Elemental Analysis of Spent Media For Cultivated Meat Media Cell Type Optimization

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Introduction

People need to consume sufficient amounts of essential macronutrients—carbohydrates, fats, and protein—to support their body's energy needs. Protein is needed for growth, development, and repair of body tissues, and is especially important for building or maintaining muscles and for bone-health. Meat, poultry, fish, dairy products, and eggs are a major source of protein while plant-based sources include soya, beans, nuts, lentils, grains, cereals, fruit, and vegetables.

Around the world, there are increasing numbers of people who are following a vegan or vegetarian diet, or who are reducing their intake of animal-based foods for ethical, dietary, or health reasons. Recent reports on the impact of intensive livestock farming on the climate and natural resources may persuade even more people to limit the amount of meat in their diets. The food industry is keenly aware of the rise in popularity of meat-free foods. This trend can be seen by the ever-increasing selection of alternative protein foods on supermarket shelves and the menus of fast-food outlets and restaurants. Some food companies, including several startups, are already selling products that are produced by cultivating "meat" from cells in a bioreactor.

To ensure that non-meat based protein and alternative protein products are safe for human consumption, manufacturers must comply with Good Manufacturing Practices (GMP). Typically, GMP guidelines provide guidance for manufacturing, testing, and quality assurance of foods. Food safety analysis includes testing for chemicals, e.g., organic contaminants such as pesticide residues, and inorganic contaminants such as heavy metals, which are controlled in foodstuffs. In the United States (US), the Food and Drug Administration (FDA) regulates a wide range of foods and publishes analytical methods that laboratories should use to help ensure food safety. For example, FDA Elemental Analysis Manual (EAM) 4.7 is a comprehensive method that describes how to determine 12 elements in food digests (prepared using microwave assisted acid decomposition) by ICP-MS. EAM 4.7 also outlines a series of quality control (QC) tests to ensure instrument performance and data accuracy (1). Companies wanting to produce, import, or export cell-based alternative meats may need regulatory approval in each target market. However, it is likely that existing analytical testing of foods, such as EAM 4.7, can be applied to any newly developed cell-cultivated food products.

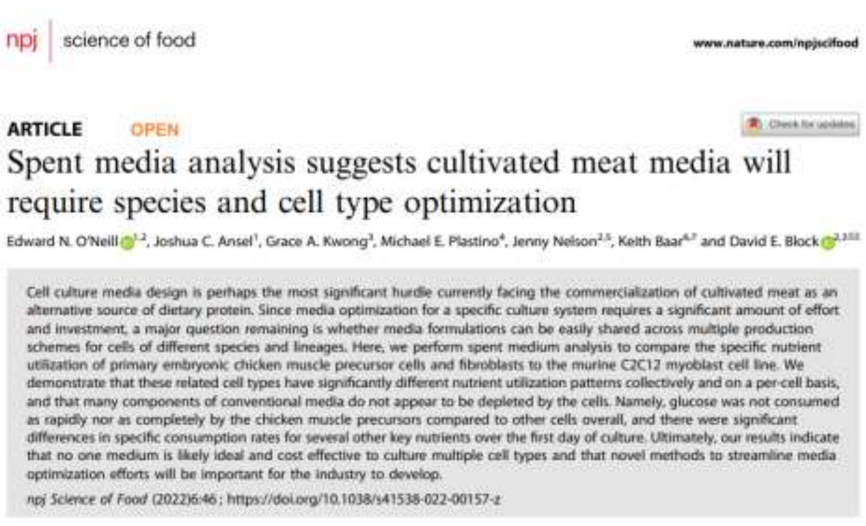
This study describes the use of the Agilent 7850 ICP-MS and Agilent SPS 4 autosampler for the analysis of 23 elements in different plant-based alternative meat samples and cell-culture solutions. The analytical method was adapted from a previous foods analysis study using the 7850 ICP-MS (2). The list of elements included the 12 elements that are specified in EAM 4.7. The elements include heavy metals and trace elements: arsenic, cadmium, chromium, copper, lead, manganese, mercury, molybdenum, nickel, selenium, thallium, and zinc. Other elements included antimony, calcium, cobalt, iron, magnesium, phosphorus, potassium, sodium, strontium, sulfur, and vanadium.

