cu		Fe		Pb	
	ICP-MS	GFAAS	ICP-MS	GFAAS	ICP-MS	GFAAS	ICP-MS	GFAAS
Mg	0.9810**	0.9817**	0.9160*	0.9301*	0.7419	0.7365	0.6975	0.6862
Pb	0.7776	0.7708	0.8922*	0.8798*	0.9541*	0.9548*		
Fe	0.8178	0.8104	0.9383*	0.9224*				
Cu	0.9657**	0.9736**						

^a*, significant at the $p < 0.05$ level; **, significant at the $p < 0.01$ level.

refining processes were lower than those permitted in PN-EN ISO 8294:2000²¹ (1500 and 5000 $\mu\text{g kg}^{-1}$ for refined and crude oils, respectively). However, the mean amounts of Fe in the discussed oils were about 2 orders lower, when compared to results observed by Rossi et al.¹¹ (62000, 66000, and 800–1690 $\mu\text{g kg}^{-1}$ for crude, degummed, and refined palm oils, respectively) and Njoku et al.¹⁶ (38300–78300 $\mu\text{g kg}^{-1}$ for crude palm oils). Moreover, the significantly higher concentrations of Fe in the digested samples of palm kernels (42700–220000 $\mu\text{g kg}^{-1}$) and seeds (25270–60350 $\mu\text{g kg}^{-1}$), as well as in the freeze-dried palm berries (44000 $\mu\text{g kg}^{-1}$) were determined by other authors.^{12,13,15,20}

It is noteworthy that the mean contents of Pb (from 1.7 ± 0.05 to $16.0 \pm 0.7 \mu\text{g kg}^{-1}$ and from 1.9 ± 0.1 to $16.5 \pm 0.8 \mu\text{g kg}^{-1}$ for ICP-MS and GFAAS methods, respectively) in analyzed palm oil samples from various stages of the refining process were much lower than the regulated value (100 $\mu\text{g kg}^{-1}$).²² Similar concentrations of Pb in the crude (12.0 $\mu\text{g kg}^{-1}$) and steam-refined (<5.0 $\mu\text{g kg}^{-1}$) palm oils were determined by Rossi et al.,¹¹ whereas amounts of this element in the degummed (49.0 $\mu\text{g kg}^{-1}$) and bleached (<5.0 $\mu\text{g kg}^{-1}$) palm oils were about 7 and 3 times higher, respectively. However, a surprisingly high content of Pb in the commercial palm oils (260–1820 $\mu\text{g kg}^{-1}$ for a stripping chronopotentiometry assay (SCP) and 280–1900 $\mu\text{g kg}^{-1}$ for the GFAAS method) was analyzed by Cypriano et al.¹⁰ Specifically, the determined mean amount of Pb in CPO can be related to the environmental (geology, rain drainage, soil erosion) conditions. On the contrary, palm berries contain only 36.77 $\mu\text{g Pb kg}^{-1}$,²⁰ whereas this toxic metal was not detected in defatted and undefatted palm kernel.¹²

On the other hand, the mean concentrations of the chelating, prooxidant, and toxic metal ions in the studied palm oil samples significantly differ from each other (Duncan test, Table 2). This variability can be explained by the impact of genetic, environmental (natural contamination, bioaccumulation from the soil, pollution), and technological factors, which can affect the trace elements content. However, the mean contents of prooxidant metals, Cu and Fe, in palm oils from the stages before degumming (CPO and POAR) did not differ significantly (Duncan test, Table 2). Also, insignificant differences in the mean amounts of Ca and Fe in DPO were found. Moreover, there were no significant differences in the mean levels of Mg and Cu in POABF, whereas metals contents determined by two analytical methods in the refined palm oil (POAD) differed significantly (Duncan test, Table 2).

The values of RSD ranged between 0.81 and 5.99% and between 1.18 and 5.26%, indicating reasonable repeatability of the digestion procedure and the used methods, ICP-MS and GFAAS, respectively, for Mg, Ca, Cu, Fe, and Pb determinations in palm oils at different stages of the refining process (Table 2). For comparison, RSD values obtained for Cu and Pb

