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Original Research Article
**Instrumental Analysis of Foods: Inductively
Coupled Plasma Mass Spectrometry for
Determination of Metals in Cereals and Fast Ion
Chromatography Analysis for
Minerals in Sport Drinks**

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ABSTRACT

Aims: Foods are analyzed for their content for important reasons that include: adherence to government regulations, nutritional labeling, authentication, inspection (for grading), safety, quality control, and detection of adulterants.

Study design: Inductively coupled plasma mass spectrometry (ICP-MS) analysis of various commercial cereals was accomplished. Commercial drinks were analyzed by ion chromatography instruments.

Place and Duration of Study: The investigation was carried out at the University of Nebraska, Omaha Nebraska from January 2014 to May 2014.

Methodology: Cereals were treated in strong acid to extract heavy metals. Following dilution steps, these extracts were injected into ICP-MS for analysis. Quantity of metals as well as isotope distribution was established. Commercial sports drinks were examined for clarification and injected into ion chromatography instruments for analysis for calcium, sodium, potassium, magnesium, chloride, phosphate, sulphate, and fluoride.

Results: (ICP-MS) analysis of various commercial cereals showed a content of chromium, nickel, copper, zinc, and traces of lead. In general, zinc was found to be highest in content within the cereal products analyzed ranging from 2.951 mg/kilogram to 90.56 mg/kilogram (mean = 58.38 mg/kilogram), followed by copper ranging from 1.218 mg/kilogram to 4.395 mg/kilogram (mean =2.756 mg/kilogram). This is followed in amounts (by average of all samples tested) by nickel, lead, and chromium. Chromium, copper, nickel, and zinc are considered human micronutrients. Sport drinks are consumed for their mineral content and enhancement of electrolytes. Various sports drinks analyzed showed both cation and anion minerals in their content, which included: sodium, potassium, calcium, magnesium, sulfate, phosphate, chloride, fluoride, and nitrate ions.

Conclusion: ICP-MS and ion chromatography are versatile approaches for analysis of food nutrient value and metals content. ICP-MS can track the absorption of heavy metals into commercial cereals. Ion chromatography can identify adulterants in sport drinks.

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Keywords: ICP-MS, cereals, mass spectrometry, food analysis, sports drinks

1. INTRODUCTION

Chromium, copper, nickel, and zinc are considered human micronutrients. Atmospheric deposition from urban and agricultural areas may be responsible for lead residue in agricultural products. The analysis of cereals utilizing ICP-MS is

a valuable and versatile application for food analysis. Many sport drinks are consumed for their mineral content and enhancement of electrolytes within the blood stream.

The analysis of foods has gained more recognition and has the need for robust and sensitive methodologies to ensure safety, quality, and compliance to government regulations and consumer demand [1]. As demand for nutritional content increases, so too must the techniques to ensure quality and compliance [1]. The mineral content of cereal grains comprise from 1% to 3% of their total weight and are concentrated in the external areas of wheat grain [2]. In many countries, cereals are the main source of food for the human diet, with wheat and rice comprising the basic food for approximately 80% of the world population [3]. The actual metallic content varies greatly and depends on the type of land cultivated, variety of grain, weather, insecticide, and fertilizer used for cultivation [4]. The concentrations of the toxic heavy metals of lead and cadmium are generally very low [5].

Micronutrients are considered to be manganese, iron, copper, zinc, chromium, and nickel; with sodium, potassium, magnesium, and calcium to be macronutrients [2]. However, lead and cadmium are considered to be trace toxic elements [2]. The variations in levels of lead and cadmium in food products are sufficiently important to require continual monitoring by some countries [6]. In general, wheat flour is considered to be the major source of manganese, copper, zinc, magnesium, and lead that is found to be in breads, but only one-half of the chromium, nickel, tin, and cadmium that is found [7]. There is usually a wide variation in the amounts of nickel, zinc, lead, tin, cadmium, and chromium [7].

Heavy metals, being a potential environmental contaminate, can cause health problems if found in excess within food products [8]. Metal toxicity are responsible for cases of disease, disorders, and malfunction of organs [9]. Atmospheric deposition from urban and agricultural areas can play an important role for enrichment of agricultural produce with lead and cadmium [10]. Examples are copper arsenate used as insecticide [11], calcium arsenate used as herbicide [12], and lead hydrogen arsenate used as insecticide [13]. Cadmium exposure causes kidney damage [14], lead in soil or water causes kidney damage [15], and excess copper or zinc is toxic [16].