The data quality obtained for these elements was assessed through the measurement of four food certified reference materials (CRMs), a fortified method blank (FMB), and four fortified analytical portions of plant-based meat alternative foods .

All the details for this study can be found from these two QR codes linking to the published paper and application note.

Experimental

All the Experimental information can be found in the below application note and published paper.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Spent media analysis suggests cultivated meat media will require species and cell type optimization

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Cell culture media design is perhaps the most significant hurdle currently facing the commercialization of cultivated meat as an alternative source of dietary protein. Since media optimization for a specific culture system requires a significant amount of effort and investment, a major question remaining is whether media formulations can be easily shared across multiple production schemes for cells of different species and lineages. Here, we perform spent medium analysis to compare the specific nutrient utilization of primary embryonic chicken muscle precursor cells and fibroblasts to the murine C2C12 myoblast cell line. We demonstrate that these related cell types have significantly different nutrient utilization patterns collectively and on a per-cell basis, and that many components of conventional media do not appear to be depleted by the cells. Namely, glucose was not consumed as rapidly nor as completely by the chicken muscle precursors compared to other cells overall, and there were significant differences in specific consumption rates for several other key nutrients over the first day of culture. Ultimately, our results indicate that no one medium is likely ideal and cost effective to culture multiple cell types and that novel methods to streamline media optimization efforts will be important for the industry to develop.

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Application Note
Foods

Determination of Heavy Metals and Trace Elements in Alternative Meats Per EAM 4.7 Method for ICP-MS

Food safety analysis of plant-based protein foods and cell cultures using ICP-MS

Experimental

Instrumentation

An Agilent 7850 ICP-MS, which includes the ORS[4] collision cell and UHMI aerosol dilution system, was used for the analysis. The ICP-MS was configured with the standard sample introduction system consisting of a Micro Mist glass concentric nebulizer, temperature-controlled quartz spray chamber, and quartz torch with 2.5 mm id injector. The interface consisted of a nickel-plated copper sampling cone and a nickel skimmer cone.

Other instrument operating settings were optimized automatically using the ICP-MS MassHunter autotune function. All analytes were acquired in helium (He) mode (enhanced He mode for Se). Operating the ORS[4] in He mode is a widely used method to remove most commonly occurring polyatomic ion interferences on analytes ions by Kinetic Energy Discrimination (KED) (3, 4). When UHMI is selected, all related settings are autotuned as appropriate for the matrix levels of the target sample types. Instrument operating conditions are listed in Table 2.

Table 2. ICP-MS operating conditions*.

ICP-MS Parameter	Setting
RF Power (W)	1600
Sampling Depth (mm)	10
Carrier Gas Flow (L/min)	0.80
Dilution (UHMI) Gas Flow (L/min)	0.15
Lens Tune	Autotune
Helium Cell Gas Flow (mL/min)	4.3 (10**)
Energy Discrimination (V)	5 (7**)

* Shaded parameters are defined in the method and HMI-4 plasma presets; all parameters were automatically optimized during start-up and autotuning. ** Enhanced He mode settings used for Se.

Results and Discussion

Typical 7850 ICP-MS instrument detection limits (DLs) calculated from the ICP-MS MassHunter calibrations are shown in Table 3. The EAM method detection (LOD) and quantification limits (LOQ) – also shown in Table 3 – were calculated based on method blanks measured at the end of the run, n=10 (8). Data was acquired for 30 elements, including the 12 elements required by EAM 4.7, using He cell gas for all analytes.

Table 3. Agilent 7850 ICP-MS detection limits and EAM 4.7 nominal analytical limits, where provided.

