Microwave Digestion Followed by ICP-OES for the Determination of Al, Cd, Cr, Cu, Fe, Ni, Pb, and Sn in Maize

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INTRODUCTION

Maize (also known as corn) (*Zea mays L.*) is a domesticated cereal grain that has been grown as food and animal feed for tens of thousands of years. It is currently the most widely grown crop in the world, and is used not only for food/feed but also to produce pharmaceuticals, ethanol, oils, and industrial starches (1). Maize is a highly valuable agricultural commodity for Turkey and is currently the third most important grain crop, following wheat and barley. Maize in Turkey is grown mainly in the regions of the Black Sea, Marmara, Aegean, and Cukurova/Mediterranean (2,3).

Most foods contain natural or synthetic chemicals that could become toxic for the consumer. The contamination of food by hazardous environmental metal pollutants is of worldwide concern and requires their detection using chemical analysis (4). Metal contamination can also take place during the handling and processing of foods, from the farm to the point of consumption.

Contact between metal and food, such as with processing equipment, packaging and storage containers, is a significant source of metal contamination. Once metals are present in food, their concentrations can rarely be modified by traditional preparation and processing techniques, although in some cases washing may decrease the

ABSTRACT

Maize (*Zea mays L.*) is currently the most widely grown crop in the world and is used not only for food and animal feed but also to produce industrial starches, biodiesel, ethanol, and oils. The present work reports the results obtained by the proposed method for the determination of metals (Al, Cd, Cr, Cu, Fe, Ni, Pb and Sn) in maize by ICP-OES, using digestion with an oxidant mixture in a closed-vessel microwave oven. The results in the maize samples (minimum-maximum in mg/kg) were: Al 6.678-7.051, Sn 2.092-3.977, Cr 0.394-0.423, Cu 21.56-24.78, Fe 93.37-102.86, Ni 1.679-1.877 and Pb 0.375-0.392. The concentration of Cd was below the detection limit (< 0.03 mg/kg). The certified reference material, CRM NIST1573a Tomato Leaves, was used in order to verify the accuracy of the microwave digestion method. The results of this study were in good agreement with the certified values.

metal content (4-7). Thus, the determination of metals in food products is of special concern because some of them are essential elements for humans, while others are highly toxic (8). For example, heavy metals such as iron (Fe), zinc (Zn), copper (Cu), chromium (Cr), cobalt (Co), and manganese (Mn) are essential, while lead (Pb), cadmium (Cd), nickel (Ni), and mercury (Hg) are toxic at certain levels (9).

The FAO (Food and Agricultural Organisation) and WHO (World

Health Organisation) Joint Committee prepared guidelines for the study of the dietary intake of chemical contaminants including heavy metals. The FAO/WHO report states that it is the responsibility of national authorities to guarantee that food products do not contain toxic chemical substances (e.g., heavy metals, pesticide residues, aflatoxins) in concentrations susceptible to affect the health status of the consumer (4).

For the determination of metals in food samples, spectroanalytical techniques require sample preparation to avoid measurement errors. Factors to be considered for sample digestion include the level of contamination, sample homogeneity, completeness of digestion, reproducibility, suitability of the analytical technique employed, time needed for sample preparation, economic aspects including labor and reagent consumption, equipment cost, etc. (1,2). Wet digestion procedures can be performed in closed or open vessels using thermal energy or microwave radiation. Microwave heating offers a considerable advantage in time over thermal heating. Many microwave-assisted sample preparation systems allow multi-sample preparation with temperature and pressure control features. For wet digestion procedures, it is important to consider the time and amount of reagents needed in comparison to the dilution methods (10). Microwave digestion provides a rapid and efficient method and also reduces volatilization loss and contamination (11-15). The common practice for digestion of plant matrices is the addition of H_2O_2 to HNO_3 . This

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procedure increases the oxidation power of HNO_3 during digestion and enhances the dissolution of the organic material present in the sample (16).

