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## I. INTRODUCTION

Dry ashing is the standard method for the preparation of organic material for electrolyte analysis; hence this method has been used in most investigations of the electrolyte metabolism of soft tissues. The chief disadvantages of the method are [1] that it requires relatively expensive apparatus (platinum crucibles and muffle furnace) and [2] that there is danger of volatilization of significant amounts of some electrolytes when the digestion temperature is kept high enough to yield a white ash. And now a day it is replaced by wet digestion method.

Wet ashing of organic material requires less expensive apparatus and there is little danger of volatilization of electrolytes. The usual oxidizing agents employed are sulfuric, perchloric, and nitric acids, hydrogen peroxide, and their various combinations. The disadvantages which are responsible for the limited use of this method arise from the fact that the final solution is either very strongly acid, so that large amounts of base are required to neutralize the excess acid, or the digestion must be continued over a relatively hot flame to drive off the excess acid. Volatilization of sulfuric or

perchloric acid over an open flame is a difficult procedure to carry out without occurrence of creeping or spattering resulting in partial loss of the sample, unless a relatively large and subsequently disadvantageous digestion tube is used. If only relatively volatile oxidizing substances, such as nitric acid [3] and hydrogen peroxide which can be removed by heating on a water bath, are used, a colored, incompletely oxidized, residue results which is not completely soluble and consequently difficult to work with.

Wet ashing is primarily used in the preparation of samples for subsequent analysis of specific minerals. It breaks down and removes the organic matrix surrounding the minerals so that they are left in an aqueous solution. A dried ground food sample is usually weighed into a flask containing strong acids and oxidizing agents (e.g, nitric, perchloric and/or sulfuric acids) and then heated. Heating is continued until the organic matter is completely digested, leaving only the mineral oxides in solution. The temperature and time used depends on the type of acids and oxidizing agents used. Typically, a digestion takes from 10 minutes to a few hours at temperatures of about 350°C. The advantage of this method is little loss of volatile minerals occurs because of the lower temperatures used, more rapid than dry ashing. The disadvantages of this method are, it is labor intensive, requires a special fume-cupboard if perchloric acid is used because of its hazardous nature, and low sample throughput [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.

The aim of this article is to present the analytical method applied for the determination of metal content in Ethiopian spices and clearly some aspects, regarding

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the sample preparation and the validation of the method.

## II. MATERIALS AND METHODS

### a) 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.

Heating digester (type DK 20) for wet ashing was used to digest the sample for the analysis of Heavy metals concentration. Sample volume, digestion times for the wet digestion, 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

### b) Reagents

All reagents were of analytical reagent grade. Double distilled deionized water (Milli-Q Millipore 18.2 M $\Omega$ -cm resistivity) was used for all dilutions. HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> were of suprapure quality (E. Merck, Darmstadt). All plastic and glassware were cleaned by soaking in diluted HNO<sub>3</sub> (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.

### c) 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.

### d) Wet Ashing (Digestion) Procedure

Wet digestion of each spice sample was performed using an oxi-acidic mixture of HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> (4:1) (10 ML for a 1.0 g sample) in a 100 ML beaker inside a hood. This mixture was heated up to 120°C for 3 hr and brought to a volume of 25 ML with de-ionized water and blank digestion was also carried out in the same way [6].

### e) Digestion Conditions

Different procedures for spices sample digestion were assessed based on varying reagent volume, digestion time and digestion temperature. For optimizing the procedure all spices were selected and it was digested with wet 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 [7]. The results are given in Table 2. The comparison of three digestion methods showed statistically significant differences in results. The recovery values were nearly ( $\geq 95\%$ ) 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 is 5hr.

### f) Evaluation of Analytical Figures

#### i. Accuracy and precision

Accuracy and precision are probably the most often quoted terms to express the extent of errors in a given analytical results. Analytical results must be evaluated to decide on the best values to report and to attempt to establish the probable limits of errors of these values [8, 9]. The analyst will thus be concerned with the question of precision (repeatability of results), that is, the agreement between a set of results for the same quantity; and also with accuracy, that is the difference between the measured value and the true value of the quantity, which is determined [8]. In this study the precision of the results were evaluated by the standard deviation of the results of triplicate samples ( $n = 3$ ), analyzed under the same condition. Standard deviation is a useful parameter in estimating and reporting the probable size of indeterminate errors. On the other hand, the accuracy and validity of the measurement were determined by analyzing spiked samples using BUCK SCIENTIFIC standard solutions.

