Dedicated to Professor Cristian Silvestru
on the occasion of his 65th anniversary

ANALITICAL PERFORMANCE AND VALIDATION OF OPTICAL EMISSION AND ATOMIC ABSORPTION SPECTROMETRY METHODS FOR MULTIELEMENTAL DETERMINATION IN VEGETABLES AND FRUITS

Eniko COVACI,[a,b] Marin SENILA,[c] Michaela PONTA[a,b] and Tiberiu FRENTIU[a,b,*]

[a] Babes-Bolyai University, Faculty of Chemistry and Chemical Engineering, Arany Janos 11, 400028 Cluj-Napoca, Romania
[b] Research Center for Advanced Chemical Analysis, Instrumentation and Chemometrics, Arany Janos 11, 400028 Cluj-Napoca, Romania
[c] National Institute for Research and Development of Optoelectronics Bucharest, Research Institute for Analytical Instrumentation, Donath 67, 400293, Cluj-Napoca, Romania

This study presents the validation of inductively coupled plasma optical emission spectrometry (ICP-OES) for the determination of macroelements and microelements, graphite furnace atomic absorption spectrometry (GFAAS) and thermal decomposition atomic absorption spectrometry (TDAAS) for essential traces and toxic elements in vegetables and fruits. The limits of detection were in the range $0.02 - 5$ mg kg^{-1} for ICP-OES, $0.004 - 0.025$ mg kg^{-1} for GFAAS and 0.004 mg kg^{-1} Hg for TDAAS. Accuracy of the methods was checked by analysis of certified reference materials that provided recovery in the range $87 - 108\%$. The precision assessed from the analysis of real samples of dried goji berry was in the range of $1.5 - 10.0\%$. The spectrometric methods fulfilled the requirements of Association of Official Agricultural Chemists (AOAC) and European Commission so that ICP-OES, GFAAS and TDAAS were considered appropriate for determination of elements in vegetables and fruits.
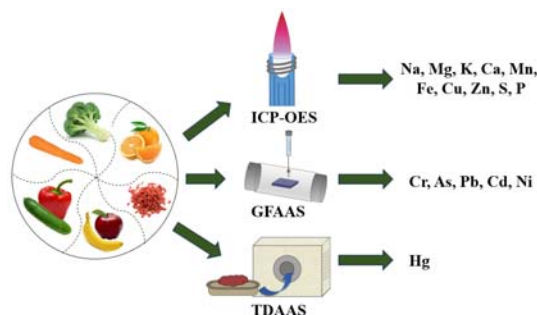
INTRODUCTION

A diet rich in fruits and vegetables has been shown to prevent numerous diseases, as they are sources of essential nutrients such as vitamins, minerals, antioxidants and fiber. Furthermore, there is an increasing interest in the so called superfruits like acai, camu-camu or goji berry, with health benefits.[1] Goji berries for example contain antioxidants like carotenoids, polyphenols, vitamins C and E, anti-inflammatory (e.g. beta sitosterol), anti-fungal and anti-bacterial agents (e.g. solavetivone), anticancer agents *(e.g.* cryperone) as well as essential fatty acids and minerals.[2] Goji dietary intake has been shown to have positive effects on human health when consumed either fresh or dried, such as vision protection, hypolipidemic, hypoglycemic, anticancer, antitumor, immunostimulatory, neurological protective, antiaging and cardiovascular protective effect.[3] Similar benefits have been found in the case of other dried fruit consumption like dates, prunes, figs, pears and raisins.[4] Therefore, there is an increasing demand

* Corresponding author: ftibi@chem.ubbcluj.ro

for healthy fruits and vegetables that are produced at large scale with the aid of fertilizers. These substances contain essential macroelements (N, P, K, S, Ca and Mg) and microelements (Cu, Fe, Zn and Mo) for plant growth, but they may contain potentially toxic elements such as As, Hg, Cd, Cr, Tl or Pb.[5,6] Vegetables and fruits can take up these metals with negative impact on human health. A recent review reported the contamination of rice and spinach with up to 0.94 mg kg^{-1} As and canned tuna up to 1.42 mg kg^{-1} Hg.[5] Currently, there is a great interest for the determination of a large number of essential macroelements, microelements and toxic elements in vegetables and fruits (tomato, asparagus, garlic, parsley, spinach, strawberry, cherry, grape, apples, etc.).[7-20] Various analytical methods such as inductively coupled plasma optical emission spectrometry (ICP-OES),[7,21-23] inductively coupled plasma mass spectrometry (ICP-MS),[24,25] flame atomic absorption spectrometry (FAAS),[13,18,26] electrothermal/graphite furnace atomic absorption spectrometry (ETAAS/GFAAS),[13,15,27-29] thermal decomposition atomic absorption spectrometry (TDAAS),[30-32] cold vapor atomic fluorescence spectrometry (CV-AFS),[33] X-ray fluorescence spectrometry (XRF),[25] proton induced X-ray emission spectrometry (PIXE)[34] and photochemical vapor generation capacitively coupled plasma microtorch optical emission spectrometry (UV-PVG-μCCP-OES)[19] were used for the determination of elements in vegetables and fruits. Until now, the ICP-OES is standardized method for the determination of selected elements in water,[35] while GFAAS for Pb, Cd, Zn, Cu, Fe, Cr and As in foodstuffs.[36-38] In the case of Hg, the standardized method for water analysis is CV-AFS.[39] An U.S. EPA procedure also exists based on TDAAS on solids and solutions.[40] Under these circumstances validation of these spectrometric methods for the analysis of vegetables and fruits has broad practical interest.