determination in palm oil samples by SCP and GFAAS methods were insignificantly higher (0.55 and 7.41%).¹⁰ Besides, similar RSD values in the determination of Mg, Ca, Cu, and Fe ions in defatted and undefatted palm kernels by AAS method (0.29–7.56%) were reported by Akpanabiutu et al.,¹² whereas significantly higher RSD values (5.80–30.39%) were calculated by Kok et al.¹³ for the analysis of these elements in kernels of the tenera and clonal palms (*E. guineensis*) using an ICP-OES method. Although results listed in Table 2 indicate that the average concentrations of metal ions in the studied palm oils and their standard deviations obtained by standard GFAAS method were somewhat lower (except c_{Mg} in CPO, c_{Cu} in POAR and POAD, c_{Pb} in CPO, POABF, and POAD) than those obtained by ICP-MS procedure, an *F* test revealed no significant difference between the variances (squares of the standard deviations) of the applied methods at $p = 0.05$. The calculated *F* values (the variance ratio of standard GFAAS and proposed ICP-MS methods for Mg, Ca, Cu, Fe, and Pb ions determinations, respectively), ranging from 1.04 to 17.12, are below $F_{\text{theor}} = 19.25$. Therefore, the proposed ICP-MS and standard GFAAS methods did not differ significantly in their precision. Also, the experimental *t* values for the studied metal ions in most palm samples were lower than the critical value $t = 2.78$ ($p = 0.05$). The results of *t* values collected in Table 2 indicate that there are significant differences between the mean content of Mg in POAD and Ca level in POABF, assayed by both analytical methods. Comparison of two experimental means of metals contents in the studied palm oils indicated that the proposed ICP-MS method in two cases is affected by systematic errors, although the results of Mg and Ca ions determinations in POAD and POABF samples obtained by the two analytical methods did not differ significantly at $p = 0.001$ because t_{calcd} values are below $t_{\text{theor}} = 8.61$ (Table 2).

Correlations between Mg, Ca, Cu, Fe, and Pb Ions Contents in Palm Oil Samples. Regression analysis was performed for the correlations among contents of metal ions in palm oils from different steps of the refining process determined according to the proposed ICP-MS and the standard GFAAS methods.

As can be seen in Table 3, significant correlations ($p < 0.01$) were found between mean amounts of Ca, Mg, and Cu in the studied palm oils (correlation coefficients ranged between 0.9657 and 0.9817). For comparison, a lower correlation coefficient ($r = 0.90$) for the relationship between Ca and Mg concentrations in dried Roselle seeds was reported by Hainida et al.²³ Moreover, the mean content of Cu in the palm oils determined by both analytical assays significantly correlated with Fe, Mg, and Pb levels in these samples ($r = 0.8798$ – 0.9383 , $p < 0.05$). The obtained results of metals concentrations in palm oils at various stages of technological processes indicated that there is a significant positive correlation between Fe and Pb amounts (Table 3). Also, linear positive correlations

between Fe and Cu ($r = 0.853$, $p < 0.001$), between Pb and Cu ($r = 0.752$, $p < 0.001$), and between Pb and Fe ($r = 0.798$, $p < 0.001$) in virgin argan oil samples were reported by Marfil et al.²⁴ Furthermore, Pb content in mushroom samples determined by the AAS assay significantly positively correlated with Cu amount ($r = 0.83$), whereas correlations among Fe–Cu and Fe–Pb were not significant and linear ($r = 0.09$ and 0.33 , respectively).²⁵

On the other hand, statistically insignificant correlations in linearity were observed between Ca and Fe, between Ca and Pb, between Mg and Fe, and between Mg and Pb contents in the studied palm oils analyzed by ICP-MS and GFAAS methods ($r = 0.6862$ – 0.8178 , $p > 0.05$). It is noteworthy that the highest correlation coefficients were calculated for the relationship among Mg and Ca concentrations in palm oil samples, whereas the lowest correlation coefficients were found between Mg and Pb contents in the discussed oils (Table 3).

The good correlation between Mg and Ca concentrations can be explained by the fact that these two elements are present together with organic compounds such as free fatty acids and phospholipids in vegetable oils, forming oil-soluble soaps and nonhydratable gums. Also, high statistically significant positive correlations between Cu and Ca, Mg, Fe, and Pb contents found in all studied samples can suggest a similarity in behavior and synergistic coexistence of those elements in palm oils. It can be noted that all relationships between the analyzed metal ions are direct. The positive correlations suggest that contents of main metals (Mg, Ca) in the palm oils from different stages of technological processes decreased with the decreasing concentrations of trace metals in those oil samples. In addition, positive, high correlations among the studied metals indicate that each step of the refining process effectively removed Mg, Ca, Cu, Fe, and Pb from palm oils.