In this study, a sample pretreatment and preparation procedure using microwave acid digestion (MW-AD), followed by inductively coupled plasma optical emission spectrometry (ICP-OES), was examined for the determination of Al, Cd, Cr, Cu, Fe, Ni, Pb, and Sn in maize.

EXPERIMENTAL

Instrumentation

A Model Optima™ 2100 DV inductively coupled plasma optical emission spectrometer (ICP-OES) (PerkinElmer, Shelton, CT, USA) was used for the determination of Al, Cd, Cr, Cu, Fe, Ni, Pb, Sn in maize in diluted solutions. The instrumental operating parameters are listed in Table I.

The most sensitive lines without spectral interferences in the sample matrix were used for the analysis.

The analytical wavelengths used are listed in Table II.

Certified standard reference material CRM SRM 1573a Tomato Leaves (National Institute of Standards and Technology, NIST, Gaithersburg, MD, USA) was used to assess the accuracy and precision of the method. All samples were analyzed in triplicate.

Samples and Reagents

For this study, 21 samples of corn/maize were obtained in 2010 from southeastern Anatolia in Diyarbakir, Turkey. In order to preserve the original quality of the maize, the samples were stored at ambient temperature in sealed plastic bags prior to any conditioning.

Nitric acid (HNO_3 , 65%) and hydrogen peroxide (H_2O_2 , 30%) used for the digestion, were of analytical grade (E. Merck, Darmstadt, Germany). Distilled water was further purified with a Milli-Q™ system (Millipore Corporation, USA) and used throughout the experiments.

Digestion Procedure

A Berghof MWS-3 model microwave digestion system (Berghof, USA) was used for digestion of the maize samples. Microwave acid digestion (MW-AD) was carried out as follows: 0.35 g of maize samples and solid reference material were weighed and transferred into pressure-resistant PTFE vessels and 5 mL of the acid mixture (HNO_3 - H_2O_2 , 2:1) was added to each sample. The vessels were closed and heated in the microwave oven as listed in Table III. The power applied was 600 W. The limit of detection (LOD) and limit of quantification (LOQ) for each metal were determined as follows: 10 independent analyses of a blank solution spiked with the metal at a level of lower concentration of the analytical curve were performed. The LOD and LOQ were calculated from the standard deviation (σ) of these

TABLE I
Instrumental Operating Conditions Using Optima 2100 DV ICP-OES

Parameters	
RF power	1450 W
Plasma gas flow rate	15 L min ⁻¹
Auxiliary gas flow rate	0.2 L min ⁻¹
Nebulizer gas flow rate	0.8 L min ⁻¹
Sample flow rate	1.5 mL min ⁻¹
View mode	Axial
Read	Peak area
Source equilibration time (s)	15 s
Read delay (s)	50 s
Replicates	3
Background correction	2-point (manual point correction)
Spray chamber	Scott type spray chamber
Nebulizer	Cross-flow GemTip™ Nebulizer (HF resistant)
Detector	Solid state (CCD) detector
Purge gas	Nitrogen
Shear gas	Air
Plasma Gas	Argon

TABLE II
Analytical Wavelengths Used

Elements	λ (nm)	LOD ($\mu\text{g mL}^{-1}$)	LOQ
Al	396.153	0.53	1.76
Cd	228.802	0.03	0.10
Cr	267.716	0.04	0.13
Cu	327.393	0.02	0.07
Fe	238.204	0.72	2.40
Ni	231.604	0.03	0.10
Pb	220.353	0.09	0.30
Sn	189.927	0.38	1.26

TABLE III
Temperature Program of the Microwave Digestion System for Maize (*Zea mays* L.) Samples

Step	1	2	3	4
T (°C)	140	160	165	100
T (min) ^a	5	5	2	5
Time (min) ^b	5	5	2	5

^a Waiting time at desired temperature.

^b Time between the two sequential temperatures..

determinations ($LOD = 3 \times \sigma$ and $LOQ=10 \times \sigma$). The vessels were removed and carefully vented in a fume hood after cooling to room temperature in a waterbath. The reaction mixture was subjected to an evaporation procedure by use of the evaporation module in order to remove the acids after the digestion. Then the residue was dissolved in Milli-Q™ water and filtered, and the filtrate diluted to a fixed volume. The ICP-OES measurements of the elements were performed for the diluted solutions.