The aim of this study was the determination of Na, Mg, K, Ca, Mn, Fe, Cu, Zn, S, P, Cr, As, Pb, Cd, Ni and Hg from goji berry using validated ICP-OES, GFAAS and TDAAS methods. The methods were characterized in terms of selectivity, specificity, limit of detection (LOD), limit of quantification (LOQ), linearity of calibration curve, precision, recovery and uncertainty. In agreement with ISO/IEC 17025[41], validation of a method is necessary to confirm fulfilling of particular requirements for a specific intended use. Thus, the analytical performances were compared with the demands of European Commission regulations for official laboratories[42-44] and

recommendations of the Association of Official Agricultural Chemists (AOAC).[45]

RESULTS AND DISCUSSION

Selectivity

Selectivity was assessed by recovery of a spike in the original samples of 1 mg L^{-1} element in ICP-OES, 1 μg L^{-1} Cd and 5 μg L^{-1} As, Cr, Ni and Pb in GFAAS and 10 μg L^{-1} Hg in TDAAS. The recoveries of spikes were within 90–110%. The good selectivity was the result of choosing interference-free working wavelengths (Table 1) and high resolution of instrumentation.

Limit of detection, quantification and linear range of calibration curve

The LOD (3σ criterion),[46,47] LOQ (3xLOD) and parameters of the calibration curves achieved by external standards in ICP-OES, GFAAS and TDAAS methods are summarized in Table 1. The LODs and LOQs in dry mass were calculated considering the sample preparation protocol. Standard deviation of blank signal was evaluated from 10 measurements of independent reagent blank solutions prepared similarly to samples.

The LODs were in the range 0.02 – 5 mg kg^{-1} element for ICP-OES and allowed quantification above 0.06 – 15 mg kg^{-1} element (Table 1). LODs for Cu, Mg, Mn, Fe, Zn, P and S in ICP-OES were found to be better than those reported by Nascimento *et al.*[48] in the analysis of goji berries (2.15 – 15.45 mg kg^{-1}).

In GFAAS, LODs for As, Cd, Cr, Ni and Pb were in the range 0.004 – 0.025 mg kg^{-1} and allowed determinations of concentrations higher than 3xLODs. The LODs for Cd, Ni and Pb were poorer than those reported by Jeszka-Skowron *et al.*[27] (0.0014 mg kg^{-1} Cd, 0.0008 mg kg^{-1} Ni and 0.0022 mg kg^{-1} Pb) and Sanchez-Moreno *et al.*[28] (0.002 mg kg^{-1} Cd, 0.018 mg kg^{-1} Ni and 0.009 mg kg^{-1} Pb). In vegetables and fruits maximum permissible limits are set only for Cd and Pb. The LODs for Cd and Pb in GFAAS were 12 and 5-15 times lower than the admitted level in different vegetables and fruits set in Commission Regulation 1881/2006.[49] Consequently, GFAAS is considered appropriate for the determination of Cd and Pb in vegetables and fruits according to the European legislation related to official control laboratories.[42-44]

Table 1

Working concentration ranges, variance ratio at the limits of the calibration range s_1^2/s_2^2, correlation coefficients (R), variation coefficients of the methods (V_{ox}),
limits of detection and quantification of the spectrometric methods used in the multielemental analysis of vegetables and fruits

