

Comparison of Microwave, Dry and Wet Digestion Procedures for the Determination of Trace Metal Contents in Spice Samples Produced in Turkey

MUSTAFA SOYLAK^{1*}, MUSTAFA TUZEN², IBRAHIM NARIN³ AND HAYATI SARI²

¹ Chemistry Department, Faculty of Science and Arts, Erciyes University, 38039 Kayseri, Turkey

² Chemistry Department, Faculty of Science and Arts, Gaziosmanpasa University, 60250 Tokat, Turkey

³ Faculty of Pharmacy, Erciyes University, 38039 Kayseri, Turkey

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ABSTRACT

The concentrations of trace metals (Pb, Cd, Cr, Ni, Fe, Cu, Mn and Zn) in spice samples from Turkey were determined because similar studies have not been published. The experiments were carried out using atomic absorption spectrometry after dry ashing, wet ashing and microwave digestion methods. The study of sample preparation procedures showed that the microwave method was the best. The proposed method showed satisfactory recovery, detection limits and standard deviation for trace metal determination in spice samples. The concentrations of Pb, Cd, Cr, Ni, Fe, Cu, Mn and Zn in the samples were in the range of 0.47-1.89, 0.10-0.93, 0.67-7.15, 0.65-8.69, 88.9-376.3, 4.1-28.7, 11.9-211.3 and 7.84-47.6 $\mu\text{g/g}$, respectively. Correlations between metal concentrations were evaluated.

Key words: trace metals, digestion, spices, atomic absorption spectrometry

INTRODUCTION

Trace heavy metals are important in daily diets, because of their essential nutritious value and possible harmful effects. Metals like iron, copper, zinc, cobalt and manganese are essential metals since they play an important role in biological systems; whereas mercury, lead, cadmium, etc. are non-essential metals which can be toxic even in trace amounts⁽¹⁻³⁾. The essential metals can also have harmful effects when their intakes exceed the recommended quantities significantly. Spices are important sources of nutrition and flavoring for humans⁽⁴⁻⁶⁾. Small amounts of heavy metals in spices have been widely reported for spices in other countries⁽⁷⁻¹⁰⁾. However, according to our literature survey, studies have not been carried out dealing with this subject matter in Turkey.

Many analytical methods including atomic absorption spectrometry for trace element determination in plant materials require the decomposition of the sample⁽¹⁰⁻¹²⁾. Hence, the mineralization procedure is of great importance for obtaining desirable results for the analytes⁽¹²⁾. The wet and dry ashing procedures are slow. Also these procedures are difficult to follow consistently. Microwave digestion is a rapid and efficient method for sample decomposition prior to the determination of trace metals⁽¹³⁻¹⁵⁾.

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 determination. Also flame and graphite furnace AAS is the main instrument in food analysis laboratories for the determination of trace heavy metal contents in the samples tested⁽¹⁶⁻²⁰⁾.

In the present study, the levels of Pb, Cd, Cr, Ni and Fe, Cu, Mn, Zn in spice samples collected from different cities of Turkey were determined by flame and graphite furnace atomic absorption spectrometry after digestion following various methods. In order to investigate the relationship of spice samples inspected, the correlation coefficients between the metal contents of the spice samples were also determined.

MATERIALS AND METHODS

I. Sampling

A total of 12 different spice samples were purchased from local markets in different cities of Turkey. The samples were dried at 105°C for 24 hr. Dried samples were homogenized using an agate pestle and stored in pre-cleaned polyethylene bottles until analysis.

II. Reagents

All reagents were of analytical reagent grade unless otherwise stated. Double deionized water (Milli-Q Millipore 18.2 M Ωcm^{-1} resistivity) was used for all dilutions. HNO₃ and H₂O₂ were of suprapur quality (E.

* Author for correspondence. Tel: +90-352-437-4933
Fax: +90-352-437-4933; E-mail: soylak@erciyes.edu.tr

Merck). All the plastic and glassware were cleaned by soaking in dilute HNO₃ (1+9) and were rinsed with distilled water and air dried before use. The element standard solutions used for calibration were prepared by diluting a stock solution of 1000 mg/L of the given element supplied by Sigma and Aldrich.

III. Apparatus

A Perkin Elmer Analyst 700 model AAS with deuterium background corrector was used in this study. Pb, Cd, Cr and Ni were determined by HGA graphite furnace using high purity argon. Other measurements were carried out in an air/acetylene flame. The operating parameters for working elements were set according to the recommendations of the manufacturer. A Milestone Ethos D closed vessel microwave system (maximum pressure 1450 psi, maximum temperature 300°C) was used for microwave digestion.

IV. Digestion Procedures

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

(I) Dry ashing

One gram of sample was placed into a high form porcelain crucible. The furnace temperature was slowly increased from room temperature to 450°C in 1 hr. The sample was ashed for about 8 hr until a white or grey ash residue was obtained. The residue was dissolved in 5 mL of HNO₃ (25%, v/v) and, when necessary, the mixture was heated slowly to dissolve the residue. The solution was transferred to a 10-mL volumetric flask and made up to volume. A blank control was carried out in the same way using the solvent alone.

