

Determination Of Pb (II), Cr (VI) And Cd (II) Contents In Some Ethiopia Spices After Microwave Digestion Using Atomic Absorption Spectroscopy

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ABSTRACT: The concentrations of Lead, Cadmium and Chromium in spice samples collected from eastern Ethiopia were determined after digesting the samples by microwave oven using an oxi acid mixture of 4:2 mL HNO₃/H₂O₂ for 2 min in 250 watt. Contents of investigated trace metal in spice samples of Lead in Fenugreek, Black cumin, garlic and ginger 0.0140, 0.0224, 0.0058, 0.0172 mg/kg respectively. Cadmium is detected only in Fenugreek in the ranged of ND to 0.0175mg/kg where as Chromium in Fenugreek, Black cumin, Garlic and Ginger 0.0219, 0.0152, 0.0016, 0.0258 mg/kg respectively.

Key words: microwave oven, fenugreek, black cumin, ginger, garlic

1. INTRODUCTION

Spices are dried parts of plants, which have been used as dietary components of food often to improve its color, aroma, palatability and acceptability. They consist of rhizomes, barks, leaves, fruits, seeds, and other parts of the plant. There have been many definitions for 'spices' which are often used interchangeably with 'herbs'. Spices are defined as the aromatic parts of any plants that are used to add flavor to food. But herbs are the aromatic leaves of any plant that can add flavor to food. The origins of herbs are leaves of plants that have soft stems or are shrubs but not trees. In general, spices are defined as un-leafy dried substances whereas herbs are leafy un-dried substances (Wahid and Durrani 1989). Heavy metals are defined as those elements with a specific density at least five times the specific gravity of water. Heavy metals include cadmium (Cd), copper (Cu), lead (Pb), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag), chromium (Cr), iron (Fe), and platinum group elements. The important heavy metals from water pollution point of view include mercury, cadmium, lead, zinc, copper, nickel and chromium. Copper and zinc are essential trace elements for living organism at low concentration (< 10 mg/L), however, they become toxic at high concentration (>10 mg/L). Most of these metal ions (Cd, Cu, Zn, Hg, As, Ag, Cr, and Fe) can be release from the industries are in simple cationic forms (Volesky, 1995). The characteristics of heavy metals are described as Wang (2006). Many analytical methods including Atomic Absorption Spectrometry for trace element determination in plant materials require the digestion of the sample (Polkowska et al., 2000). Because of its sensitivity, specificity, simplicity and precision, Atomic Absorption Spectrometry (AAS) is the most widely recommended instrument utilized in analytical procedures for trace heavy metal analysis. In order to separate the analyte from the matrix and to avoid organic matter which may react with the metal ions or chemical reagents and interfere with the analyte in acid digestion methods are very important step.

The most commonly used methods for the sample treatment of spices are dry ashing, wet ashing and microwave assisted treatment. Microwaves are non-ionizing electromagnetic radiation. Molecules exposed to microwave radiation undergo molecular motion by the migration of ions and the rotation of dipoles without changing the structure. Microwave energy has the frequency range from 300 to 300,000 MHz. Most industrial and scientific microwave ovens use four different frequencies: 915±25, 2450±13, 5800±75, and 22,125±125 MHz (Vandecasteele and Block, 1993). The advantages Microwave digestion over more traditional procedures: a shorter acid digestion time; a supposed better recovery of volatile elements; lower contamination levels; minimal volumes of reagents are required, more reproducible procedures; and a better working environment (White and Douthit 1985). It has also its own disadvantage: the microwave itself is expensive and overall analysis time is not much longer. This is due to the sample vessel handling. Opening and closing of the sample vessel is a step that is not necessary using glass beakers with the hot plate digestion (Kingston and Jassie, 1986). In this study, the authors compare the efficacy microwave digestion on commercial spice samples. The contents of Lead, Chromium and Cadmium in spice samples produced in Ethiopia were determined by flame atomic absorption spectrometry after microwave digestion.