Element	λ (nm)	Method	Calibration range	s_1^2/s_2^2	R	V_{ox} (%)	LOD[a] (mg kg^{-1})	LOQ[b] (mg kg^{-1})
Na	588.995	ICP-OES	0.009 - 6 mg L^{-1}	6.12	0.9995	5.65	0.15	0.45
Mg	285.213		0.003 - 6 mg L^{-1}	5.90	0.9999	3.75	0.05	0.15
K	766.491		0.12 - 6 mg L^{-1}	6.42	0.9994	4.95	2	6
Ca	422.673		0.003 - 6 mg L^{-1}	4.05	1.0000	2.72	0.05	0.15
Mn	257.610		0.003 - 6 mg L^{-1}	6.51	0.9999	0.86	0.06	0.18
Fe	259.940		0.0012 - 6 mg L^{-1}	6.40	0.9995	1.26	0.02	0.06
Cu	324.754		0.06 - 6 mg L^{-1}	6.19	1.0000	1.68	1	3
Zn	213.856		0.18 - 6 mg L^{-1}	6.90	1.0000	1.23	3	9
S	181.975		0.3 - 6 mg L^{-1}	6.25	0.9999	5.75	5	15
P	213.618		0.24 - 6 mg L^{-1}	7.25	0.9998	5.89	4	12
Cr	357.87	GFAAS	0.008 - 20 µg L^{-1}	2.26	0.9997	1.70	0.013	0.039
As	193.70		0.0004 - 10 µg L^{-1}	2.04	0.9994	2.59	0.006	0.018
Pb	283.31		0.0012 - 20 µg L^{-1}	5.71	0.9996	2.46	0.020	0.060
Cd	228.80		0.0003 - 5 µg L^{-1}	2.04	0.9994	2.59	0.004	0.012
Ni	232.00		0.0015 - 20 µg L^{-1}	4.99	0.9996	1.25	0.025	0.075
Hg	253.65	TDAAS	0.0003 – 0.050 µg	6.23	0.9995	3.12	0.004	0.012

[a] LOD was calculated based on (3σ) criterion and parameters of the calibration curves; LOD in dry sample corresponds to 500 mg digested sample diluted to 25 mL for ICP-OES and GFAAS and 200 mg solid sample in TDAAS
[b] LOQ was calculated as 3xLOD

Table 2

Results obtained in the repeatability and reproducibility assay by ICP-OES, GFAAS and TDAAS in the multielemental analysis of goji sample (n=10 parallel samples)

Element	Method	Repeatability			Reproducibility		
		Mean result (mg kg^{-1})	s_r (%)	r (%)	Mean result (mg kg^{-1})	s_R (%)	R (%)
Na	ICP-OES	3520	5.5	15.4	3490	8.0	22.4
Mg		1190	3.1	8.7	1200	6.9	19.3
K		18300	6.2	17.4	17800	8.5	23.8
Ca		910	3.5	9.8	1010	7.0	19.6
Mn		20.1	2.7	7.6	19.5	5.5	15.4
Fe		58.7	2.5	7.0	58.3	7.1	19.9
Cu		11.5	7.0	19.6	11.3	10.5	29.4
Zn		21.8	6.5	18.2	22.3	10.5	29.4
S		222	3.1	8.7	215	6.7	18.8
P		3870	2.8	7.8	3800	5.8	16.2
Cr	GFAAS	0.250	6.8	19.0	0.230	9.8	27.4
As		0.018	8.0	22.4	0.017	10.0	28.0
Pb		0.210	8.0	22.4	0.195	10.5	29.4
Cd		0.090	7.2	22.2	0.100	9.5	26.6
Ni		1.060	6.7	18.8	1.020	9.5	26.6
Hg	TDAAS	0.010	8.5	23.8	0.011	10.5	29.4

s_r – standard deviation of repeatability; r – limit of repeatability (2.8xs_r); s_R – standard deviation of reproducibility; R – limit of reproducibility (2.8xs_R)

The LOD/LOQ for Hg in TDAAS of 0.004/0.012 mg kg^{-1} respectively, are higher than previously reported by Senila *et al.*[33] as a results of a that lower mass of sample subjected to analysis. In the same time, LOD in TDAAS was higher than in CV-AFS (0.0025 mg kg^{-1}), which is recognized for its high sensitivity for Hg.[33] The maximum level of Hg is provided for seafood (0.5-1 mg kg^{-1}) and food supplements (0.1 mg kg^{-1}).[49,50] Compared to the threshold in food supplements, LOD/LOQ in TDAAS are 25/12 fold lower, so that TDAAS is able to quantify Hg in vegetables and fruits according to demands in European legislation.[42-44]

Variance ratio at the limits of working range, correlation coefficient and variation coefficients (V_{ox}) for ICP-OES, GFAAS and TDAAS methods according to SR ISO 8466-1:1999[51] are presented in Table 1. Good linearity over the calibration range with correlation coefficients of 0.9994 – 1.000 was obtained in all three methods. The ratios s_1^2/s_2^2 calculated for measurements of concentrations corresponding to LOQ and highest standard were below the critical value $F_{6;6,0.99}$=8.47 for ICP-OES and $F_{5;5,0.99}$=10.97 for GFAAS and TDAAS demonstrating dispersion homogeneity. The variation coefficient was better than 6% in ICP-OES and better than 4% in GFAAS and TDAAS.

Precision

Internal repeatability and reproducibility for the three spectrometric methods were evaluated by analyzing a test goji sample and reagent blank.[52]

Table 2 summarizes the results for the internal repeatability assay on 10 parallel samples performed by a single operator and those for the reproducibility study on 10 replicate samples carried out by different operators in different days.