Principal Component Analysis. PCA was applied to observe any possible groups within the analyzed palm oil samples from different stages of technological processes. A set of five orthogonal variables (PCs) was generated by PCA. The first principal component (PC1) had the highest eigenvalue of 4.47 and accounted for 89.40% of the variability in the data set. The remaining four generated PCs (PC2, PC3, PC4, and PC5) yielded progressively lower eigenvalues (<1 , 0.47, 0.047, 0.014, and 0.0002, respectively) and did not explain the variability in the data ($<10.60\%$ total). Therefore, according to Kaiser's rule,²⁶ only the first two PCs were used for further study. The calculated results suggested that PC1 inversely correlated with all variables, Mg (-0.918), Ca (-0.961), Cu (-0.997), Fe (-0.939), and Pb (-0.910), whereas PC2 was highly contributed by Mg (-0.386) and Pb (0.386). Evidently, PC1 is generally more correlated with the variables than PC2. The distribution of the most significant variables (Mg, Ca, Cu, Fe, and Pb amounts determined by ICP-MS and GFAAS methods) along the two first principal components and the groupings and/or the differences among palm oil samples are presented in the biplot (Figure 1).

It is noteworthy that Pb and Fe were the variables with negative loadings on PC1 and positive loadings on PC2. However, Cu, Ca, and Mg were the features with negative loadings on PC1 and PC2.

The PCA graph revealed that the crude palm oil (CPO-ICP, CPO-AAS) and palm oil after rinsing (POAR-ICP, POAR-AAS) with higher metals levels were located to the left in the score biplot and had negative values for PC1, whereas degummed, bleached, and deodorized palm oils (DPO-ICP,

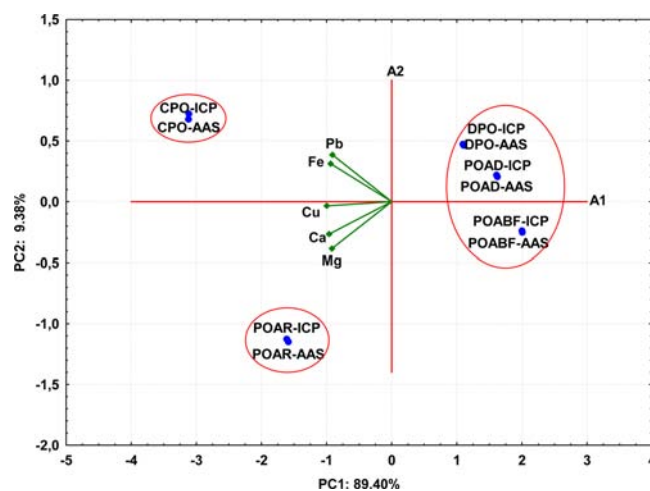


Figure 1. Biplot of scores and loadings of data obtained from Mg, Ca, Cu, Fe, and Pb contents in palm oils from various stages of the refining process determined by the proposed ICP-MS and the standard GFAAS methods.

DPO-AAS, POABF-ICP, and POABF-AAS; and POAD-ICP and POAD-AAS) with lower concentrations of the determined metal ions were situated at the right in the diagram and had positive values for PC1.

Furthermore, degummed and deodorized palm oils with similar contents of Pb and about 2 times higher amounts of prooxidants metals (Cu and Fe) in DPO than in POAD were located above the A1 axis. Also, levels of Cu and Fe in CPO did not differ significantly.

It should be noted that the studied palm oils fell into three distinct groups, respectively (Figure 1). CPO with the longest distance from other oil samples revealed the highest concentrations of Ca, Cu, Fe, and Pb. POAR created an evidently distinct cluster. This oil generally had similar amounts of Ca, about 2 times lower contents of Cu, Fe, and Pb and 1.5 times higher levels of Mg than in CPO. However, DPO, POABF, and POAD with low concentrations of metal ions determined by two analytical methods (ICP-MS and GFAAS) were separated from other studied samples (Figure 1).

It is noteworthy that there are differences in amounts of the analyzed metal ions in palm oils from different stages of the refining process.

Hierarchical Cluster Analysis. HCA was applied to group the studied palm oil samples on the basis of similarities in mean concentrations of metal ions determined by the proposed ICP-MS and the standard GFAAS methods. Also, HCA was performed to reveal relationships between the analyzed elements and element groups in palm oils from different stages of the refining process. The obtained results are presented as dendrograms in Figure 2, which display some results also found by PCA.