Chromium, copper, nickel, and zinc are considered human micronutrients. Atmospheric deposition from urban and agricultural areas may be responsible for lead residue in agricultural products. Since sport drinks are consumed for their mineral content, there are concerns about nutritional content and adulterants, which is a further encouragement for ion chromatography methodologies for analysis. A particular advantage of ion chromatography over traditional techniques is the capability for simultaneous determination of several species [17]. Ion chromatography has been shown to be a simple, fast method of proving identity or brand of many types of spirits on the basis of anion composition [18]. This will enable the testing of suspicions that arise during food control [18]. Ion chromatography is shown to be a simple, rapid, and accurate method for the quantitative determination of fluoride, chloride, nitrate, phosphate, and sulphate ions in green, black, and white tea infusions [19]. Ion chromatography will like-wise be shown to be accurate for analysis of sport-type drinks in this study.

Clearly, a continual need for versatile and accurate methodologies for monitoring metals in food products is warranted. New methods or extension of known methods would be advantageous and assist in commercial compliance for safe and nutritious foods.

2. MATERIAL AND METHODS

2.1 Instrumentation and Reagents

The Varian Model ICP-MS (inductively coupled plasma mass spectrometry) is utilized in this study. This model uses argon gas as carrier of sample liquid with interface with a Dell computer for operation and Varian liquid sampler. For ion chromatography a 792 Basic IC instrument was utilized for cation and anion identification. Mobile solvents utilized for cation chromatography are 70% ethanol in distilled water, with Metrosep column 1-2. Mobile solvents utilized for anion chromatography are 0.10 molar sulfuric acid, 1.8 millimolar sodium carbonate, 1.7 millimolar sodium bicarbonate in distilled water, with IC SEP AN1 column. The 792 Basic IC instrument uses a thermostatted conductivity detector. The thermostatted detector maintains a highly stable temperature with minimal variation from the operating temperature. Actual cell volume of detector is 1.5 microliter. All reagents used were analytical grade and supplied by Sigma-Aldrich, PO Box 14508, St Louis Missouri 63178 USA.

2.2 Preparations

Commercial products obtained were taken from regular shelf locations for public consumption. Products were kept separate in suitable plastic containers and labeled appropriately. For each food product a known amount was placed into clean mortar and pestle for grinding to fine powder-like consistency. From this powder-like material then, into plastic weighing boats an amount very close to one gram, recorded, and measured to three decimal places is obtained. This amount is placed into clean Erlenmeyer flask (with Tuttle lid) with ten milliliters of distilled water and ten milliliters of 15.9 molar nitric acid. The contents are heated to about 50 °C for one hour. Remove from heat and cool, followed by addition of 25 milliliters of distilled water. The mixture is filtered through Whatman #1 filter paper into a cleaned 100 milliliter volumetric flask (filtering through filter paper can be repeated if necessary). Sample is diluted to volume with distilled water then aliquot placed into Varian sampling tubes and submitted for analysis. Data acquired and received after analysis by ICP-MS is in units of parts per billion and requires conversion to milligram per kilogram for each metal by calculation including mass of sample and dilution step, equation [1]:

$$\text{Milligram of metal per kilogram} = \frac{(\text{amount in parts per billion})(100)}{(\text{Mass for analysis})(1000)} \quad [1]$$

For comparison and internal control, distilled water was included, which contained zero amounts of all metals targeted, and was treated exactly the same as test cereal samples.

2.3 Software and Numerical Analysis

Summary statistical analysis was accomplished utilizing EXCEL (Microsoft Office Professional plus 2013 Windows 7) and PAST multivariate analysis version 3.01 (copyright O. Hammer and D.A.T. Harper 1999-2013). Analysis for t-test, Kolmogorov-Smirnov, and Fligner-Killeen coefficient of variation was done by PAST version 3.01.

3. RESULTS AND DISCUSSION

3.1 ICP-MS Analysis of Cereals

Wheat is one of the most consumed cereals among those that are the main source of food world-wide [2]. The metallic content of wheat is highly variable and depends on the type of land of cultivation, variety of wheat, and types of insecticides/herbicides applied [2]. The careful monitoring of mineral content as well as those metals considered toxic (lead and cadmium) is a necessary endeavour to assure safety and appropriate nutritional content. The analysis of food by ICP-MS is an effective and sensitive methodology for assuring quality and safety.