Data show that standard deviation of repeatability/limit of repeatability were better than 7.0/20.0% in ICP-OES, 8.0/23.0% in GFAAS and 9.0/24.0% in TDAAS. Standard deviation of reproducibility/limit of reproducibility were better than 11.0/30.0 % for all three methods. The precision of measurements expressed as relative standard deviation on the test goji sample in ICP-OES, GFAAS and TDAAS was below 10.0% in compliance with the AOAC guidelines.[45]

Recovery

The accuracy of the methods was checked by analyzing four vegetable and fruit certified reference materials. According to AOAC, a spectrometric method should provide recovery within the range 80 – 120% to be suitable for elemental analysis.[45] In the same time the European legislation states that recovery provided by certain methods for the determination of Cd, Pb and Hg contaminants in food should be in the range 100±10% against the target value.[42-44] The results obtained in the analysis of certified reference materials presented in Table 3 show good recoveries in the range of 87 – 108% for the three methods used in this study, while the *t-test* indicates no significant difference between the found and certified values ($t_{calc,95\%}$: 0–4.303).

Table 3

Results obtained in the multielemental analysis of certified reference materials using ICP-OES, GFAAS and TDAAS

Element	Certified Reference Material	Certified value ± U[a] (mg kg⁻¹)	Found value U[b] (mg kg⁻¹)	Recovery ± U[b] (%)	$t_{calc, n=3; 95\%}$
Na	IAEA-359 Cabbage	580 ± 13	536 ± 50	92 ± 9	3.387
	NIM-GBW-10019 Apple	1160 ± 90	1190 ± 90	103 ± 10	1.434
Mg	IAEA-359 Cabbage	2160 ± 50	1980 ± 180	92 ± 9	4.303
	NIST-RM 8413 Corn	990 ± 82	900 ± 90	91 ± 10	4.303
K	IAEA-359 Cabbage	32500 ± 690	30800 ± 1840	95 ± 6	3.976
	NIM-GBW-10019 Apple	7700 ± 400	7100 ± 720	92 ± 10	3.586
	NIST-RM 8413 Corn	3570 ± 370	3800 ± 380	106 ± 10	2.604
Ca	IAEA-359 Cabbage	18500 ± 510	17850 ± 1000	96 ± 6	2.797
	NIST-RM 8413 Corn	42 ± 5	45± 4	107 ± 9	3.227
Mn	IAEA-359 Cabbage	31.9 ± 0.6	31.0 ± 1.2	97 ± 4	3.228
Fe	IAEA-359 Cabbage	148 ± 3.9	145 ± 4.9	98 ± 3	2.635
Cu	IAEA-359 Cabbage	5.67 ± 0.18	5.58 ± 0.16	98 ± 3	2.420
	NIM-GBW-10019 Apple	2.5 ± 0.2	2.2 ± 0.3	88 ± 14	4.303
	NIST-RM 8413 Corn	3.0 ± 0.6	2.6 ± 0.4	87 ± 15	4.304
Zn	IAEA-359 Cabbage	38.6 ± 0.7	37.1 ± 2.1	96 ± 6	3.074
	NIST-RM 8413 Corn	15.7 ± 1.4	16.0 ± 1.2	102± 8	1.076
S	NIM-GBW-10019 Apple	630 ± 40	592 ± 39	94 ± 7	4.193
P	NIM-GBW-10019 Apple	660 ± 40	668 ± 46	101± 7	0.748
Cr	IAEA-359 Cabbage	1.3 ± 0.06	1.4 ± 0.17	108 ± 12	2.531
	NIM-GBW-10019 Apple	0.30 ± 0.06	0.30 ± 0.05	100 ± 17	0
As	IAEA-359 Cabbage	0.10 ± 0.004	0.10 ± 0.009	100 ± 9	0
	CS-SR-2 Carrot Root Powder	0.050 ± 0.008	0.046 ± 0.005	91 ± 11	3.442
Pb	CS-SR-2 Carrot Root Powder	0.579 ± 0.040	0.547 ± 0.044	94 ± 8	3.129
	NIM-GBW-10019 Apple	0.084 ± 0.032	0.080 ± 0.010	95 ± 12	1.721
Cd	CS-SR-2 Carrot Root Powder	0.196 ± 0.020	0.190 ± 0.019	97 ± 10	1.359
	IAEA-359 Cabbage	0.12 ± 0.005	0.12 ± 0.007	100 ± 6	0
	NIM-GBW-10019 Apple	0.0058 ± 0.0012	0.0054 ± 0.0007	93 ± 14	1.721
Ni	IAEA-359 Cabbage	1.05 ± 0.05	1.08 ± 0.09	103 ± 8	1.434
	NIM-GBW-10019 Apple	0.14 ± 0.05	0.12 ± 0.03	86± 25	2.869
Hg	IAEA-359 Cabbage	0.013 ± 0.002	0.013 ± 0.001	100 ± 8	0

[a] U is the expanded uncertainty for 95 % confidence level (k=2)

[b] n = 3 complete extraction/analysis for each sample

Recoveries were similar to those reported in literature (80 – 121%) in ICP-OES, ICP-MS and high resolution continuum source electrothermal atomic absorption spectrometry (HR-CS-ETAAS).[24,27,28] The overall recoveries of Pb, Cd and Hg were in the range 95±10%; 94±10% and 100±10% respectively, for 95% confidence level (n=3). Therefore, GFAAS and TDAAS are suitable for Cd and Pb, and Hg determination respectively, in vegetables and fruits according to European legislation.[42-44] Moreover, the three spectrometric methods satisfied the recommendation of AOAC.[45]

Standard uncertainty

The individual standard uncertainties, composed uncertainties and expanded uncertainties consistent with the specific protocol are presented in Tables 4 and 5.