2. MATERIALS AND METHODS

2.1. Apparatus

Buck scientific 210 VGP flame atomic absorption spectrometer was used in the experiments. A deuterium background corrector was used for background corrections. The operating parameters for the elements were set as recommended by the manufacturer (Table 1). For flame measurements, a 10-cm long slot-burner head, a lamp and an air-acetylene flame were used. Microwave oven (type MX 335 7JBG400414) for microwave digestion was used to

digest the sample for the analysis of Heavy metals concentration. Sample volume, digestion times for the drying, ashing, atomization and cleaning temperatures were

optimized prior to analysis in order to obtain the maximum absorbance with minimum background.

Element	Wavelength nm	Slit Width nm	Lamp Current mA	IDL mg/L	MDL mg/L	Flame type
Cd	228.9	0.7	2	0.005	0.0062	Rich/yellow
Cr	357.9	0.7	2	0.05	0.051	Lean/blue
Pb	217.0	1.0	5	0.01	0.016	Lean/blue

Table 1: Working Conditions of Atomic Absorption Spectroscopy

2.2 Reagents

All reagents were of analytical reagent grade. Double distilled deionized water (Milli-Q Millipore 18.2 MΩ-cm resistivity) was used for all dilutions. HNO₃ and H₂O₂ were of suprapure quality (E. Merck, Darmstadt). All plastic and glassware were cleaned by soaking in diluted HNO₃ (10% v/v) and rinsed with distilled water prior to use. The element standard solutions used for calibration were prepared by diluting stock solutions of 1000 mg/L of each element.

2.3 Sampling

Total of four spice sample types were collected randomly from different traders and Samples were washed thoroughly with tap water followed by de-ionized water and dried in the oven at a temperature of 105 °C for 24 hr for Fenugreek, Ginger, and Garlic. But black cumin was dried at a temperature of 105 °C for 48 hr. The dried samples were ground in a stainless steel mill till obtaining fine particles that pass through a 0.5 mm mesh and kept dry in a polyethylene bag in desiccators until analysis.

2.4 Digestion Procedure

Three types of digestion procedures (dry, wet and microwave) were applied to the spice samples produced in Ethiopia. Optimum digestion conditions are given below.

2.4.1 Microwave Digestion

One gram of each sample was digested with 4 mL of HNO₃ (65%) and 2 mL of H₂O₂ (30%) in microwave digestion system. After digestion, the solution was diluted with 10 ml de-ionized water. A blank digest was carried out in the same way. The samples were digested in 250 W Power for 2 min (Mustafa et al., 2004).

2.5 Digestion Conditions

Different procedures for spices sample digestion were assessed based on varying reagent volume, digestion time and digestion temperature. For optimizing the procedure Fenugreek (*Trigonella foenumgraecum* L.) was selected and it was digested with the three digestion methods. The selection for the respective optimum digestion methods was made based on the particular procedure which resulted to the following conditions: clear digestion solution, minimal reflux time/digestion time, minimal reagent volume consumption, absence of undigested spice samples (Mustafa et al., 2004). The results are given in Table 2. The comparison of three digestion methods showed statistically

significant differences in results. The recovery values were nearly (≥ 95%) for all digestion methods. **ANOVA** was used in this study (p < 0.05). The relative standard deviations were less than 10% for all elements. The approximate time required for dry, wet and microwave digestions were 10 hr, 5 hr and 40 min, respectively. In light of these results, the microwave digestion procedure was best for the digestion of all the spice samples, because of shorter required time and smaller deviations than dry and wet digestions. The effect of reagents used for the microwave digestion was investigated. When concentrated HNO₃ and H₂O₂ were separately used as the digestion agent, it was observed that the samples did not dissolve sufficiently. Subsequently, for the wet ashing and microwave digestion procedure, different combinations of these two reagents were tested. HNO₃/H₂O₂ 4/2 (10 mL for a 1.0 g sample) and 4:1 (10 mL for a 1.0 g sample) mixture was used for spice digestion resulted in shortened digestion time, excellent recovery and precision than other combinations for microwave digestion and wet ashing respectively.