The basic schematic plan for the departmental ICP-MS instrument arranged and managed for general application is presented in Figure 1. The organization follows an overall general set-up for analytical applications [20]. The components indicated are in the same order in which a test sample flows and are arranged in sequence [20]: 1) Sample conversion to suitable form for introduction into a plasma; 2) Ionization by the plasma; 3) Extraction of ions from plasma; 4) Focusing of ions into mass spectrometer; 5) Mass spectrometer separation of ions based on mass-to-charge ratio (m/z); 6) Counting of ions to quantify. Test sample preparation for this study solubilized all metals present in cereals into an acidic aqueous solution that was carefully filtered and efficiently passed through the Varian sampler to be followed by nebulization for introduction into the plasma. All cereal tests samples ran accordingly with the expected outcome of measured elements as with other applications known for this instrument (i.e. no difficulties).

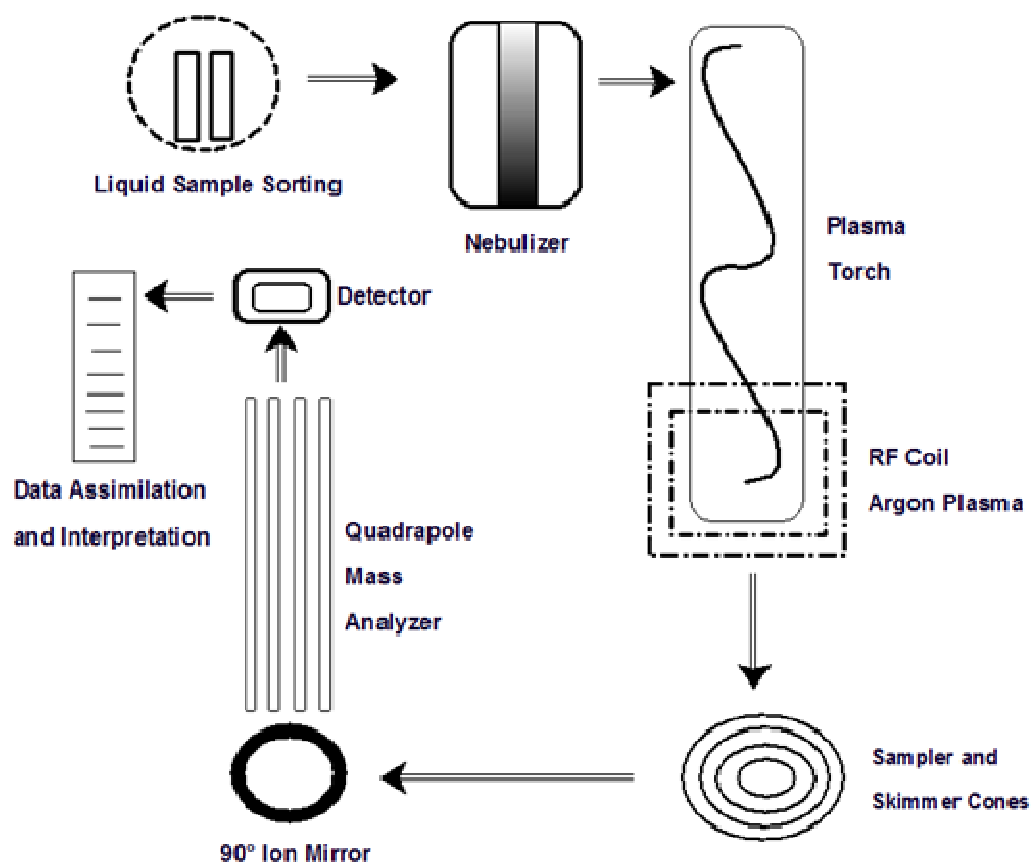


FIG. 1. Schematic of ICP-MS instrument utilized for cereals analysis. Preparation of samples were sufficient to avoid corruption of any step of instrumentation and eliminate potential background interferences. Steps followed: 1) Sample conversion; 2) Ionization by the plasma; 3) Extraction from plasma; 4) Focusing into mass spectrometer; 5) Mass spectrometer separation by mass-to-charge ratio (m/z); 6) Counting of ions to quantify.

The outcome found for the specific determined metallic elements are presented in Table 1. All test cereal samples of 1 through 6 showed some level of metals present in the format of milligram of metal per kilogram of cereal product (i.e. mg/kilogram). Metals chromium, nickel, copper, and zinc is considered to be micronutrients; however, lead is generally considered to be a toxic contaminant [2, 5, 6, 10]. It is generally considered that a weekly intake of lead should not exceed 0.05 milligram per kilogram of body weight in order to avoid health issues [10]. An individual weighing 77.1 kilograms (170 pounds) should not consume more than 3.855 milligrams of lead per week to avoid health issues. Easily identified is the very broad range of amounts of zinc in cereal test samples (range is 2.951 mg/kilogram to 90.56 mg/kilogram). Statistical analysis of outcome will be discussed next.