The relative expanded uncertainty (k=2, 95%) was in the range 9-25% in ICP-OES, 7-10% in GFAAS and 10% for Hg determination in TDAAS. The highest value for Cu in ICP-OES (25%) is attributed to fitting. The major contributions to combined standard uncertainty in the three spectrometric methods come from replicate analysis, fitting and standard preparation.

Analysis of dried Goji berry samples

The results obtained in the analysis of 10 dried Goji berry samples are summarized in Table 6. The relative standard deviation in multielemental determination by ICP-OES was in the range 1.5 – 10.0% for Na, K, Ca, Mg, Mn, Fe, Cu, Zn, P and S, 6.3 – 10.0% in GFAAS for Cr, As, Pb, Cd, Ni and 7.5 – 10.0% in TDAAS for Hg.

Traceability chain	Unit	Element									
		Na	**Mg**	**K**	**Ca**	**Mn**	**Fe**	**Cu**	**Zn**	**S**	**P**
Weighting	g	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
ICP standard 1000 mg L^{-1}	mg L^{-1}	0.0057	0.0057	0.0057	0.0062	0.0058	0.0058	0.0062	0.0057	0.0057	0.0057
Preparation of standard	mg L^{-1}	0.0125	0.0125	0.0125	0.0125	0.0126	0.0126	0.0126	0.0125	0.0125	0.0125
Sample dilution	mg L^{-1}	0.0035	0.0028	0.0046	0.0035	0.0005	0.0005	0.0005	0.0005	0.0023	0.0028
Fitting	mg L^{-1}	0.0490	0.0329	0.0335	0.0491	0.0354	0.0334	0.1078	0.0337	0.0335	0.0335
Replicate analysis	mg kg^{-1}	0.0550	0.0310	0.062	0.0350	0.0270	0.0250	0.0700	0.0650	0.0310	0.0280
Composed uncertainty (u_c)	mg kg^{-1}	0.0750	0.0473	0.0719	0.0620	0.0466	0.0440	0.1293	0.0745	0.0477	0.0459
Expanded uncertainty (U=2xu_c) 95% confidence level	mg kg^{-1}	0.1500	0.0947	0.1439	0.1240	0.0933	0.0879	0.2586	0.1490	0.0954	0.0918
Relative expanded uncertainty (U_{rel})	(%)	15	10	15	13	10	9	25	15	10	10

		GFAAS					TDAAS	
Traceability chain	**Units**	**Cr**	**As**	**Pb**	**Cd**	**Ni**	**Units**	**Hg**
Weighting	g	0.0001	0.0001	0.0001	0.0001	0.0001	g	0.0001
ICP standard 1000 mg L^{-1}	mg L^{-1}	0.0058	0.0058	0.0058	0.0058	0.0058	µg L^{-1}	0.0058
Preparation of standard	µg L^{-1}	0.0154	0.0154	0.0095	0.0095	0.0095	µg L^{-1}	0.0095
Sample dilution	□g L^{-1}	0.0060	0.0060	0.0060	0.0060	0.0060	µg L^{-1}	0.0037
Fitting	□g L^{-1}	0.0188	0.0210	0.0235	0.0095	0.0178	□g	0.0220
Replicate analysis	mg kg^{-1}	0.0445	0.0390	0.0427	0.0299	0.0325	mg kg^{-1}	0.0441
Composed uncertainty (u_c)	mg kg^{-1}	0.0514	0.0476	0.0504	0.0338	0.0392	mg kg^{-1}	0.0510
Expanded uncertainty (U=2xu_c), 95%	mg kg^{-1}	0.1028	0.0953	0.1007	0.0676	0.0783	mg kg^{-1}	0.1010
Relative expanded uncertainty (U_{rel})	(%)	10	10	10	7	8	(%)	10

Table 6

Results (mg kg^{-1}) obtained in the multielemental analysis of dried Goji berry using ICP-OES, GFAAS and TDAAS