(II) Wet ashing

Wet digestion of spice samples was performed using an oxi-acidic mixture of HNO₃/H₂O₂ (2/1) (12 mL for a 1.0 g sample) in a 100-mL beaker inside a hood. This mixture was heated up to 130°C for 4 hr and brought to a volume of 25 mL with deionized water. A blank was carried out in the same way.

(III) Microwave digestion

One gram of sample was digested with 4 mL of HNO₃ (65%) and 2 mL of H₂O₂ (30%) in microwave digestion system and diluted to 10 mL with deionized water. A blank digest was carried out in the same way. All sample solutions were clear. Digestion conditions are given in Table 1.

V. Digestion Conditions

The conditions for the digestion of the spice samples were investigated first. For that purpose, a spice sample (*Nigella sp.*) was used. *Nigella sp.* was digested with the dry, wet and microwave digestion methods given above. The results are given in Table 2. The comparison of three digestion methods showed no statistically significant differences in results. The recovery values were nearly quantitative ($\geq 95\%$) for all digestion methods. *T*-test 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 8 hr, 4 hr and 35 min, respectively.

In light of these results, the microwave digestion procedure was chosen 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 by using *Nigella sp.* 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 microwave digestion procedure, different combinations of these two reagents were tested. The HNO₃/H₂O₂ (4/2) mixture used for spice digestion resulted in shortened digestion time, excellent recovery and precision than other combinations. The same results for the

Table 1. Operating conditions for microwave digestion system

Steps	Time (min)	Power (W)
1	2	250
2	2	0
3	6	250
4	5	400
5	8	550
6	8	Vent

Table 2. Comparison of trace metal contents ($\mu\text{g/g}$) in *Nigella sp.* determined by AAS after digestion using three different methods, n = 5

Metal	Dry ashing	Recovery (%)	Wet ashing	Recovery (%)	Microwave	Recovery (%)
Cu	10.2 ± 1.1	96	11.4 ± 1.0	97	12.8 ± 0.6	101
Cd	0.56 ± 0.10	95	0.67 ± 0.11	96	0.61 ± 0.05	99
Pb	0.98 ± 0.11	95	1.12 ± 0.14	95	1.23 ± 0.10	98
Zn	25.4 ± 2.3	96	22.7 ± 1.8	97	27.2 ± 1.3	100
Mn	65.2 ± 5.4	97	71.4 ± 6.6	98	62.6 ± 3.2	98
Fe	247 ± 19	96	238 ± 21	98	265 ± 15	103
Cr	2.76 ± 0.25	97	2.94 ± 0.32	99	2.62 ± 0.12	100
Ni	4.83 ± 0.45	95	4.68 ± 0.39	97	4.97 ± 0.27	99

HNO₃/H₂O₂ (4/2) mixture were obtained for other 11 samples for microwave digestion procedure.

RESULTS AND DISCUSSION

The instrument detection limit (IDL) is defined as the concentration corresponding to three times the standard deviation of 10 blanks. Instrument detection limit values of the investigated elements for AAS were found to be 0.22 mg/L for Pb, 0.08 mg/L for Cd, 0.11 mg/L for Mn, 0.09 mg/L for Cu, 0.14 mg/L for Zn, 0.13 mg/L for Fe, 0.18 mg/L for Cr and 0.16 mg/L for Ni.

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 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⁽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.*

The manganese levels in the samples varied from 11.9 µg/g in *Capsicum annuum* to 211.3 µg/g in *Diantus sp.* Trace metal levels in spices of India have been reported in the range of 2.94-14.4 µg/g for copper, 0.41-7.68 µg/g for nickel, 30.2-101 µg/g for manganese and 8.96-71.4 µg/g for zinc⁽¹¹⁾. The lowest and highest contents of iron were found as 88.9 µg/g for *Diantus sp.* and 376.3 µg/g for

Menta sp., respectively. The concentration of chromium varied from 0.67 µg/g in *Papaver somniferum* to 7.15 µg/g in *Capsicum annuum*. Chromium contents in spices of Spain have been reported in the range of 0.11-1.14 µg/g⁽²¹⁾. Our values for chromium were generally agreeing with the results given in literature 21 with some exceptions.

The spices analyzed are widely consumed in Turkey for adding flavors and provide sources of some important minerals. The fact that toxic metals are present in high concentrations in spices is of particular importance in relation to the WHO⁽²²⁾ standards for Pb and Cd as toxic metals. The maximum permissible doses for an adult are 10 mg/kg Pb and 0.3 mg/kg Cd in medicinal plants. While our values for lead for all the samples analyzed below WHO's value, the levels of cadmium in *Menta sp.*, *Cocos nucifera*, *Papaver somniferum*, *Cuminum cyminum*, *Capsicum annuum*, *Piper nigrum* were higher than WHO's value.