Element	ICP-MS MassHunter		Calculated Based on EAM 4.7 Analytical Limits		EAM 4.7 Nominal Analytical Limits	
	DL (µg/kg)	BEC (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)
9 Be	0.000	0.000	0.011	0.037	-	-
11 B	4.290	8.808	1.501	5.002	-	-
23 Na	7.410	275.1	7.505	25.02	-	-
24 Mg	0.140	0.384	0.141	0.471	-	-
27 Al	0.100	0.423	0.204	0.680	-	-
31 P	1.650	3.475	2.372	7.908	-	-
34 S	242.0	911.3	212.9	709.8	-	-
39 K	13.58	152.0	4.311	14.37	-	-
43 Ca	6.450	8.585	5.955	19.85	-	-
47 Ti	0.219	0.110	0.289	0.962	-	-
51 V	0.012	0.060	0.015	0.049	-	-
52 Cr	0.035	0.433	0.032	0.107	5.390	48.90
55 Mn	0.021	0.032	0.010	0.033	2.330	21.20
56 Fe	0.005	0.787	0.053	0.175	-	-
59 Co	0.001	0.002	0.001	0.003	-	-
60 Ni	0.024	0.024	0.006	0.020	6.380	58.00
63 Cu	0.006	0.055	0.018	0.060	6.020	54.70
66 Zn	0.159	1.003	0.116	0.387	37.40	340.0
75 As	0.029	0.043	0.004	0.014	1.270	11.60
78 Se	0.166	0.412	0.088	0.292	7.280	66.10
88 Sr	0.004	0.008	0.002	0.006	-	-
95 Mo	0.005	0.002	0.003	0.012	5.180	47.10
107 Ag	0.001	0.002	0.002	0.005	-	-
111 Cd	0.003	0.003	0.003	0.010	0.408	3.710
118 Sn	0.011	0.129	0.008	0.025	-	-
121 Sb	0.013	0.033	0.007	0.024	-	-
137 Ba	0.017	0.008	0.017	0.058	-	-
201 Hg	0.006	0.006	0.012	0.039	0.861	7.820
205 Tl	0.001	0.004	0.013	0.044	*0.281	*2.100
Pb**	0.002	0.024	0.001	0.005	1.200	10.90

Verification of instrument calibration and sample digestion process

As part of the method quality control procedure specified in EAM 4.7, and to ensure the ongoing validity of the calibration, a CCV standard was analyzed five times during the analytical sequence. Most tested elements reported recoveries within the EAM acceptance criteria of ±10% of the actual concentration of the CCV (results not shown).

To verify the sample digestion process and the accuracy of the analytical method, two sets of the four NIST SRMs were analyzed in duplicate using the 7850 ICP-MS. As shown in Table 4, the mean concentrations were in good agreement with the certified concentrations, meeting the QC criteria requirements of the FDA EAM method of 80–120%. Since not all SRMs are certified for all analytes, blank cells indicate the absence of a certified or reference value.

Table 3 & 4. Mean measured concentrations of four food-based NIST SRMs using the Agilent 7850 ICP-MS. Mean calculated from triplicate sample digestion, each run in triplicate, n=9.