Value	ICP-OES										GFAAS					TDAAS
	Na	K	Ca	Mg	Mn	Fe	Cu	Zn	P	S	Cr	As	Pb	Cd	Ni	Hg
Min	1450	10800	585	1100	10.0	49.5	10.5	20.0	3580	220	0.015	0.006	0.020	0.004	0.025	0.004
Max	6150	28300	1100	1500	21.8	80.0	14.5	28.5	4940	450	0.760	0.019	0.200	0.050	0.810	0.018
Mean	3425	23000	800	1225	18.7	65.3	12.5	23.5	4100	300	0.225	0.014	0.070	0.025	0.280	0.015
RSD(%)	5.4-8.9	5.7-7.8	3.2-6.6	2.2-6.7	1.5-4.5	1.8-6.0	6.5-10.0	5.8-9.5	2.1-4.2	2.2-5.1	6.4-8.8	7.5-9.8	7.6-10.0	6.3-8.7	6.7-9.1	7.5-10.0

Data in Table 6 show that goji berry is a rich source of K, P, Mg, Ca, Fe, Zn, Mn and Cu, which have similar levels to those reported in the literature.[27,53] The concentrations of hazardous elements (Ni, Pb, Cd, As and Hg) in the analyzed Goji berry were similar to those in Goji berry marketed in Spain[24] and Poland[27] and lower than in Italian Goji.[53] Lead was below the maximum value of 0.2 mg kg^{-1} in berries and small fruits, while Cd below 0.050 mg kg^{-1} in vegetables and fruits.[49] There is no maximum value for Hg in vegetables and fruits, instead a content of 0.1 mg kg^{-1} Hg is the maximum accepted in food supplements.[50] The very low concentrations of Cd, Pb and Hg found in goji berry pose no risk to consumers.

The Total Target Hazard Quotient (TTHQ) for Cd, As, Hg, Pb and Ni was 0.087±0.036 (95% confidence level), calculated for a 30 g serving of dried goji berry per day, 365 days per year exposure frequency, 60 kg average body weight, 70 exposure years and oral reference doses (RfDs) in μg/kg per day of 1 (Cd), 0.3 (As), 0.3 (Hg), 4 (Pb) and 20 (Ni).[54] Since TTHQ is much lower than 1, adverse health hazard is unlikely to happen.

EXPERIMENTAL

Materials

Nitric acid 60% (w/w) ultrapure, hydrochloric acid 30% (w/w) ultrapure, hydrogen peroxide 30% (w/w) pro-analysis, ICP multi-elemental standard solution IV 1000 mg L^{-1}, single element standard solutions 1000 mg L^{-1} of As, Hg, P and S purchased from Merck (Darmstadt, Germany) were used. Matrix modifiers 10% $NH_4H_2PO_4$, 1% $Mg(NO_3)_2$ and 1% $Pd(NO_3)_2$ used in GFAAS measurements were purchased from Perkin Elmer (Shelton, USA) and prepared before analysis, as recommended by the manufacturer. Four certified reference materials were analyzed to validate the multielemental determinations by ICP-OES, GFAAS and TDAAS: IAEA-359 Cabbage (International Atomic Energy Agency, Vienna, Austria), NIM-GBW-10019 Apple (Institute of Geophysical and Geochemistry Exploration, Langfang, China), NIST-RM 8413 Corn (Zea Mays) Kernel (National Institute of Standards and Technology, Gaithersburg, USA) and CS-SR-2 Carrot Root Powder (Institute of Nuclear Chemistry and Technology, Warsaw, Poland). Ultrapure water (18 MΩ cm resistivity) prepared with Milli-Q system (Millipore, Bedford, USA) was used throughout the experiments.

Sample preparation

Amounts of 500 mg dried CRMs or real samples (dried goji fruits commercially available) were subjected to microwave-assisted digestion in a mixture of 9 mL 65% HNO_3 and 3 mL 30% H_2O_2 using the MWS3+ Berghof microwave digester (Berghof, Germany) according to the five stage temperature program previously used for the preparation of food samples.[55] Prior to digestion, samples were left in the HNO_3-H_2O_2 mixture at room temperature for 12 h for a preliminary oxidation. The digest was diluted to 25 mL with 2% (v/v) HNO_3 and passed through the 0.45 μm Whatman membrane filter nylon (Merck, Darmstadt, Germany). The elemental determination was carried out in the original filtrate or after an appropriate dilution with 2% (v/v) HNO_3. Reagent blank was prepared using the same microwave-assisted digestion procedure.

Instrumentation

The concentration of Na, Mg, K, Ca, Mn, Fe, Cu, Zn, S and P was determined by ICP-OES using the SPECTRO CIROS[CCD] spectrometer (Spectro, Kleve, Germany) under the following conditions: 1400 W plasma power, 27.12 MHz radiofrequency, 12 L min^{-1} outer Ar, 0.6 L min^{-1} auxiliary Ar, 1 L min^{-1} nebulization Ar, axial plasma viewing (X=-3.9 mm, Y=+3.6 mm, Z=+2.6 mm torch position), 2 mL min^{-1} sample flow fed by a peristaltic pump, cross-flow nebulizer (40 s flushing time, 20 s delay time), optical detection with a double grating Paschen Runge polychromator with chamber filled with N_2 (160 – 800 nm) and 22 charge coupled device (CCD) detector. Quantification was based on external calibration (7-point linear calibration curve starting from LOQ up to 6 mg L^{-1} element in 2% HNO_3). Peak heights were measured for 48 s integration time and two points model background correction approach. The most sensitive analytical lines free of spectral interferences were selected.

