

Validation of a Method for Determining Heavy Metals in Some Ethiopian Spices By Dry Ashing Using Atomic Absorption Spectroscopy

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ABSTRACT: The optimal conditions for determination of Pb, Cd and Cr in spices sample by FAAS after dry mineralization 1g spice samples for 5hr in a temperature of 500 °C followed dissolution in 4 mL HNO₃ in Fenugreek, Black cumin, garlic and ginger. Working linear ranges are given: for Cd, 0 -6mg/l, for Cr, 0.2-1mg/l and for Pb, 2-20µg/l. Found detection limits are 0.2mg/l, 0.13mg/l and 0.02µg/l for Cd, Cr and Pb, respectively. The result obtained Lead in Fenugreek, Black cumin, garlic and ginger ranged from 0.0126 to 0.0155, 0.0205 to 0.0254, 0.0046 to 0.0066 and 0.0161 to 0.0178 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 ranges from 0.0187 to 0.0219, 0.0134 to 0.0152, 0.0014 to 0.0016 and 0.0258 to 0.0346 mg/kg respectively which is under the values recommended for concentration levels present in the analyzed samples.

KEYWORDS: dry ashing, fenugreek, black cumin, ginger, garlic, atomic absorption spectrometry.

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 [1].

In Ethiopia, there are between 6,000 to 7,000 higher plant species, out of about 500 (about 8%) are edible [1]. According to [2]. on 50 species of aromatic and medicinal plants collected from eastern Ethiopia, it was indicated that a sizable number of local merchants were involved in the trading of spices along with other aromatic and medicinal plants. Of these plant species studied as such, a significant number of the plants (over 25) were spices.

Heavy metals are those metals which are toxic for our body even if at their lower concentration include cadmium (Cd), copper (Cu), lead (Pb), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag), and chromium (Cr). 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 [3], [4].

Many analytical methods including Atomic Absorption Spectrometry for trace element determination in plant materials require the digestion of the sample [5]. 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.

Dry ashing procedures use a high temperature muffle furnace. Most minerals are converted to oxides, sulfates, phosphates, chlorides or silicates. The advantage of Dry ashing is safe, few reagents are required, many samples can be analyzed simultaneously and not labor intensive. The disadvantage of this method is, it requires relatively expensive apparatus (platinum crucibles and muffle furnace), losses due to volatilization, resistance to ash by some materials, difficult dissolution of ashed material and high risk of contamination[6].

In this study, the authors compare the efficacy of differing digestion procedures dry ashing 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 dry ashing.

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.

Furnace (type 1500) for dry ashing was used to ash the sample for the analysis of Heavy metals concentration. Platinum and porcelain Crucibles was used to dry the sample at Furnace. 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.

Table 1. Working Conditions of Atomic Absorption Spectroscopy

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

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 was 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

Dry ashing was applied for digesting the spice samples produced in Ethiopia. Optimum digestion conditions are given below.

2.5 DRY ASHING

One gram of each spice sample was placed into porcelain crucible. The furnace temperature was slowly increased from room temperature to 500°C. The sample was ashed for about 5 hr until a white or grey ash residue was obtained. The residue was dissolved in 5 mL of HNO₃ (25%, v/v). The solution was transferred to a 10 mL volumetric flask and made up to the mark [7].

2.6 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 [8]. 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 ashing was 10 hr.

2.7 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 [9].

2.8 VALIDATION OF EXPERIMENTAL RESULTS

2.8.1 DETERMINATION OF DETECTION LIMITS

Detection limit is the lowest concentration level that can be determined at 95% confidence level [10] or the minimum concentration that can be detected by the analytical method with a given certainty [11] 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 [12]. In this study the detection limit of each element was calculated as three times the standard deviation of the blank (3σ blank, n = 5).

2.8.2 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 %. The results are given in Table 3. 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.

$$\% \text{ Recovery} = \frac{\text{Amount after Spike} - \text{Amount before Spike}}{\text{Amount Added}} \times 100 \quad [2]$$

2.8.3 OPTIMIZATION OF WORKING PROCEDURE

The optimal procedure chosen on the basis of these criteria for dry ashing 5 hr digestion time at a temperature 500°C in 4 mL HNO₃ was employed 4 mL HNO₃ was added after white ash is obtained. 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.