Element	NIST 1577c Bovine Liver					NIST 1947 Lake Michigan Fish Tissue				
	Conc Unit	Certified Conc	Mean Measured Conc	Recovery (%)*	QC Criteria (80–120%)**	Conc Unit	Certified Conc	Mean Measured Conc	Recovery (%)*	QC Criteria (80–120%)**
^{23}Na	mg/kg	2033	2039	100	Pass	-	-	-	-	-
^{24}Mg	mg/kg	620	614	99	Pass	-	-	-	-	-
^{31}P	mg/kg	11,750 R	12,189	104	Pass	-	-	-	-	-
^{34}S	mg/kg	7490	7541	101	Pass	-	-	-	-	-
^{39}K	mg/kg	10,230	10,195	100	Pass	-	-	-	-	-
^{44}Ca	mg/kg	131	115	88	Pass	-	-	-	-	-
^{51}V	µg/kg	8.17	8.52	104	Pass	-	-	-	-	-
^{52}Cr	µg/kg	53	57	107	Pass	-	-	-	-	-
^{55}Mn	mg/kg	10.46	10.18	97	Pass	mg/kg	0.076	0.071	93	Pass
^{56}Fe	mg/kg	197.94	199.86	101	Pass	mg/kg	3.79	3.38	89	Pass
^{59}Co	mg/kg	0.300	0.307	102	Pass	-	-	-	-	-
^{60}Ni	µg/kg	44.5	49.3	111	Pass	-	-	-	-	-
^{63}Cu	mg/kg	275.2	256.8	93	Pass	mg/kg	0.411	0.356	87	Pass
^{66}Zn	mg/kg	181.1	181.7	100	Pass	mg/kg	2.66	2.44	92	Pass
^{75}As	µg/kg	19.6	22.7	116	Pass	mg/kg	0.732	0.672	92	Pass
^{78}Se	mg/kg	2.031	2.182	107	Pass	mg/kg	0.475	0.426	90	Pass
^{88}Sr	µg/kg	95.3	96.8	102	Pass	-	-	-	-	-
^{95}Mo	mg/kg	3.30	3.49	106	Pass	-	-	-	-	-
^{107}Ag	µg/kg	5.9	6.1	104	Pass	-	-	-	-	-
^{111}Cd	µg/kg	97.0	98.4	101	Pass	-	-	-	-	-
^{121}Sb	µg/kg	3.13 R	3.74	120	Pass	-	-	-	-	-
^{201}Hg	µg/kg	5.36 R	5.93	111	Pass	mg/kg	0.254	0.274	108	Pass
Pb	µg/kg	62.8	63.6	101	Pass	-	-	-	-	-

Element	NIST Whole Milk Powder SRM 1549a					NIST Rice Flour SRM 1568b				
	Conc Unit	Certified Conc	Mean Measured Conc	Recovery (%)*	QC Criteria (80–120%)**	Conc Unit	Certified Conc	Mean Measured Conc	Recovery (%)*	QC Criteria (80–120%)**
^{23}Na	mg/kg	3176	3648	115	Pass	-	-	-	-	-
^{24}Mg	mg/kg	892	1018	114	Pass	mg/kg	559	525	94	Pass
^{31}P	mg/kg	7600	8792	116	Pass	mg/kg	1530	1711	112	Pass
^{39}K	mg/kg	11920	13673	115	Pass	mg/kg	1282	1307	102	Pass
^{44}Ca	mg/kg	8810	10195	115	Pass	mg/kg	118.4	125.0	105	Pass
^{52}Cr	-	-	-	-	-	mg/kg	118.4	124.5	105	Pass
^{55}Mn	mg/kg	0.184	0.189	103	Pass	-	19.2	19.2	100	Pass
^{56}Fe	mg/kg	1.85 R	2.12	115	Pass	-	7.42	7.68	104	Pass
^{63}Cu	-	-	-	-	-	mg/kg	2.35	2.39	102	Pass
^{66}Zn	mg/kg	33.8	34.7	103	Pass	mg/kg	19.42	18.55	96	Pass
^{75}As	-	-	-	-	-	mg/kg	0.285	0.335	118	Pass
^{78}Se	mg/kg	0.242	0.288	119	Pass	mg/kg	0.365	0.425	116	Pass
^{111}Cd	-	-	-	-	-	mg/kg	0.0224	0.0201	90	Pass
^{201}Hg	-	-	-	-	-	µg/kg	5.91	6.00	107	Pass

Matrix effects and spike recoveries

To test for non-spectral interferences (matrix effects), an FMB was prepared by spiking the blank at 1 µg/kg for most trace elements, 50 µg/kg for Al, Fe, Cu, Zn, and 4000 µg/kg for major elements including K, P, and S. The FMB was analyzed periodically throughout the entire analysis run. All recoveries were within the EAM 4.7 method acceptable % recovery range of 90–110%, as shown in the application note.