TABLE 1. METALS DETECTED IN COMMERCIAL CEREALS BY ICP-MS

Sample Tested	METALS DETECTED				
	Chromium (mg/kilogram)	Nickel (mg/kilogram)	Copper (mg/kilogram)	Zinc (mg/kilogram)	Lead (mg/kilogram)
1 Large granular chunks, multiple colored product cereal	0.5737	0.9842	2.557	82.17	0.415
2 Light brown powder to small granules wheat bran cereal flakes Cereal	0.6561	0.2266	1.218	2.951	1.181
3 Small powder to small granules, multiple colored Cereal	0.6224	0.1988	2.332	38.31	0.3856
4 Brown to light brown powder Cereal	0.6050	0.4461	1.849	90.56	0.2784
5 Brown to dark brown granule high wheat fiber Cereal	0.6002	0.5635	4.187	77.91	1.753
6 Brown flaked whole wheat bran Cereal	0.6560	0.6121	4.395	No result	0.2682
7 Distilled Water	0	0	0	0	0

Statistical analysis for metals determination is presented in Table 2, and indicate useful information for consideration of nutrition content of foods as well as for the lead metal content. Save for the metal zinc, the median values of metals content (mg metal per kilogram of cereal food product) are close to the mean values for these six cereals. The variation of zinc is striking, having a mean of 58.38 mg/kilogram but median of 77.91 mg/kilogram and standard deviation (37 mg/kilogram) that is 63% of the mean value. The minimum, maximum, and range of values are presented in Table 2.

TABLE 2. STATISTICAL PARAMETERS OF HEAVY METALS DETECTED IN CEREALS

	METALS DETECTED				
Statistic	Chromium (mg/kilogram)	Nickel (mg/kilogram)	Copper (mg/kilogram)	Zinc (mg/kilogram)	Lead (mg/kilogram)
Mean	0.6189	0.5052	2.756	58.38	0.7135
Median	0.6137	0.5048	2.445	77.91	0.4003
Standard Deviation	0.033	0.29	1.3	37	0.61
Range	0.0824	0.7854	3.177	87.61	1.485
Minimum	0.5737	0.1988	1.218	2.951	0.2682
Maximum	0.6561	0.9842	4.395	90.56	1.753
95%	0.03436	0.3039	1.339	45.87	0.64441
Confidence Interval					
†Kurtosis	-1.29	0.483	-1.57	-0.521	0.213
†Skewness	0.00869	0.750	0.412	-1.03	1.29

†Not for mass per kilogram of material

Kurtosis values are negative for chromium (-1.29), copper (-1.57), and zinc (-0.521), which indicates a distribution that is flatter than a Guassian distribution. Positive kurtosis for nickel (0.483) and lead (0.213) indicates a distribution of greater peakness than a Guassian distribution [21]. Skewness values are positive for chromium (0.00869), nickel (0.750), copper (0.412), and lead (1.29), which indicates asymmetric distribution with higher values [21]. However, a skewness of -1.03 for zinc indicates asymmetric distribution with lower values.

Previous studies of agricultural cereals bound for marketing and from middle-east nations found the metal lead in rice, maize, barley and wheat [10]. The amounts of lead detected, also measured as mg/kilogram of product, showed amounts to be [10]: 0.239 mg/kilogram, 0.116 mg/kilogram, 0.131 mg/kilogram, and 0.398 mg/kilogram, respectively. These findings are generally similar to that found in the six cereal food products analyzed and presented in Table 1. When applying the t-test for unpaired data, it is found that the mean level of lead in middle-east cereals (inclusive of rice, maize, barley, and wheat) is equal to the mean level of lead in cereals 1 to 6 (P = 0.16) [21]. In addition, the result of Kolmogorov-Smirnov test showed that the two populations of lead analysis showed equal distributions (P = 0.10). Results of Fligner-Killeen test showed the two populations of cereals have equal coefficients of variation (P = .54). Such outcome suggests that lead is a common species found in cereal grains; however, this is not an indication that the cereal grains examined in this study have the same origin.