GFAAS measurements for As, Cd, Cr, Ni and Pb quantification were carried out using the PinAAcle 900T Perkin Elmer spectrometer (Norwalk, CT, USA). Aliquots of 20 μL calibration standard/digested sample mixed with 5 μL appropriate chemical modifier (Cr: 0.3% $Mg(NO_3)_2$; As: 0.1% $Pd(NO_3)_2$ + 0.06% $Mg(NO_3)_2$; Pb and Cd: 1% $NH_4H_2PO_4$ + 0.06% $Mg(NO_3)_2$; Ni: without matrix modifier) were automatically injected into the graphite tube. The longitudinal Zeeman background correction was used. The temperature program is summarized in Table 7. The external radiation sources were electrodeless discharge lamps (EDLs) for As (380 mA, 193.759 nm), Pb (400 mA, 283.305 nm) and Cd (230 mA, 228.802 nm) and hollow cathode lamps (HCLs) for Cr (25 mA, 367.868 nm) and Ni (25 mA, 232.003 mA).

Quantitative measurements were based on 6-point calibration curves over the range LOQ–20 μg L^{-1} Cr, Pb and Ni, LOQ–10 μg L^{-1} As and LOQ–5 μg L^{-1} Cd.

Mercury determination was carried out using the Automated Direct Mercury Analyzer Hydra-C (Teledyne Instruments, Leeman Labs, USA).[30] Aliquots of 200±0.1 mg solid samples or 200 μl standard solution were subjected to combustion in the furnace module, the generated Hg vapor was concentrated *via* gold amalgamation and the absorption signal was measured as peak area at 253.652 nm. Operating conditions were as follows: 300 °C drying temperature for 35 s, 800 °C decomposition temperature for 150 s, 600 °C catalyst temperature for 60 s, 700 °C gold trap temperature for 30 s, 90 s measuring time under 300 mL min^{-1} oxygen flow. The 6-point calibration curve in the range of LOQ – 0.050 μg Hg was generated by injecting different aliquots of 100 or 1000 μg L^{-1} Hg aqueous standards prepared in 5% (v/v) HCl into the nickel boats.

Corrections for reagent blanks were made in each spectrometric method.

Table 7

Furnace program for multielemental determination using PinAAcle 900T Perkin Elmer GFAAS spectrometer

Furnace program	Cr	As	Pb	Cd	Ni
Drying					
Temperature (ºC)	110	110	110	110	110
Ramp (s)	1	1	1	1	1
Hold (s)	30	30	40	40	30
Ar (mL min^{-1})	250	250	250	250	250
Drying					
Temperature (ºC)	130	130	130	130	130
Ramp (s)	15	15	15	15	15
Hold (s)	30	30	40	40	30
Ar (mL min^{-1})	250	250	250	250	250
Pyrolysis					
Temperature (ºC)	1500	1200	850	500	1100
Ramp (s)	10	10	10	10	10
Hold (s)	20	20	20	20	20
Ar (mL min^{-1})	250	250	250	250	250
Atomization					
Temperature (ºC)	2300	2000	1600	1500	2300
Ramp (s)	0	0	0	0	0
Hold (s)	5	5	5	5	5
Ar (mL min^{-1})	0	0	0	0	0
Cleaning					
Temperature (ºC)	2450	2450	2450	2450	2450
Ramp (s)	1	1	1	1	1
Hold (s)	3	3	3	3	3
Ar (mL min^{-1})	250	250	250	250	250

Validation of spectrometric methods

The spectrometric methods were characterized in terms of selectivity, LOD, LOQ, linearity of calibration curves, precision, recovery and uncertainty.[41,46]

Selectivity, defined as the ability of a method to accurately quantify the analyte in the presence of interferences is related, in case of spectrometric methods, to possible interferences at working wavelength of analyte.[46] Firstly the analyte was determined in a test sample of goji using the standard addition approach, then selectivity was assessed by recovery of a spike of 1 mg L^{-1} element in ICP-OES, 1 μg L^{-1} Cd and 5 μg L^{-1} As, Cr, Ni and Pb in GFAAS and 10 μg L^{-1} Hg in TDAAS based on the external calibration. The self-imposed target was spike recovery of 100±10% for all the elements under study.

Limit of detection was calculated using the (3σ) criterion and parameters of the calibration curve.[47]

$$LOD = \frac{3s_b}{m} \qquad (1)$$

where (s$_b$) is the standard deviation of background assessed from 10 measurements of reagent blank, while (m) the slope of the calibration curve.