The procedure of spiking was as follows: for the determination of the validity of the developed optimized procedures used for determination of metals in raw and roasted coffee bean samples, known concentration of standard solutions (that is 100 mg/L of Cr, Pb, and 10

mg/L of Cd) were prepared. From these solutions based upon the amount that make the concentration the final solution 0.20 mg/L (Cr and Pb) and 0.02 mg/L (Cd); 0.15, 0.10 and 0.10 mL, respectively, were added to 0.50 g of spice samples. Then they were digested with the developed digestion Procedures. After diluting the spiked samples to the required volume with deionized water, they were analyzed with the same procedure followed for the analysis of coffee samples. Triplicate samples were prepared and triplicate readings were obtained.

ii. *Determination of detection limits*

Detection limit is the lowest concentration level that can be determined to be statistically Different from an analyte blank [9, 10] or the minimum concentration that can be detected by the analytical method with a given certainty [9]. For a measurement, detection limit can be properly estimated from the standard deviation of several blank determinations [11]. There are numerous ways of determining detection limits of a given measurement [12]. 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 [14, 15]. In this study the detection limit of each element was calculated as three times the standard deviation of the blank ( $3\sigma_{\text{blank}}$ ,  $n = 5$ ).

Table 2 : Optimum working conditions for the digestion methods used

Digestion Methods	Previous work optimum parameters [13]			Current work optimum Parameters			Difference		
	T (°C)	V(mL)	Time(hr)	T (°C)	V(mL)	Time(hr)	T(°C)	V(mL)	Time (hr)
Wet digestion	130	2:1(12)	4	120	4:1(10)	3	10	2	1

Note; T = temperature; V = volume WA= wet ashing

III. 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 Lead and chromium in fenugreek, Black Cumin, Garlic and Ginger were found 0.0140, 0.0224, 0.0058, 0.0172 and 0.0219, 0.0152, 0.0016, 0.0258 mg/kg and cadmium in fenugreek is 0.0175 mg/kg but in other spices ND.

a) *Optimization of Working Procedure*

The optimal procedure chosen on the basis of these criteria for wet ashing required 3 h at a

iii. *Determination of limits of quantitation*

Limit of quantitation (or limit of determination) is the lowest concentration of the analyte that can be measured in the sample matrix at an acceptable level of precision and accuracy. An acceptable level of precision is typically 10 to 20 % of relative standard deviation depending upon the concentration level measured. However, in the absence of specified precision, the limit of quantification is the same as the concentration that gives a signal 10 times the standard deviation of the blank [12]. Limit of quantitation is the lowest limit for precise quantitative measurements [14]. The quantitation limit of each element was calculated as ten times the standard deviation of the blank ( $10\sigma_{\text{blank}}$ ,  $n = 5$ ).

g) *Optimization of Working Procedure*

The optimal procedure chosen on the basis of these criteria for wet ashing required 3 h at a temperature of 1200C for complete digestion of 1 g dried sample with 8 mL 70% HNO3 and 2 mL 30% H2O2 .

The optimized operating conditions were compared with literature report on similar study made by [13]. The work had improved for wet digestion methods employed there significantly as far as digestion time is concerned.st listed in table 2.

temperature of 1200C for complete digestion of 1 g dried sample with 8 mL 70% HNO3 and 2 mL 30% H2O2.

b) *Recovery Tests*

The efficiency and accuracy of the optimized methods were evaluated by analyzing the digests of spiked samples. 0. 2, ppm of Pb, Cr and 0.02 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 95 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 4* : Metal Concentration (mg/kg) in Spices from Dire Dawa Market (Mean± S.D)

Digestion Methods	Spices	Pb	Cr	Cd
WA	Fenugreek	0.0134±0.0003	0.0269±0.0016	0.0138± 0.0016
	Black cummin	0.0215±0 .0010	0.0150±0.0004	ND
	Garlic	0.0057±0.0009	0.0014±0.0002	ND
	Ginger	0.0168±0.0004	0.0270±0.0024	ND

Note; WA = wet ashing and ND = not detected at  $P \leq 0.05$

#### IV. 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<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> 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.

But dry ashing method is more time consuming and complicated than the wet digestion method in terms of digestion efficiency. The use of wet digestion system in spice samples provides very fast, safer, simple and cleaner method of sample preparation, increases analyte recoveries and useful volatile elements relative to dry ashing method. Effects of HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> 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<sub>3</sub> 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.

The aim of this study was to prove the analytical performances of a quantitative method for metal determination in spice samples by FAAS method after their wet digestion. The method, previously optimized, was validated. The performance parameters obtained such as linearity, detection and determination limits, trueness, followed as recovery and, precision, evaluated as repeatability and intermediate precision of the spice samples investigated, respect the theoretical values provided by specialty literature permitting, in this way, validation of the method. This study confirms the suitability of the proposed methods for determination metal content in spice for routine and quality control laboratories.

#### V. ACKNOWLEDGMENTS

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