Metals are ubiquitous in nature with lead being the heaviest of the non-radioactive metals that naturally occur in substantial quantities in the earth's surface. Lead is present in all soils, rivers, lakes, and seawater. Lead occurs naturally on Earth exclusively in the form of four isotopes: lead-204, -206, -207, and -208 [22]. In addition, the instrumental settings for the ICP-MS analysis allowed the computation of the predominant isotope of lead to be determined, which for lead found in this study was consistently 207 amu (see Table 3).

TABLE 3. AVERAGE ATOMIC MASS UNITS (AMU) FOR LEAD DETECTED IN CEREALS

Sample Tested	AVERAGE ATOMIC MASS OF LEAD DETECTED (atomic mass units)
1	207
2	207
3	207
4	207
5	207
6	207
7 Distilled Water	none present

The isotope Lead-207 is 22.1 % of natural abundance of all lead isotopes (with lead-208 the highest at 52.4 %) [23]. Therefore it is reasonable to find this level of lead isotope. The analysis of cereal samples by ICP-MS enabled the determination of multiple and various metallic elements. Some elements determined are considered to be micronutrients

for human health. Also, the results included the determination of lead which is generally considered to be a nondesired and toxic component of many cereal grain agricultural products. The monitoring of these metallic elements then would be highly beneficial for numerous reasons, including the enhancement of human nutrition and health.

3.2 Analysis by Ion Chromatography

Electrolytes within the body are minerals that carry an electric charge. Electrolytes affect the amount of water in the body, acidity of blood (pH), muscle function, and other important processes. Common electrolytes include: calcium, chloride, magnesium, phosphorous, potassium, and sodium. In addition, electrolytes can be acids, bases, as well as salts [24]. The measurement of electrolytes consumed for medical care of disease is an important aspect in clinical treatment. Therefore, the measurement of types and quantity of electrolytes can have great clinical importance.

The process to adulterate a consumer product (e.g. to make impure by adding extraneous, improper, or inferior ingredients) can be a costly infraction to patents and a gross misfeasance of consumer trust. Furthermore, the adulteration of a product necessary for clinical treatment of a disease can injure patient health. Therefore, monitoring of quality of product and assessment of minerals consumed is highly important. The use of ion chromatography has important advantages, which include: speed, sensitivity, selectivity, simultaneous detection, and stability of the separator columns.

Various consumer products known or believed to be a significant source of electrolytes were analyzed by ion chromatography to demonstrate the efficacy of the method. The drinks are clarified by manufacturer and presented free of bacterial or fungal contamination. The advantage of ion chromatography here, is that such a product can be directly injected into the instrument for fast examination.

Results for cation determination of various electrolyte mixtures are presented in Table 4. The cations sodium, potassium, calcium, and magnesium are generally recognized as electrolyte minerals. There is a broad range of ion concentration throughout these mixtures (e.g. for sodium a 2590xfold range, for potassium a 99xfold range, for calcium a 4xfold range, for magnesium a 45xfold range) . The instrument was able to quickly (less than 15 minutes) complete the total assay, and report the wide range of concentrations. Hence, fast and versatile assay of ions was accomplished for a variety of products by this instrumentation and solvent conditions (see MATERIALS AND METHODS).

TABLE 4. CATIONS DETECTED IN SPORT DRINKS

SAMPLE	SODIUM (MOLAR)	POTASSIUM (MOLAR)	CALCIUM (MOLAR)	MAGNESIUM (MOLAR)
POWERADE ZERO MIXED BERRY	2.367×10^{-3}	4.056×10^{-4}	4.860×10^{-5}	7.816×10^{-5}
SOBE LIFEWATER PEAR FUJI APPLE	3.017×10^{-4}	1.570×10^{-3}	1.016×10^{-3}	not detected
WATER COOLER LIME	5.672×10^{-4}	3.547×10^{-3}	not detected	not detected
POWERADE ZERO LIME	1.916×10^{-2}	2.081×10^{-3}	5.232×10^{-4}	3.900×10^{-4}
NUTRA H ₂ O	5.019×10^{-4}	2.165×10^{-3}	4.726×10^{-4}	2.035×10^{-4}
BRISK ICE TEA	1.060×10^{-2}	3.529×10^{-3}	2.807×10^{-4}	2.285×10^{-4}
SOBE PINEAPPLE PEAR	4.641×10^{-4}	1.906×10^{-3}	1.118×10^{-3}	7.232×10^{-5}
VITAMIN WATER POWER-C ZERO	7.395×10^{-6}	3.550×10^{-5}	not detected	9.190×10^{-6}
POWERADE ZERO ORANGE	not detected	2.568×10^{-3}	5.613×10^{-4}	4.195×10^{-4}