RESULTS AND DISCUSSION

In this study, wet acid microwave digestion was used as the sample preparation method. The ICP-OES analytical method was applied due to its simplicity and reliability. It also offers high selectivity and has low spectral interference problems from other elements. As can be seen in Table IV, the method was validated through the analysis of CRM NIST 1573a Tomato Leaves (National Institute of Standards and Technology, Gaithersburg, MD, USA) with good recoveries (17,18).

Optical emission spectrometry (OES) with a plasma excitation source uses quantitative measurement of the optical emission from excited atoms to determine analyte concentration. The high-temperature atomization source provides sufficient energy to promote the atoms into high energy levels. The atoms decay back to lower levels and emit light. Since the transitions are between distinct atomic energy levels, the emission lines in the spectra are narrow. The spectra of multi-element samples can be very complex, and spectral separation of nearby atomic transitions requires a high resolution spectrometer. Since all atoms in a sample are excited simultaneously, they can be detected simultaneously, which is the major advantage of OES compared to AAS. ICP-OES provides a

higher reproducibility and quantitative linear range than FAAS (19-22). It is a convenient technique for this type of analysis because of its capability for simultaneous, rapid, and precise determination with a wide dynamic range and low limits of detection. These features allow it to compete successfully with the more traditional techniques of flame atomic emission spectrometry, atomic absorption spectrometry, instrumental neutron activation analysis, and X-ray fluorescence (16,23).

Maize is the world's most widely grown food crop which is used not only as human food, animal feed or for commodities, but also for advanced biochemical factories to produce valuable biomolecules, such as pharmaceuticals, industrial enzymes, nutrients, chemical precursors, and fuels. A continuous surveillance system of contaminants in food is, therefore, crucial for consumer protection and the facilitation of international trade (24). Risk assessment is an evolving process and new information on contaminants, their implicated health effects, and their occurrence in food should be continuously studied and monitored (4).

Of the heavy metals, Hg, Cd, and Pb are most toxic to humans. The bio-accumulation of these elements in the organism leads to a number of diverse and deleterious effects, both in the short and the long term, and varies according to the type of metal. They can cause damage to the kidneys, the immune and nervous systems, and in some cases can have cancerous effects. The classic symptoms of heavy metal intoxication include irritability, headaches, depression, mood changes, tremor, loss of memory, and reduced capacity of sight (25).

In contrast, metals such as Cu, Fe, and Zn are essential for humans. For example, Cu is important in respiration for the synthesis of

TABLE IV
Accuracy Assessment of Analysis of CRM NIST1573a Tomato Leaves

Elements	Certified (mg/kg)	Found (mg/kg)	Recovery (%)
Al	598±12	595±3	99.5
Cd	1.52±0.04	1.53±0.01	100.7
Cr	1.99±0.06	2.02±0.03	101.5
Cu	4.70±0.14	4.68±0.16	99.6
Fe	368±7	364±2	98.9
Ni	1.59±0.07	1.61±0.06	101.2

hemoglobin, the protein that transports oxygen in the blood stream. Moreover, it participates in the synthesis of collagen and the neurotransmitter noradrenalin, is one of the most important blood antioxidants, and helps to prevent breakdown of polyunsaturated fatty acids to keep the cell membrane healthy. Iron is also necessary for the synthesis of collagen and is present in the brain as a co-factor in the synthesis of serotonin, dopamine, and noradrenalin neurotransmitters. Its principal function is through its interaction with proteins and copper in the formation of hemoglobin (26,27).

On the other hand, metals such as Pb, Cd, As, Cu, Al, Fe, and Zn are known to become potentially toxic when present at high concentrations (27,28). The Food and Agriculture Organization/World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives has defined the provisional tolerable weekly intake (PTWI) for a chemical with no intended function as an estimate of the amount of the chemical that can be ingested weekly over a lifetime without appreciable health risk (29-31).

