Determination of Metal and Mineral Content in Plant-Based Protein Supplements using Inductively Coupled Plasma Mass Spectroscopy Techniques

University of Manitoba
Richardson Centre for Food Technology and Research

Atanas Karamanov, Michael C. Janzen, Rotimi E. Aluko

Richardson Centre for Food Technology and Research, University of Manitoba

Introduction

- The dietary supplement industry continues to expand with an economic impact >$100 billion in the USA alone
- The industry however lacks regulation and incidents of contamination, including with toxic heavy metals, can place consumers at potential risk
- In this study, concentration levels of 13 metals (Na, Mg, K, Ca, P, Mn, Fe, Cu, Zn, As, Cd, Pb, Hg) were determined by ICP-MS after microwave-assisted digestion or dry ashing
- A NIST protein powder standard reference material and six (6) commercial plant-based protein supplements were selected for testing

Experimental

Calibration Standards

- The calibration standards were prepared in 2% nitric acid and 0.5% hydrochloric acid
- Calibration standards were prepared from Agilent standard solutions including environmental calibration standard, p/n 5183-4688
- Agilent single calibration standards were used for P (p/n 015-25) and Hg (p/n 5190-8575)
- Elements Na, K, Mg, Ca and P were calibrated from 100 to 2,000 ppb
- Elements Cu, Zn and Mn were calibrated from 10 to 200 ppb
- Heavy metals As, Cd, Pb and Hg were calibrated from 0.1 to 1.0 ppb

Calibration Standards

- NIST Standard Reference Material 3252 Protein Drink Mix and six (6) common commercial plant-based protein supplements were sourced for this study

Standard and Sample Preparation

- The NIST Protein Drink Mix and six (6) commercial plant-based protein supplements were prepared as received and digested using either microwave (Table 1) or dry ashing methods according to internal SOPs

Table 1. *MARS microwave digestion parameters*

Parameter	Setting
Power (W)	1600
Ramp Time (min)	20
Hold Time (min)	15
Temperature (°C)	200

- The analytical sequence is shown in Figure 1. The sample block was analyzed repeatedly with automatic insertion of the periodic QC block after every 15 samples

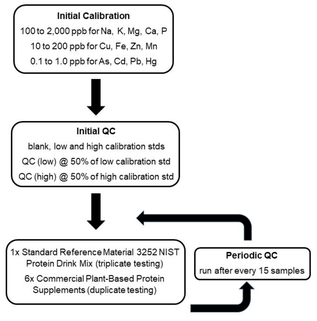

Figure 1. *Analytical sequence*

Instrumentation

- An Agilent 7850 ICP-MS was used for analysis using a sample introduction system consisting of a MircoMist glass concentric nebulizer, temperature-controlled quartz spray chamber, and quartz torch with 2.5mm id injector
- A nickel-plated copper sampling cone and a nickel skimmer cone were used

Table 2. *Agilent 7850 ICP-MS operating conditions*

ICP-MS Parameter	Setting
RF Power (W)	1550
Sampling Depth (mm)	10
Carrier Gas Flow (L/min)	0.90
Helium Cell Gas Flow (mL/min)	5.0
Lens Tune	Autotune
Autosampler Type	SPS4
Ion Lenses Model	X-Lens

Results and Discussion

- Representative calibration curves are presented in Figure 2. The plots for Ca and Pb show excellent linearity across the calibrated range, with correlation coefficients of 0.9999 or better

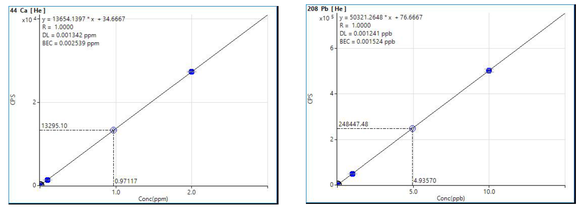

Figure 2. *Representative calibration curves for calcium and lead*

Verification of instrument calibration and sample digestion process

- To ensure quality control, a CCV standard was analyzed during the analytical sequence; all elements in the CCV and the initial ICV were recovered within ±10% of the actual concentration (Fig. 3)