2.6 Preparation of Standard solutions

Determination of the metal concentration in the experimental solution was based on the calibration curve. In plotting the calibration curves lead, cadmium and chromium stock solutions of 1000 ppm were prepared by dissolving 1.6 g of Pb(NO₃)₂, 2.74 g Cd(NO₃)₂·4H₂O and 2.83 g K₂Cr₂O₇ in de-ionized water respectively. Blank solutions were prepared for the methods and, for the standard working solutions, to prepare 100 ppm, 10 mL of the standard Pb(NO₃)₂, Cd(NO₃)₂·4H₂O and K₂Cr₂O₇ stock solution were pipetted and added into 100 mL calibrated flasks finally diluted with de-ionized water and the solution was mixed thoroughly. Next, to prepare 50 ppm standard solution of each metal, 50 mL of each of 100 ppm stock solution was pipetted into 100 mL volumetric flasks and diluted with de-ionized water. Finally to prepare 0.0, 0.5, 1.0, 2.0, 4.0, 6.0 ppm aliquots of this standard working solution 0.0, 0.5, 1.0, 2.0, 4.0, 6.0 mL was pipetted from 50 ppm standard solution into 50 mL calibrated flasks and made up to volume with De-ionized water (AOAC, 1971).

2.7 Validation of Experimental Results

2.7.1 Determination of Detection Limits

Detection limit is the lowest concentration level that can be determined at 95% confidence level (Bassett et al., 1978) or the minimum concentration that can be detected by the analytical method with a given certainty (Christian., 2004). A general accepted definition of detection limit is the concentration that gives a signal three times the standard deviation of the blank or background signal (Bassett et al., 1978). In this study the detection limit of each element was calculated as three times the standard deviation of the blank (3σ blank, n = 5).

2.7.2 Recovery Tests

The efficiency and accuracy of the optimized methods were evaluated by analyzing the digests of spiked samples. 0.02, 0.02 ppm of Pb, Cr and 0.2 ppm Cd, respectively, were taken from stock solution of each metal and spiked in a 250 mL Erlenmeyer flask containing 1g spice sample. The recoveries of metals in the spiked spice samples were 92 to 103 %. Generally, good recoveries were obtained for all metals, (particularly in Garlic for metals like Cd, Pb and Cr). In Cd the percentage recovery for all samples except

Fenugreek were not calculated due to results obtained was not within the method detection limit. Each determination was carried out at least three times in order to ensure precision. The relative standard deviations were less than 10% for all measurements.

$$\% \text{ Recovery} = \frac{\text{Amount after Spike} - \text{Amount before Spike}}{\text{Amount Added}} \times 100$$

(AOAC, 1995)

2.8 Optimization of Working Procedure

The optimal procedure chosen on the basis of these criteria is 2 min digestion time at 250 W in 4 mL 70% HNO₃ and 2 mL 30% H₂O₂ volume ratio for 1 g spice sample was used in the case of microwave digestion method. The optimized operating conditions were compared with literature report on similar study made by Mustafa et al., (2004). The work had improved especially dry ashing and wet digestion methods employed there significantly as far as digestion time is concerned. as it listed in Table 2

Digestion Methods	Previous work optimum parameters (Mustafa et al., 2004)			Current work optimum Parameters			Difference		
	T (°C)	V(mL)	Time(hr)	T (°C)	V(mL)	Time(hr)	T(°C)	V(mL)	Time (hr)
Microwave digestion	250W	6:2	2 min	250 W	4:2	2min	-	2mL	-

Table 2: Optimum working conditions for the digestion methods used
Note; T = temperature; V = volume MD= microwave digestion

3. RESULTS AND DISCUSSION

The method detection limit (MDL) is defined as the concentration corresponding to three times the standard deviation of blanks. method detection limit values of the investigated elements for AAS were found to be 0.016 mg/L for Pb, 0.0062 mg/L for Cd and 0.051 mg/L for Cr.

