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Comparison of Digestion Methods for Determination of Pb (II), Cr (VI) and Cd (II) Contents In Some Ethiopia Spices 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 different digestion methods using FAAS. Oven-dried 1 g spice samples were first Wet-digested in 8 mL of (69–70%)* HNO_3 *and 2 mL of (30%)* H_2O_2 *for 3 hr at a temperature of 120 °C and the second is dry ashed for 5 hr in a temperature of 500 °C followed dissolution in 4 mL* HNO_3*. The third digestion method is microwave oven by using an oxi acid mixture of 4:2 mL* HNO_3/H_2O_2 *for 2 min in 250 watt. We compared wet, dry and microwave digestion procedures for the digestion of the spice samples. Microwave oven digestion method showed very fast, safer, simple and cleaner and also gives satisfactory recovery, detection limits and standard deviation. Contents of investigated trace metal in spice samples of 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*.

Key words: *trace metals, wet digestion, dry ashing, microwave oven, fenugreek, black cumin, ginger, garlic, atomic absorption spectrometry*

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).

In Ethiopia, there are between 6,000 to 7,000 higher plant species, out of about 500 (about 8%) are edible (Asfaw and Tadesse, 2001). According to Letchamo and Storck (1991) 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 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 it's 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).

Dry ashing procedures use a high temperature muffle furnace capable of maintaining temperatures of between 200 and 600 oC. Water and other volatile materials are vaporized and organic substances are burned in the presence of oxygen in air to CO2, H2O and N2. 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) and there is danger of volatilization of significant amounts of some electrolytes when the digestion temperature is kept high enough to yield a white ash (Tuzen et al., 2004).

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 350oC. 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 throughout (Tuzen, 2002).

In this study, the authors compare the efficacy of differing digestion procedures (wet and dry ashing and 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 dry ashing, wet ashing and microwave digestion.

MATERIALS AND METHODS

I. Experimental Site

Spice samples were collected from Dire Dawa market which is located in Eastern Ethiopia, 40 km from Haramaya and 542 km away from Addis Ababa with latitude of $9^0 35' 0''$N and longitude of $41^0 52' 0''$E.

II. 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, heating digester (type DK 20) for wet ashing and Microwave oven (type MX 335 7JBG400414) for microwave digestion was used to digest 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

III. 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. HNO_3 and H_2O_2 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.

IV. 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 $105^{o}C$ for 24 hr for Fenugreek, Ginger, and Garlic. But black cumin was dried at a temperature of $105^{o}C$ 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.

V. 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.

a. Microwave Digestion

One gram of each sample was digested with 4 mL of HNO_3 (65%) and 2 mL of H_2O_2 (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).

b. Wet Ashing

Wet digestion of each spice sample was performed using an oxi-acidic mixture of HNO_3/H_2O_2 (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 (AOAC, 1985).

c. 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 HNO_3 (25%, v/v). The solution was transferred to a 10 mL volumetric flask and made up to the mark (AOAC, 1985).

VI. 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 ($\geq 95\%$) 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 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 standard deviations of the dry and wet digestion methods are considerably higher than those of the microwave digestion method. The effect of reagents used for the microwave digestion was investigated. When concentrated HNO_3 and H_2O_2 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. HNO_3/H_2O_2 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.

VII. 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 $Pb(NO_3)_2$, 2.74 g $Cd(NO_3)_2.4H_2O$ and 2.83 g $K_2Cr_2O_7$ 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 $Pb(NO_3)_2$, $Cd(NO_3)_2.4H_2O$ and $K_2Cr_2O_7$ 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).

VIII. Validation of Experimental Results

a. 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).

b. 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 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.

$$\% \text{ Recovery} = \frac{\text{Amount after Spike} - \text{Amount before Spike}}{\text{Amount Added}} \text{ x } 100$$ (AOAC, 1995)

Optimization of Working Procedure

The optimal procedure chosen on the basis of these criteria for wet ashing required 3 h at a temperature of 120^{0}C for complete digestion of 1 g dried sample with 8 mL 70% HNO_3 and 2 mL 30% H_2O_2. For dry ashing 5 hr digestion time at a temperature 500^{0}C in 4 mL HNO_3 was employed 4 mL HNO_3 was added after white ash is obtained. Finally 2 min digestion time at 250 W in 4 mL 70% HNO_3 and 2 mL 30% H_2O_2 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.

Table 2. Optimum working conditions for the digestion methods used

Digestion Methods	Previous work optimum parameters (Mustafa *etal*., 2004)			Current work optimum Parameters			Difference		
	T (°C)	V(mL)	Time(hr)	T (°C)	V(mL)	Time(hr)	T(°C)	V(mL)	Time (hr)
D A	450	5	8	500	4	5	50	1	3
WA	130	2:1(12)	4	120	4:1(10)	3	10	2	1
	Power (W)			Power (W)					
M D	250W	6:2	2 min	250 W	4:2	2min	-	2mL	-

Note; T = temperature; V = volume DA= dry ashing WA= wet ashing MD= microwave digestion

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 annuum* 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 (Tuzen *et al*., 2004). 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 (Vandecasteele *et al*.,1993). The concentration of lead varied from 0.47 µg/g in *Rhus coriaria* to 1.89 µg/g in *Nigella sp.*

I. Optimization of Working Procedure

The optimal procedure chosen on the basis of these criteria for wet ashing required 3 h at a temperature of 120^0C for complete digestion of 1 g dried sample with 8 mL 70% HNO_3 and 2 mL 30% H_2O_2. For dry ashing 5 hr digestion time at a temperature 500^0C in 4 mL HNO_3 was employed 4 mL HNO_3 was added after white ash is obtained. Finally 2 min digestion time at 250 W in 4 mL 70% HNO_3 and 2 mL 30% H_2O_2 volume ratio for 1 g spice sample was used in the case of microwave digestion method as given in Table 2.