A spike recovery (FAP) test was carried out to check the accuracy of the 7850 ICP-MS method for the analysis of the plant-based (meat-substitute) food products. Each sample was spiked with all elements at 1 or 50 µg/kg and measured using the 7850 ICP-MS. For samples that had naturally occurring elemental concentrations below 1 µg/kg, a 1 µg/kg spike is reported. For samples with higher naturally occurring concentrations, the 50 or 4000 µg/kg spike results are reported. The recoveries for all elements in the fortified plant-based beef-substitute food samples were within the EAM 4.7 method QC criteria of ±20%, as shown in the application note.

Results and Discussion

Calibration Curves

Representative calibration curves are presented in Figure 2. The plots for Na, Mg, Mn, Cu, As, and Hg show excellent linearity across the calibrated range, with correlation coefficients of 0.9999 or better.

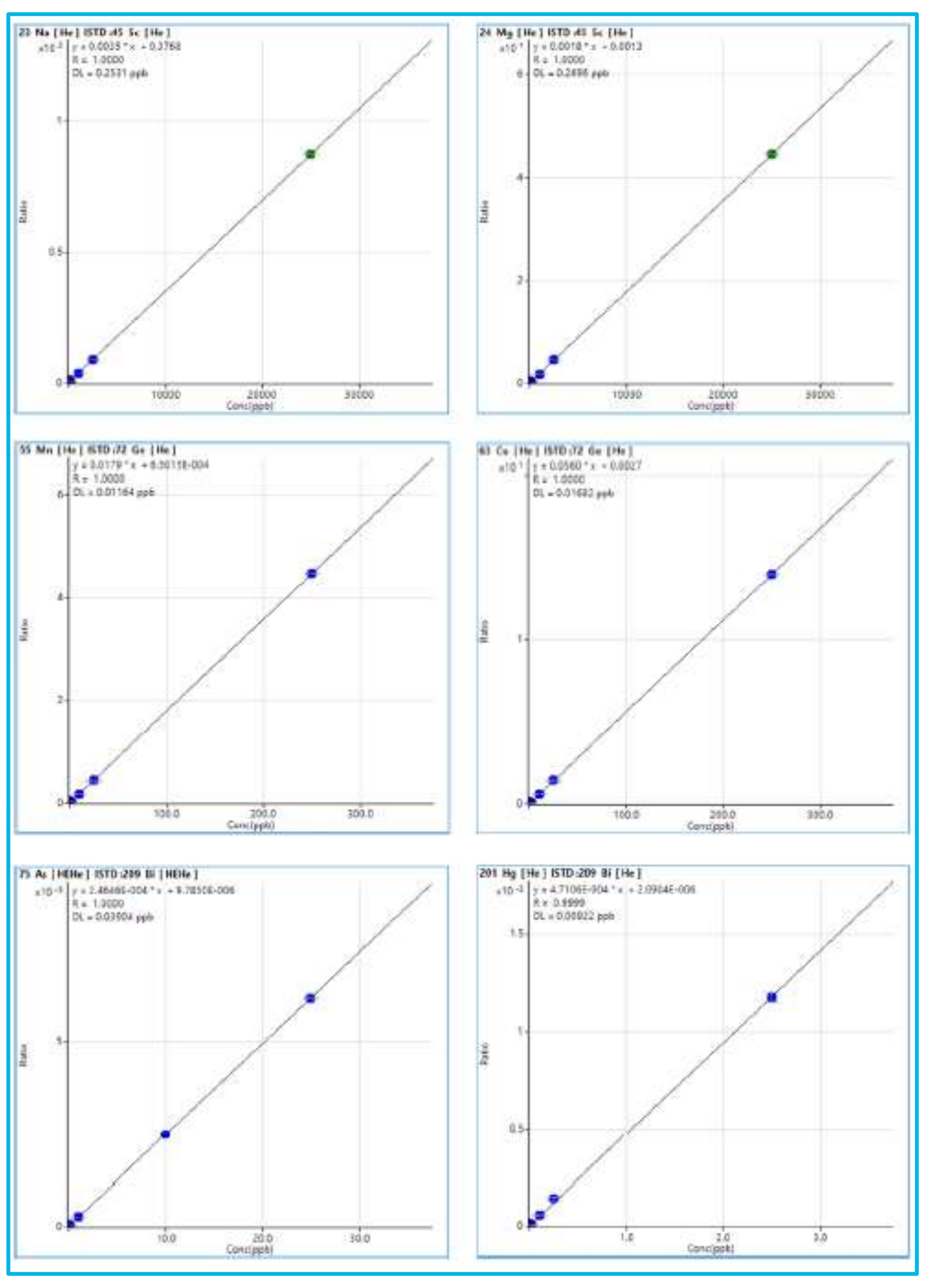