Trace metal levels in the analyzed samples are given in Table 3. The metal contents in the samples studied depended on the specific species. Levels of the essential metals in the spice samples were found to be higher than those of the non-essential metals. The lowest and highest contents of copper were found in 4.1 µg/g for *Diantus* sp. and 28.7 µg/g for *Rhus coriaria*, respectively. The lowest and highest levels of zinc were found as 7.84 µg/g for *Capsicum annum* and 47.6 µg/g for *Papaver somniferum*, respectively. Copper and zinc values in spices of Nigeria have been reported in the range of 0.40-13.3 µg/g and 0.20-53.7 µg/g, respectively. The highest cadmium level was found as 0.93 µg/g for *Piper nigrum*, whereas the lowest cadmium level was 0.10 µg/g in *Nigella* sp. The lowest and highest contents of nickel were found as 0.65 µg/g for *Cassia* sp. and 8.69 µg/g for *Menta* sp, respectively. The concentrations of cadmium and nickel in spices of Nigeria have been reported in the range of 0.12-0.36 µg/g and 1.03-3.47 µg/g, respectively. The

concentration of lead varied from 0.47 µg/g in *Rhus coriaria* to 1.89 µg/g in *Nigella* sp.

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3.2 Recovery Tests

The efficiency and accuracy of the optimized methods were evaluated by analyzing the digests of spiked samples. 0.02, 0.02 ppm of Pb, Cr and 0.2 ppm Cd respectively, were taken from stock solution of each metal and spiked in a 250 mL Erlenmeyer flask containing 1g spice sample. The recoveries of metals in the spiked spice samples were 92 to 103 %. Generally, good recoveries were obtained for all metals, (particularly in Garlic for metals like Cd, Pb and Cr). In Cd the percentage recovery for all samples except Fenugreek were not calculated since results obtained was not within the method detection limit. Each determination was carried out at least three times in order to ensure precision. The relative standard deviations were less than 10% for all measurements.

Metal		Microwave digestion			
		Fenugreek	Black Cumin	Garlic	Ginger
Pb	Amount Recovered	0.034	0.042	0.026	0.037
	percentage recovery	99	98	103	100
	concentration in spice	0.0140	0.0224	0.0058	0.0172
Cr	Amount Recovered	0.041	0.034	0.021	0.045
	concentration in spice	0.0219	0.0152	0.0016	0.0258
	percentage recovery	95	97	99	96
Cd	Amount Recovered	0.215	0.186	0.196	0.188
	concentration in spice	0.0175	-	-	-
	percentage recovery	99	-	-	-

Table 3. Recovery test of the methods at $P \leq 0.0$ Since Cd is not detected in all except fenugreek the %R is also not calculated

Digestion Methods	Spices	Pb	Cr	Cd
MW	Fenugreek	0.0140±0.0013	0.0219±0.0018	0.0175±0.0023
	Black cumin	0.0224±0.0026	0.0152±0.0006	ND
	Garlic	0.0058±0.0090	0.0016±0.0001	ND
	Ginger	0.0172±0.0009	0.0258±0.0009	ND

Table 4. Metal Concentration (mg/kg) in Spices from Dire Dawa Market (Mean± S.D)
Note MW = microwave oven digestion; ND = not detected at $P \leq 0.5$

Conclusions

This study is focused on check the extraction efficiency of Microwave oven digestion methods in different spices including Garlic, Ginger, Black Cumin and Fenugreek for the determination of heavy metals like Cd, Pb and Cr by using Atomic Absorption spectrometry (AAS). Spice samples were digested by using three digestion methods by using HNO_3 and H_2O_2 used as digestion reagents. The concentrations of these reagents were optimized to minimize possible matrix interferences. Different temperature programs, reagent volume and digestion time were investigated to obtain maximum digestion efficiency with minimum digestion reagent consumption for all digestion methods. All methods gave almost similar results for the elements studied. microwave digestion method is the most efficient digestion method. The use of microwave digestion system in spice samples provides very fast, safer, simple and cleaner method of sample preparation, increases analyte recoveries and useful volatile elements. Effects of HNO_3 and H_2O_2 concentrations on Pb (II), Cd (II) and Cr (VI) signals were investigated to see the effect of matrix matching on AAS and it was found that increasing concentrations of HNO_3 has suppression effect on Pb and Cr concentration whereas the effect on Cd concentration were not detected by using neither direct calibration method or standard addition method by AAS. Based on this knowledge direct calibration method was used for Pb and Cr determination whereas for Cd determination standard addition method was applied but similar result was

obtained. In the last part of the study the results of previous analyses for Cd, Cr and Pb determination in spice samples were evaluated together with the results of this study for statistical analysis. For each spice sample the results of all elements obtained by different digestion methods were compared by using ANOVA at 95% confidence interval.

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