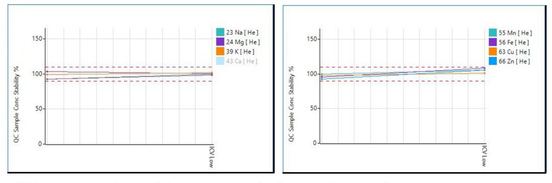

Figure 3. *ICV and CCV data for selected elements*

Quantitative results for commercial plant-based protein supplement powders

- Table 3 shows quantitative results for six (6) commercial plant-based protein supplement powders for elements Na, Mg, K, Ca, P, Mn, Fe, Cu, Zn, As, Cd, Pb, Hg
- Lead concentration in sample #6 was 1,405 ppb, considerably higher compared to other samples tested

Table 3. *Quantitative results (average of n=2) measured using the Agilent 7850 ICP-MS for six (6) plant-based commercial protein supplement powders*

Sample No.	Concentration Value of Analyzed Elements													
	Parts Per Million									Parts Per Billion				
	^{23}Na	^{24}Mg	^{31}P	^{39}K	^{43}Ca	^{55}Mn	^{56}Fe	^{63}Cu	^{66}Zn	^{75}As	^{111}Cd	^{208}Pb	^{201}Hg
1	9125.33	369.44	6689.50	395.80	1261.54	4.63	221.52	10.20	88.17	26.83	35.78	24.12	<LOD
2	3068.93	493.82	3082.40	2160.72	287.66	14.63	53.48	5.77	21.85	27.91	17.55	17.83	<LOD
3	12542.70	662.95	8717.89	1403.55	976.81	17.89	186.22	7.31	111.62	17.02	55.22	26.86	<LOD
5	8881.41	2180.62	12280.60	3575.50	5277.93	38.89	175.74	6.77	127.16	14.08	57.81	30.18	<LOD
6	634.93	626.11	1460.32	9005.66	2400.08	45.95	448.65	5.82	21.22	85.18	37.01	1405.17	<LOD
7	4142.06	1523.53	12613.83	11962.75	5321.02	33.49	167.48	9.36	115.49	20.52	51.16	104.49	<LOD

Quantitative results for standard reference material 3252 Protein Drink Mix

Table 4 shows quantitative results (average of n=3) for NIST 3252 Protein Drink Mix for elements Na, Mg, K, Ca, P, Mn, Fe, Cu, Zn, As, Cd, Pb, Hg

Table 4. *Quantitative results (average of n=3) measured using the Agilent 7850 ICP-MS for NIST 3252 Protein Drink Mix and comparison to its Certificate of Analysis*

Element	Average Result ± SD (n=3)	RSD (%)	Protein Drink Mix 3252 CoA	'3252 CoA' minus 'Average Result'	Percent Difference b/w '3252 CoA' and 'Average Result'
^{23}Na	5676 ± 586 ppm	10.33	6820 ppm	1144 ppm	16.77 %
^{24}Mg	5795 ± 107 ppm	1.85	6337 ppm	542 ppm	8.55 %
^{31}P	14750 ± 485 ppm	3.29	17210 ppm	2460 ppm	14.29 %
^{39}K	9966 ± 317 ppm	3.19	11550 ppm	1584 ppm	13.71 %
^{43}Ca	16482 ± 549 ppm	3.33	17840 ppm	1358 ppm	7.61 %
^{55}Mn	9.87 ± 0.07 ppm	0.67	11.12 ppm	1.25 ppm	11.20 %
^{56}Fe	338.3 ± 10.16 ppm	3.00	318.00 ppm	-20.3 ppm	11.20 %
^{63}Cu	37.65 ± 4.82 ppm	12.81	36.36 ppm	-1.29 ppm	-3.55 %
^{66}Zn	221.1 ± 5.48 ppm	2.48	235.00 ppm	13.9 ppm	5.91 %
^{75}As	33.17 ± 5.62 ppb	16.93	NR	NA	NA
^{111}Cd	37.08 ± 3.86 ppb	10.42	40.78 ppb	3.70 ppb	9.07 %
^{208}Pb	43.85 ± 3.31 ppb	7.54	40.30 ppb	-3.55 ppb	-8.81 %
^{201}Hg	<LOD	NA	NA	NA	NA

- Results for NIST 3252 Protein Drink Mix yielded RSD values <17%, with 10/12 elements yielding RSD values ≤10%
- The percent difference in concentration values between the NIST 3252 Protein Drink Mix CoA and the 'Average Result' were all <17%, with 6/11 elements <10%

Conclusion

- The RCFTR ICP-MS Facility has capacity to accurately test a range of metals in plant-based protein supplements. Accurate metal testing is essential for a healthy sustainable protein ecosystem ensuring safe public consumption of food products