TABLE 5. ANION SPECIES DETECTED IN SPORT DRINKS

SAMPLE	FLUORIDE (MOLAR)	CHLORIDE (MOLAR)	NITRATE (MOLAR)	PHOSPHATE (MOLAR)	SULFATE (MOLAR)
POWERADE ZERO MIXED BERRY	1.400×10^{-5}	1.666×10^{-2}	2.900×10^{-5}	not detected	2.165×10^{-4}
SOBE LIFEWATER PEAR FUJI APPLE	1.421×10^{-6}	6.371×10^{-4}	1.280×10^{-5}	not detected	8.150×10^{-5}
WATER COOLER LIME	7.368×10^{-7}	not detected	2.867×10^{-5}	not detected	7.913×10^{-5}
POWERADE ZERO LIME	1.600×10^{-5}	1.718×10^{-2}	8.870×10^{-6}	4.009×10^{-3}	2.213×10^{-4}
NUTRA H ₂ O	5.416×10^{-5}	7.681×10^{-5}	5.773×10^{-6}	1.950×10^{-5}	not detected
BRISK ICE TEA	8.053×10^{-6}	4.716×10^{-4}	7.483×10^{-6}	6.435×10^{-3}	1.279×10^{-3}
SOBE PINEAPPLE PEAR	7.200×10^{-4}	not detected	5.580×10^{-6}	not detected	1.478×10^{-4}
VITAMIN WATER POWER-C ZERO	1.937×10^{-5}	1.254×10^{-5}	not detected	1.493×10^{-5}	1.065×10^{-4}
POWERADE ZERO ORANGE	1.453×10^{-5}	1.446×10^{-2}	8.740×10^{-6}	5.301×10^{-3}	2.349×10^{-4}

Results for anion determination of various electrolyte mixtures are presented in Table 5. The anions fluoride, chloride, nitrate, phosphate, and sulfate are generally recognized as ions utilized for various biological purposes. Natural sources of water and tap water contain chloride, nitrate, phosphate and sulfate. The anion fluoride is generally viewed to be an aid for inhibiting tooth decay and placed in public water. There is a broad range of ion concentration throughout these mixtures (e.g. for fluoride a 73xfold range, for chloride a 1370xfold range, for nitrate a 5xfold range, for phosphate a 431xfold range, for sulfate a 3xfold range). The instrument was able to quickly (less than 15 minutes) complete the total assay, and report the wide range of concentrations. Hence, fast and versatile assay of ions was accomplished for a variety of products by this instrumentation and solvent conditions (see MATERIALS AND METHODS). Ion chromatography is a versatile and useful tool for determination of medicinal and commercial electrolyte products found in general application today.

4. CONCLUSION

In many countries, cereals are the main source of food for the human diet. Analysis for heavy metals found in cereal grain type food products, whether the micronutrient or toxic contaminants, can be accomplished with high sensitivity by ICP-MS. The amounts of micronutrient metals found in wheat-based flour products can vary widely. The average amounts of the micronutrients chromium, nickel, copper, and zinc found in analyzed commercial cereals were (mg/kilogram): 0.6189, 0.5052, 2.756, and 58.38, respectively. The metal lead was found in all cereals of this study but with a wide variation in amount, noting that the standard deviation of 0.61 mg/kilogram is 85% of the mean value. Of the trace metals determined, zinc was found to be highest in content within the cereal products analyzed, followed by copper, this followed by nickel, lead, and chromium, respectively. The widest variation of micronutrient metal in cereals was zinc with nearly a 31x fold span from 2.951 mg/kilogram to 90.56 mg/kilogram. Current ion chromatography instrumentation can quickly and with versatility assay a broad concentration of cations and anions in electrolyte mixtures. The monitoring of electrolyte content is an important aspect for the clinical use of such mixtures for the treatment of disease. Sensitive and versatile methodologies for monitoring nutrients inside food products will enhance their safety, government compliance, nutritional value, and the demands from consumers.

258
259 **CONSENT (WHERE EVER APPLICABLE)**
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261 None.
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263 **ETHICAL APPROVAL (WHERE EVER APPLICABLE)**
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265 Not applicable.
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DEFINITIONS, ACRONYMS, ABBREVIATIONS

Term: ICP-MS, inductively coupled plasma mass spectrometry; mg, milligrams; amu, atomic mass units.