It can be noted that the five palm oil samples determined by two analytical methods, ICP-MS and GFAAS, were classified into two main clusters (Figure 2A). The dendrogram depicted clear separations of CPO-ICP and CPO-AAS and POAR-ICP and POAR-AAS, with the highest metal amounts from the other palm oil samples. The second group including two palm oils, POAD-ICP and POAD-AAS and POABF-ICP and POABF-AAS, was quite separated, because these samples had the low concentrations of the analyzed metal ions. However, degummed palm oil (DPO-ICP, DPO-AAS) was arranged in

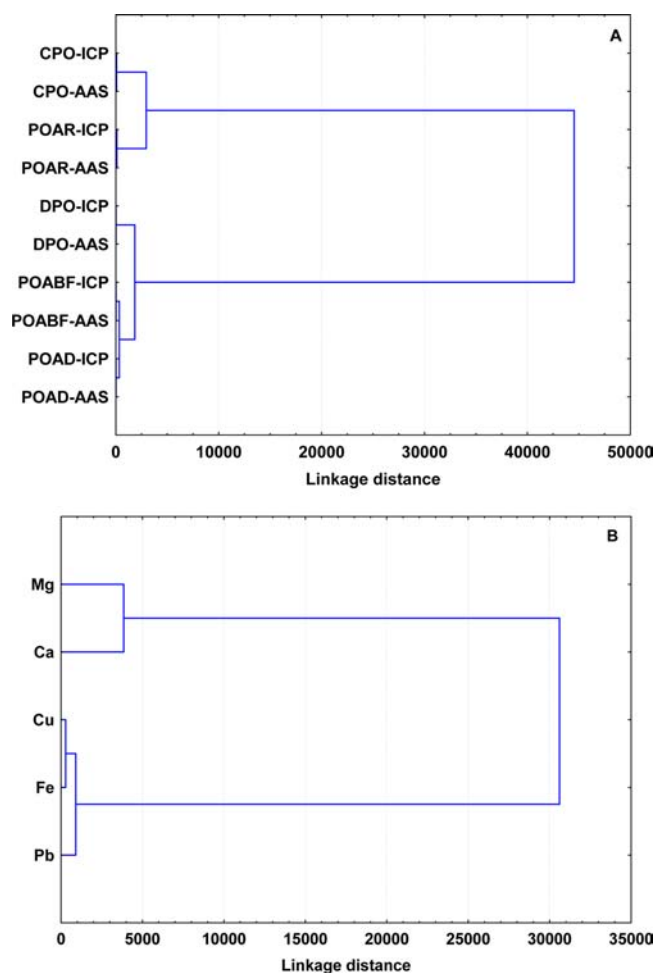


Figure 2. Dendrograms of hierarchical cluster analysis for (A) the studied palm oil samples and (B) metals concentrations in palm oil samples.

this group characterized by similar levels of Ca and Pb. It is noteworthy that mean contents of metal ions in the studied palm oils at various steps of refining processes are different from one another. The dendrogram (Figure 2A) revealed that the grouping of the palm oil samples is dependent on the technological stages, which would affect the total level of metals.

On the other hand, the results of cluster analysis indicate that the determined metal ions comprised two main groups. The main metal ions, Mg and Ca, formed the first cluster. However, the second group was composed of two subgroups consisting trace metal ions, (I) Cu and Fe and (II) Pb only (Figure 2B).

The proposed ICP-MS method is relatively simpler and shorter than the standard GFAAS method; therefore, it can be useful for the determination of main elements such as Mg and Ca as well as trace metals, Cu, Fe, and Pb, in palm oils at various steps of the refining process after microwave digestion. Moreover, the proposed procedure of sample preparation required shorter treatment time and smaller amounts of reagents than the traditional methods (wet digestion), thus significantly reducing potential environmental contamination. However, ICP-MS is a more expensive spectrometric technique than GFAAS with regard to both purchase price and running cost. It is noteworthy that each step of the refining process caused a decrease of metals contents in the studied palm oils.

The results of PCA and HCA indicated that the concentrations of metals in palm oils from various stages of technological processes differ significantly. CPO revealed the highest content of the analyzed elements, whereas the lowest amounts of metal ions among five palm oils were determined for POAD. Moreover, there are linear correlations between the metal ions determined by two analytical methods in the studied palm oils (0.6862–0.9817).

Metals composition in vegetable oils is an important decisive factor for the assessment of their quality, because they affect their rate of oxidation, nutritional value, and shelf life. Therefore, the proposed ICP-MS method can be usefully employed by the processing industry in assessing the metals concentrations in palm oils during different processing stages to guarantee a consistent product.

AUTHOR INFORMATION

Corresponding Author

*Phone: +48 56 611 47 86. Fax: +48 56 654 24 77. E-mail: olasz@umk.pl.

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Notes

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