

Exploration of Metal Concentrations in Some Local Spices by Microwave Plasma Atomic Emission Spectrometry after Microwave Digestion

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ABSTRACT: The innovative microwave plasma - nitrogen plasma - atomic emission spectrometry was used for the determination of ten metals in five spices (black pepper, chili, cinnamon, cumin, and curcumin). These measurements were preceded by microwave digestion of the spice samples. The total average of aluminium contents in the spices is 130 µg/g with a violation percentage of 68.57% of the maximum permissible limits (MPL). Arsenic was found only in curcumin with an average of 0.4286 µg/g. All curcumin samples have less As than the MPL. The total average concentration of Cd in all the spice samples is 0.0906 µg/g. Cadmium was not detected in 12 spices, but 10 other samples had higher levels than the MPL. Chromium has higher levels in almost all spice samples than the MPL with a total average of 0.3737 µg/g. All the copper values were within the maximum allowed limits. Total Cu average in the samples is 1.397 µg/g. Half of the samples have higher contents of Fe than the MPL. Iron total average is 159 µg/g. Lead was not violating any of MPL in the studied spices with a total average of 0.6811 µg/g. Manganese in all the samples was far lower than the MPL. The total average of Mn is 41.04 µg/g. Neither the levels of nickel nor of zinc in spices were higher than the MPL. The total averages of Ni and Zn are 0.3143 and 3.323 µg/g, respectively.

KEYWORDS: Spices, Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn, Microwave Plasma Atomic Emission Spectrometry, Microwave Digestion.

1. INTRODUCTION:

A spice is a vegetable substance of indigenous or exotic origin, which is or has a hot, pigment taste, used to enhance taste of foods or to add to them the stimulant ingredients contained in them (Abban, 2009). The use of spices dates back to ancient times. Archaeological excavation has revealed that prehistoric man used the leaves of certain plants to enhance the flavour of the half-cooked foods, which he ate (Baillon, 1877). The Phoenix (Greek and Roman ancient mythology) states: "From the hot hills, and with rich spice frames, A pile shall burn, and Hatch him with his flames" (Peter, 2013). Spices are of increasing importance in Egyptian markets (Hassan and Abd El-Rahman, 2013). Contamination of crude medicinal plants and spices as well as their products has increasingly been reported (Abdel .Megeed *et al.*, 2014). This has brought concerns and fears regarding practitioner's professionalism and quality, efficacy and safety of their treatment methods and products from herbal and natural sources available in market.

The microwave plasma atomic emission spectrometry (MP-AES) achieves lower detection limits than Flame Atomic Absorption Spectrometry (FAAS), the first enables the analysis of extra elements (Cauduro, 2013). The MP-AES features continuous wavelength coverage, which allows the analyst to select wavelengths that are appropriate for the expected concentration range, and free from spectral interferences.

The aim of this study was the determination of metals in some local spices by the innovative microwave plasma atomic emission spectrometry after microwave digestion of the spice samples.

2. MATERIALS AND METHODS:

The determination of metals in spice samples were performed according to the method of the Association of Official Analytical Chemistry (AOAC, 1995).

2.1. Sampling

Thirty-five samples (five different spices; viz: black pepper, chili, cinnamon, cumin, and curcumin; seven samples each) were randomly collected from local markets during 2013. Samples were collected in polyethylene bags labeled then transferred immediately to the Lab. All samples were maintained at 2-5°C until analysis. About 0.5 g (accurately weighed) dry samples (at 70°C overnight) of the collected samples were digested by the microwave digestion system. The samples were transferred quantitatively into 25 mL volumetric flasks and completed to the mark with ultrapure water.

2.2. Chemicals and Standards

All chemicals and standards are of Analytical grade. Metals stock standards of Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn were obtained from Merck, Darmstadt, Germany (1000 µg/mL).

2.3. Sample preparation

Microwave digestion was used to prepare the spice samples. Ten mL of HNO₃ was added to accurately weighed ≈ 0.5 g of the sample. A preloaded method for the MARS6 (CEM, Corporation, USA) microwave was used to digest the samples. Once cooled, the solution was diluted quantitatively to 25 mL using ultrapure water. The microwave digestion parameters were according to Table 1.

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Table 1: Microwave Digestion System Parameters

Options	
Temperature Pressure Time Power Vessels	Internal Fiber Optic Temperature Control
	Internal Pressure Control
	DuoTemp Control 210°C
	800
	Ramp : 21 min Hold : 15 min
Power	400- 1800
	EasyPrep Full Starter Set, P/T Control