Table 2. Optimum working conditions for the digestion methods used

Digestion Methods	Previous work optimum parameters (Mustafa <i>et al.</i> , 2004)			Current work optimum Parameters			Difference		
	T (°C)	V(mL)	Time(hr)	T (°C)	V(mL)	Time(hr)	T(°C)	V(mL)	Time (hr)
Dry Ashing	450	5	8	500	4	5	50	1	3

Note; T = temperature; V = volume DA= dry ashing

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 4. 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(7). 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(8). The concentration of lead varied from 0.47 µg/g in *Rhus coriaria* to 1.89 µg/g in *Nigella sp* [13]

3.1 OPTIMIZATION OF WORKING PROCEDURE

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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 %. The results are given in Table 3. 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.

Table 3. Recovery test of the methods at $P \leq 0.0$

Metal		Dry Ashing			
		Fenugreek	Black Cumin	Garlic	Ginger
Pb	Amount Recovered	0.032	0.040	0.025	0.035
	percentage recovery	95	96	97	96
	concentration in spice	0.0132	0.0209	0.0055	0.0154
Cr	Amount Recovered	0.037	0.032	0.021	0.054
	concentration in spice	0.0187	0.0134	0.0016	0.0346
	percentage recovery	94	93	95	96
Cd	Amount Recovered	0.196	0.168	0.178	0.180
	concentration in spice	0.0125	-	-	-
	percentage recovery	92	-	-	-

Since Cd is not detected in all except fenugreek the %R is also not calculated

Table 4. Metal Concentration (mg/kg) in Spices from Dire Dawa Market (Mean \pm S.D)

Digestion Methods	Spices	Pb	Cr	Cd
DA	Fenugreek	0.0132 \pm 0.0006	0.0187 \pm 0.0008	0.0125 \pm 0.0016
	Black cumin	0.0209 \pm 0.0003	0.0134 \pm 0.0005	ND
	Garlic	0.0055 \pm 0.0006	0.0016 \pm 0.0002	ND
	Ginger	0.0154 \pm 0.0002	0.0346 \pm 0.0018	ND

Note; DA= dry ashing and ND = not detected at $P \leq 0$.

4 SUMMARY AND CONCLUSION

4.1 SUMMARY

The general objective of the study was to compare the efficiency of digestion methods for determination of Cr (VI) Pb (II) and Cd (II) in some selected Ethiopian spices using AAS. All experiments were done at Haramaya University soil science and central laboratory. The Pb concentrations were found to be in the range of 0.0132 to 0.0140, 0.0209 to 0.0224, 0.0055 to 0.0058, and 0.0154 to 0.0172 mg/kg for Fenugreek, Black Cumin, Garlic and Ginger respectively. Whereas Cr concentrations ranged from 0.0187 to 0.0269, 0.0134 to 0.0152, 0.0014 to 0.0016, 0.0258 to 0.0346 mg/kg in Fenugreek, Black Cumin, Garlic and Ginger respectively, Cd was not detected in any of the samples except in Fenugreek which is ranging from 0.0125 to 0.0175 mg/kg. The results obtained showed that the spices in this study are not likely to pose any health risk to the public through consumption of the spices for both the toxic elements determined. The recoveries of the trace metals were in the range of 92 to 103% and the standard deviations were less than 10%.

4.2 CONCLUSIONS

This study is focused on check the extraction efficiency of dry ashing 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 ashed by using furnace and digested by using HNO_3 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.

But dry ashing methods are more time consuming and complicated than the other digestion methods in terms of digestion efficiency. Example the use of microwave digestion system in sample digestion provides very fast, safer, simple and

cleaner method of sample preparation, increases analyte recoveries and useful volatile elements. Effects of HNO₃ and H₂O₂ 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 HNO₃ 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.

ACKNOWLEDGMENTS

The financial support provided by the Ministry of Education, and the guidance given by Dr. Abi Tadesse College of natural and computational science (Haramaya University) to conduct the metal analysis are gratefully acknowledged. This work is a part of the research carried for postgraduate programme (MSc) at faculty of Graduate Studies, Haramaya University.

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