LOQ was considered as 3xLOD. Values of LOD and LOQ in solid sample were calculated taking into account the sample preparation protocol. The suitability of GFAAS and TDAAS methods for the determination of Cd, Pb and Hg as problematic contaminants was established by comparing analytical performances with the demands in European legislation,[42-44] which requests that LOD/LOQ to be 10/5 times lower than maximum admitted value in food.

Statistical evaluation of the linear calibration function was made according to SR ISO 8466-1.[51] The parameters under study were homogeneity of dispersion at the limits of the calibration range and variation coefficient of the method (V$_{ox}$). The target was to obtain V$_{ox}$ better than 6% in all spectrometric methods.

Homogeneity of dispersion was evaluated as variance ratio at the limits of working range so that the value to be supraunitary:

$$PG = \frac{s_1^2}{s_2^2} \quad or \quad PG = \frac{s_2^2}{s_1^2} \qquad (2)$$

where s$_1^2$ and s$_2^2$ are variances of measurements corresponding to concentrations of LOQ and highest standard or reverse.

For $PG < F_{n_1-1,n_2-1;0.99}$ the difference between variances is not significant and the preliminary working range is considered as linear. Otherwise, the working range should be narrowed until the condition is met. The target was to achieve a correlation coefficient ≥0.995, usually considered for linear calibration.

The variation coefficient of the method (V$_{ox}$) was calculated as:

$$V_{ox} = \frac{\sqrt{\frac{\sum_{i=1}^{n}\left(y_i - \hat{y_i}\right)^2}{n-2}}}{m \times \bar{c}} \times 100 \qquad (3)$$

where y$_i$ is the signal value for (i) calibration standard, $\hat{y_i}$ is the signal of (i) standard calculated from the calibration curve, (n) is the number of standards, (m) is the slope of calibration curve and $\bar{c}$ is the mean of standard concentrations.
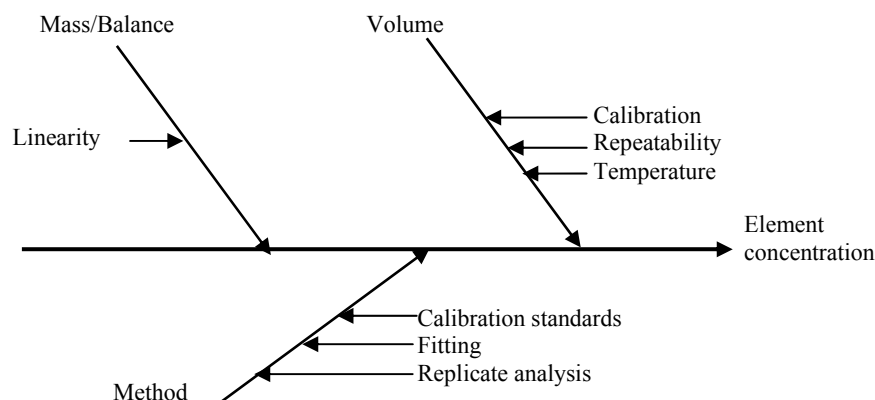
Fig. 1 – Bonefish diagram for uncertainty contributions in the measurement of element concentration
by ICP-OES, GFAAS and TDAAS.

Precision was evaluated as internal standard deviation of repeatability (s_r, %) and standard deviation of reproducibility (s_R, %), limit of repeatability ($r=2.8 x s_r$) and limit of reproducibility ($R=2.8 x s_R$).[52]

The accuracy of the spectral methods was assessed through a recovery assay by comparing measurement results with certified values of elements in several vegetable and fruit CRMs for 95% confidence level and ($n=3$ parallel measurements) (*t*-test).[47] Recovery and precision were compared with the demands of European legislation[42-44] related to official control laboratories and recommendations of Association of Official Agricultural Chemists (AOAC).[45]

Estimation of uncertainty followed the Eurachem guide.[46] The uncertainty sources are presented in Fig. 1.

The combined standard uncertainty (u_c) was calculated as:

$$u_c = \sqrt{\sum_i^n u_i^2} \qquad (4)$$

where u_i are individual standard uncertainties according to the analysis protocol.

The expanded uncertainty (U_c) was obtained as $2 x u_c$, which provided a 95% confidence interval of results. The relative expanded uncertainty (U_{rel}) was expressed as $U_c x 100$. The target value for (U_{rel}) was 25% for ICP-OES and 15% for GFAAS and TDAAS.

CONCLUSIONS

The ICP-OES, GFAAS and TDAAS methods were validated for the analysis of vegetables and fruits. It was demonstrated that ICP-OES is suitable for the determination of macroelements and microelements (Na, Mg, K, Ca, Mn, Fe, Cu, Zn, S, P), GFAAS for microelements and toxic elements (Cr, As, Pb, Cd, Ni) and TDAAS for Hg in vegetables and fruits. The analytical performance fulfills the requirements of the European Commission legislation and recommendations of AOAC. The methods were successfully applied for the analysis of goji berry.

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