A linear regression correlation test was performed to investigate correlations between the metal contents in all tested spice samples. The entire data were subjected to a statistical analysis and correlation matrices were produced to examine the inter-relationship between the investigated metal concentrations. The values of correlation coefficients between metal concentrations are given in Table 4. There are positive correlations of nickel-lead, iron-cadmium, chromium-cadmium, chromium-iron and nickel-iron with corresponding r-values of 0.511, 0.515, 0.679, 0.644, and 0.644, respectively. Chromium has significant positive relationships with cadmium (0.538). According to the data given (correlation coefficient > 0.50) in literatures 23-24, these correlations were significant. The correlations between Ni-Pb, Fe-Cd, Cr-Cd, Cr-Fe, Ni-Fe and Cr-Cd show that the spice samples with significant correlations among the concentrations of the elements are from the same sources in Turkey.

CONCLUSIONS

The dry and wet digestion methods are more time-consuming and complicated than the microwave digestion

Table 3. Trace metal contents (µg/g) in various spices determined by AAS after digestion using the microwave digestion method (mean ± SD), n = 4

Spices	Cu	Cd	Pb	Zn	Mn	Fe	Cr	Ni
<i>Nigella sp.</i>	13.6 ± 1.2	0.10 ± 0.06	1.89 ± 0.15	41.5 ± 3.2	33.3 ± 2.7	149.8 ± 11.2	1.18 ± 0.10	7.02 ± 0.51
<i>Menta sp.</i>	9.7 ± 1.0	0.50 ± 0.05	1.14 ± 0.12	21.7 ± 2.2	78.9 ± 6.3	376.3 ± 25.3	5.93 ± 0.48	8.69 ± 0.74
<i>Cocos nucifera</i>	5.2 ± 0.9	0.47 ± 0.06	1.36 ± 0.10	17.1 ± 1.5	29.9 ± 2.4	201.4 ± 19.3	1.16 ± 0.10	3.17 ± 0.22
<i>Thymus sp.</i>	4.8 ± 0.6	0.12 ± 0.10	0.85 ± 0.07	20.7 ± 2.0	87.8 ± 6.6	269.8 ± 20.2	1.05 ± 0.11	1.98 ± 0.14
<i>Cinnamomum sp.</i>	4.7 ± 0.5	0.14 ± 0.12	0.76 ± 0.05	12.4 ± 1.1	159.7 ± 14.1	158.9 ± 12.7	0.70 ± 0.10	0.77 ± 0.17
<i>Rhus coriaria</i>	28.7 ± 2.6	0.11 ± 0.10	0.47 ± 0.06	19.9 ± 1.4	13.4 ± 1.2	247.7 ± 20.4	1.21 ± 0.11	3.64 ± 0.30
<i>Papaver somniferum</i>	17.2 ± 1.4	0.33 ± 0.07	0.88 ± 0.05	47.6 ± 3.1	69.1 ± 5.3	97.1 ± 8.5	0.67 ± 0.14	2.36 ± 0.25
<i>Cuminum cyminum</i>	9.1 ± 0.8	0.61 ± 0.05	0.66 ± 0.05	22.9 ± 2.0	39.1 ± 2.7	305.1 ± 17.2	3.85 ± 0.42	4.50 ± 0.50
<i>Diantus sp.</i>	4.1 ± 0.5	0.17 ± 0.07	1.12 ± 0.10	10.4 ± 1.1	211.3 ± 15.4	88.9 ± 6.5	0.70 ± 0.10	0.74 ± 0.11
<i>Capsicum annuum</i>	4.2 ± 0.3	0.65 ± 0.05	0.79 ± 0.08	7.84 ± 0.6	11.9 ± 1.2	234.1 ± 19.8	7.15 ± 0.83	2.92 ± 0.43
<i>Cassia sp.</i>	14.7 ± 1.1	0.20 ± 0.04	0.68 ± 0.07	13.1 ± 1.4	53.5 ± 4.3	126.5 ± 10.5	1.95 ± 0.15	0.65 ± 0.14
<i>Piper nigrum</i>	9.4 ± 1.0	0.93 ± 0.10	1.44 ± 0.15	15.5 ± 1.2	14.9 ± 1.2	281.8 ± 20.5	3.44 ± 0.22	4.99 ± 0.32

Table 4. Correlation coefficients between metal concentrations determined by microwave digestion-atomic absorption spectrometry combination

	Cu	Cd	Pb	Zn	Mn	Fe	Cr	Ni
Cu	1.000							
Cd	-0.272	1.000						
Pb	-0.256	0.155	1.000					
Zn	0.429	-0.207	0.325	1.000				
Mn	-0.419	-0.455	-0.069	-0.206	1.000			
Fe	-0.046	0.515	-0.076	-0.193	-0.418	1.000		
Cr	-0.226	0.679	-0.069	-0.312	-0.394	0.644	1.000	
Ni	0.170	0.355	0.511	0.369	-0.451	0.644	0.478	1.000

method without any advantages in terms of digestion efficiency. The use of microwave digestion system in spice samples provides a better, safer and cleaner method of sample preparation. The recoveries of the trace metals were in the range of 95-103%. The standard deviations were less than 10%.

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