Figure 2. Representative calibrations for major and trace elements.


Figure 3. Stability of ISTD measurements over 48 hours. The ISTD recoveries have been normalized to the calibration blank for all samples.

IntelliQuant data

In this study, IntelliQuant data was acquired for each plant-based food sample and SRM with the 7850 ICP-MS operating in He mode. The data can be displayed in a periodic table heat map view, as shown for the plant-based "minced beef" sample in Figure 4. The color intensity heat map shows the approximate concentration of up to 78 elements in each sample, with a darker color indicating a higher concentration of an element. The IntelliQuant data is a quick and simple way to get an overview of the elemental content of a sample and identify the presence of any unexpected elements.

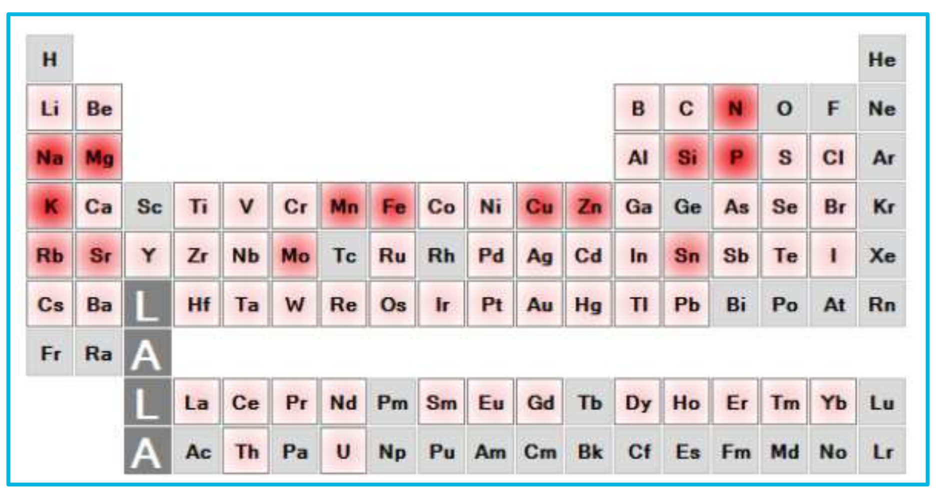
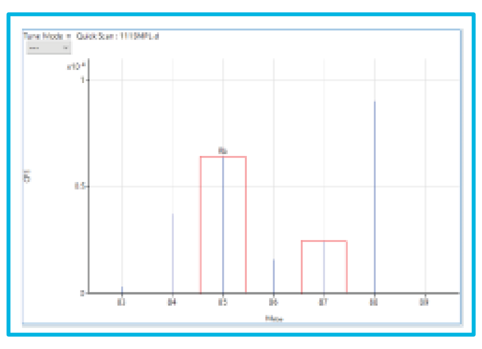


Figure 4. Periodic table heat map view of ICP-MS IntelliQuant data acquired for the plant-based "minced beef" sample.