In this paper, the ICP-OES technique was used for the determination of Al, Cd, Cr, Cu, Fe, Ni, Pb, and Sn in 21 maize samples obtained from SE Anatolia, Diyarbakir, Turkey, in 2010. The concentrations of Al, Sn, Cr, Cu, Fe, Ni, and Pb

TABLE V
Mean, Maximum, and Minimum Levels (mg/kg)
and Relative Standard Deviations (%) of Metals in Maize

	Al	Cd	Cr	Cu	Fe	Ni	Pb	Sn
Min	6.678	N.D	0.394	21.56	93.37	1.679	0.375	2.092
Max	7.051	N.D	0.423	24.78	102.86	1.877	0.392	3.977
Mean	6.864	N.D	0.409	23.17	98.11	1.778	0.384	3.069
RSD (%)	0.235	N.D	0.096	1.024	5.09	0.265	0.013	0.354

in 21 maize samples ranged from 6.678 to 7.051 mg/kg Al, 2.092 to 3.977 mg/kg Sn, 0.394 to 0.423 mg/kg Cr, 21.56 to 24.78 mg/kg Cu, 93.37 to 102.86 mg/kg Fe, 1.679 to 1.877 mg/kg Ni, and 0.375 to 0.392 mg/kg Pb; Cd was not detected (< 0.03 mg/kg). The relative standard deviation of most elements was below 10%. The recoveries of all elements are mostly in the range of 98% to 103%, which shows that this method is accurate (see Tables IV and V).

A study of the mean metal composition of the maize samples showed that Fe (98.11 mg/kg) and Cu (23.17 mg/kg) were the most abundant elemental species. The metals with next higher concentrations were Al (6.864 mg/kg), Sn (3.069 mg/kg), and Ni (1.778 mg/kg), followed by Fe (98.11 mg/kg) and Cu (23.17 mg/kg). The elements found to be at lower concentrations were Cr (0.409 mg/kg) > Pb (0.384 mg/kg) > Cd (< 0.03 mg/kg). The reason for the high Cu content (23.17 mg/kg) is because the maize was grown near the world's oldest and Turkey's largest open cast copper mine, located close to Diyarbakir (32).

The low levels of toxic elements found in these samples may be due to the fact that their concentrations are low in the soil and/or the environment and that the forms of these elements in the soil are not active and difficult to absorb by plants (33,34). It can therefore be concluded that the maize grown in Turkey has very low levels of toxic

heavy metals, is safe for human consumption, and is a highly nutritional and healthy food source.

CONCLUSION

Maize is the world's most widely grown food crop which is used not only as food, feed, or as commodity but also in advanced biochemical factories to produce the valuable biomolecules required for pharmaceuticals, industrial enzymes, nutrients, chemical precursors, and fuels.

Heavy metals are extremely toxic because they tend to bioaccumulate in the human body. This is one of the most important factors on deciding the quality of foods, because there are no standard permissible limits. The maximum allowable heavy metal content depends on soil characteristics, irrigation water quality, and crop type, etc.

In this study, the elements Al, Cd, Cr, Cu, Fe, Ni, Pb, and Sn in maize were determined using microwave digestion followed by simultaneous ICP-OES. The concentrations (in mg/kg) found were: Al 6.678-7.051, Sn 2.092-3.977, Cr 0.394-0.423, Cu 21.56-24.78, Fe 93.37-102.86, Ni 1.679-1.877, and Pb 0.375-0.392. All of these detection limits were within tolerable limits, except for Cd which was below at < 0.03 mg/kg.

The analytical results show that maize grown in Turkey contains many wholesome elements, such as Fe and Cu, but very low-level toxic

elements, which makes it an unpolluted and safe source of food.

The analytical ICP-OES technique which has been developed and validated for maize samples proved to be simple, rapid, accurate, and precise for the determination of eight essential and non-essential elements. It can also be considered a useful method for the routine analysis of trace elements in foods and possibly similar matrices, and for quality control purposes.

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