II. 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 4. 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.

III. Precision and Accuracy

In this study the precision of the results were evaluated by the pooled standard deviation and relative standard deviation of the results of triplicate digests. Triplicate measurements of each sample (n=9) were used for the analysis of trace metals in spice samples the values of relative standard deviations (%RSD) are less than 10% for most of the mean concentrations of metals and the relative error is less than 2 which is the measurement of accuracy.

Table 3. Recovery test of the methods

Metal		**Dry Ashing**				**Wet Ashing**				**Microwave Digestion**			
		F	BC	Ga	Gi	F	BC	Ga	Gi	F	BC	Ga	Gi
Pb	AR	0.032	0.040	0.025	0.035	0.033	0.041	0.025	0.036	0.034	0.042	0.026	0.037
	%R	95	96	97	96	96	97	99	98	99	98	103	100
	C^s	0.0132	0.0209	0.0055	0.0154	0.0134	0.0215	0.0057	0.0168	0.0140	0.0224	0.0058	0.0172
Cr	AR	0.037	0.032	0.021	0.054	0.046	0.034	0.021	0.046	0.041	0.034	0.021	0.045
	C^s	0.0187	0.0134	0.0016	0.0346	0.0269	0.0150	0.0014	0.0270	0.0219	0.0152	0.0016	0.0258
	%R	94	93	95	96	96	95	98	97	95	97	99	96
Cd	AR	0.196	0.168	0.178	0.180	0.206	0.180	0.196	0.180	0.215	0.186	0.196	0.188
	C^s	0.0125	-	-	-	0.0138	-	-	-	0.0175	-	-	-
	%R	92	-	-	-	96	-	-	-	99	-	-	-

Note; AR= Amount Recovered; %R=percentage recovery; C^s = concentration in spice; F= Fenugreek; BC= Black Cumin; Ga = Garlic; Gi=Ginger at $P \leq 0.05$ 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± S.D)

Digestion Methods	Spices	Pb	Cr	Cd
DA	Fenugreek	0.0132±0.0006	0.0187±0.0008	0.0125±0.0016
	Black cumin	0.0209±0.0003	0.0134±0.0005	ND
	Garlic	0.0055±0.0006	0.0016±0.0002	ND
	Ginger	0.0154±0.0002	0.0346±0.0018	ND
WA	Fenugreek	0.0134±0.0003	0.0269±0.0016	0.0138± 0.0016
	Black cumin	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
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
Egypt	Fenugreek	0.0152±0.0003	1.122	0.005±0.004
	Black cumin	0.0532±0.0022	ND	0.008
	Garlic	0. 200±0.001	2.12	ND
	Ginger	0.0881±0.009	3.43	0.022
France	Fenugreek	-	0.004±0.008	ND
	Black cumin	0.008±0.001	0.0134±0.0012	-
	Garlic	-	-	0.005±0.005
	Ginger	0.1345±0.013	0.0072±0.004	-
India	Fenugreek	ND	0.0089	ND
	Black cumin	3.44	ND	0.067±0.004
	Garlic	1.564	0.0026±0.006	ND
	Ginger	0.004±0.0003	0.0476±0.01	ND
WHO permissible limits (mg/kg)	Spices	0.030	0.040	0.090

Note; MW = microwave oven digestion; WA = wet ashing; DA= dry ashing and ND = not detected at $P \leq 0.05$ Source: values compiled are after different authors (Wang, JL,*et al.*, 2006. Volesky *et al.,* 1995, Mustafa S. *et al.*, 2004, Asfaw Z. *et al.*, 2001, Letchamo *et al.*, 1991).

SUMMARY, CONCLUSION AND RECOMMENDATION

I. 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%.

II. Conclusions

This study is focused on comparing the extraction efficiency of Dry Ashing, Wet Ashing and Microwave oven digestion methods in different spices including Garli*c*, 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. But dry and wet digestion methods are more time consuming and complicated than the microwave digestion method in terms of digestion efficiency. 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.

III. Recommendations

The following recommendations are made as a result of the outcome of this study

- This study might be repeated with ICP-OES to compare the metal contents of the selected spices.
- If it is possible spices would be directly sampled from the areas of cultivation
- Monitoring of the levels of heavy metals in spices should be encouraged.
- Efficiency of other extractants like HNO_3:H_2SO_4, HNO_3:$HClO_4$, H_2SO_4:$HClO_4$ should also be checked.

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