Figure 4 shows that the plant-based "minced beef" sample contained a relatively high concentration of Rb. Rb wasn't calibrated as part of the quantitative study, so the natural isotope template feature of IntelliQuant was used to check the Quick Scan spectrum to confirm its identity. Figure 5 shows a good fit to the natural isotope template for Rb, confirming its presence in the sample.

Figure 5. Rb in the plant-based minced beef sample was confirmed using the isotope template fit function in the IntelliQuant Quick Scan mass spectrum.



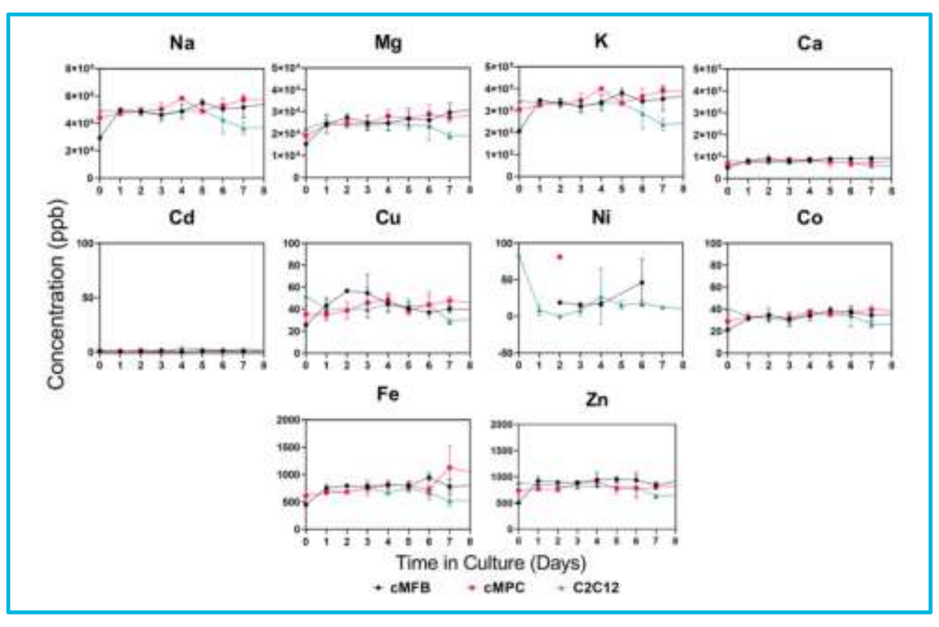


Figure 6. Elemental analysis of spent media from cultures of three cultivated meat-relevant cell types, using inductively coupled plasma-mass spectrometry. Data points represent the mean of three biological replicates ± standard deviation. The y-axis scales vary widely between the graphs, so the figure does not directly indicate relative abundances of the different elements in the spent media samples. Several other elements were targeted for analysis but their concentrations in the samples were too low for detection. cMFB primary embryonic chicken muscle fibroblasts. cMPC primary embryonic chicken muscle precursor cells. C2C12 murine myoblast-like cell line..

Conclusions

- The Agilent 7850 ICP-MS was used to analyze 30 elements in a range of plant-based protein foods and 29 elements in a range of cell culture media.
- The analysis was done in accordance with US FDA EAM method 4.7 for food and related products and included the 12 elements specified in the 4.7 method. All the food samples were prepared in the same batch using a single microwave digestion method, while the cell media samples were simply diluted before analysis.
- The 7850 ICP-MS method was predefined based on a previous EAM 4.7 food analysis batch, and the instrument was autotuned, saving development time. All elements were measured using a single data acquisition mode, with effective removal of polyatomic interferences ensured by operating the ORS[4] collision cell in He-KED mode.

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