2.4. Instrumentation

All measurements were performed using the innovative Agilent microwave plasma atomic emission spectrometry model 4200 MP-AES with nitrogen gas plasma supplied via an Agilent 4107 Nitrogen Generator. The generator alleviates the need and expense of sourcing analytical grade gases. The sample introduction system comprised a double-pass cyclonic spray chamber and the OneNeb nebulizer. The innovative 4200 MP-AES features a second-generation waveguide and torch, with mass flow controlled nebulizer gas flow (Cauduro, 2013). The 4200 MP-AES has a robust toroidal plasma with a central channel temperature of $\approx 5,000$ K that eliminates many of the chemical interferences that are present in FAAS and expands the concentration working range of the 4200 MP-AES when compared with the FAAS. This means that the element specific

sample preparation that is commonplace when using FAAS is not necessary when using the 4200 MP-AES, improving ease of use and reducing cost. Some modification has been performed with the first emerging model 4100 MP-AES into model 4200 MP-AES to enhance the performance and to resolve some drawbacks. An Agilent SPS 3 autosampler was used to deliver samples to the instrument, allowing the system to be operated unattended. The instrument operated in a fast sequential mode and featured a Peltier-cooled CCD detector. Background and spectral interferences could be simultaneously corrected easily and accurately using Agilent's MP Expert software. Method parameters are given in Tables 2 and 3. The limit of detection and limit of quantification of the determination method of metals in Spices are shown in Table 4.

Table 2: Metals Determined in Spices and their Wavelengths and Calibration Ranges.

Element	Wavelength (nm)	Calibration Range ($\mu\text{g/mL}$)	Correlation Coefficient
Al	396.2	0 – 2.000	0.9981
As*	193.7	0 – 0.020	0.8719
Cd	228.8	0 – 2.500	0.9992
Cr	425.4	0 – 2.000	0.9998
Cu	324.8	0 – 2.500	0.9992
Fe	372.0	0 – 2.500	0.9992
Mn	403.1	0 – 2.000	0.9999
Ni	352.5	0 – 2.000	0.9999
Pb	405.9	0 – 2.500	0.99996
Zn	213.9	0 – 1.500	0.9904

* As is determined by HG-AAS

Arsenic is determined by Thermo Elemental model: Solar M Atomic Absorption Spectrophotometer (AAS) with Hydride Generation kit (HG) model: VP100. The current,

wavelength and slit bandwidth of As were adjusted automatically by the instrument software.

Table 3: Microwave Plasma Atomic Emission Parameters

Parameter	Value
Replicates	3
Pump rate	15 rpm
Sample uptake delay	15 seconds
Rinse time	30 seconds
Stabilization time	15 second
Fast Pump during Uptake and Rinse	On (80 rpm)
Nebulizer	OneNeb
Spray chamber	Double pass cyclonic
Autosampler	Agilent SPS 3
Sample pump tubing	Orange/green
Waste pump tubing	Blue/blue

Table 4: Limit of Detection (LOD) and Limit of Quantification (LOQ) of Metals Determined in Spices.

Element	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)
Al	0.400×10^{-3}	1.333×10^{-3}
As*	0.044×10^{-3}	0.1467×10^{-3}
Cd	2.100×10^{-3}	7.000×10^{-3}
Cr	0.100×10^{-3}	0.333×10^{-3}
Cu	0.700×10^{-3}	2.333×10^{-3}
Fe	4.600×10^{-3}	15.333×10^{-3}
Mn	0.200×10^{-3}	0.667×10^{-3}
Ni	0.900×10^{-3}	3.000×10^{-3}
Pb	3.300×10^{-3}	0.011
Zn	4.500×10^{-3}	0.015

LOD: The Limit Of Detection (=3X standard deviation of 10 measurements in blank matrix).

LOQ: The Limit Of Quantification (= 3XLOD).

* As is determined by HG-AAS

3. RESULTS and DISCUSSION:

Analysis of Chinese herbal medicines by microwave plasma-atomic emission spectrometry (Agilent model: 4100 MP-AES) was conducted by Chunhua Wu *et al.* (2012). They mentioned that: "preparing samples by microwave digestion and subsequent analysis by microwave plasma-atomic emission spectrometry, three typical Chinese herbal medicines, can be analyzed for trace and major concentration elements with good accuracy". Furthermore, the MP-AES has the lowest operating costs of comparable techniques such as flame AA, and by using non-flammable gases, removes safety concerns associated with acetylene and nitrous oxide. The addition of the Nitrogen Generator is significantly lower gas costs or for analysis in remote locations or where sourcing of gases is costly or difficult.

Tables 5 – 14 describe the detailed results of Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn determinations in five spices (black pepper, chili, cinnamon, cumin, curcumin), respectively.

Aluminium concentrations in 24 spice samples of the

35 (68.57%) were above the maximum limit of permission which was issued by the Ministry of Health and the Standardization Administration of China (Yokel, 2012). Although arsenic was determined by the lowest detection limit method (hydride generation - atomic absorption spectrometry), it was only detected in curcumin samples (Table 6). None of the curcumin showed higher As concentrations than the maximum permissible limits, set by International / National Standards for Heavy Metals in Food (Choi, 2011). From Table 7 it was shown that Cd was not detected in 12 spice samples. Nevertheless, 10 samples have higher concentrations of Cd than the permissible limits (Yokel, 2012). It can be seen from Table 8 that Cr has higher levels in almost all spices than the maximum allowed limits (Yokel, 2012). All the copper values (Table 9) were within the maximum allowed limits approved by WHO and FAO (Nkansah and Amoako, 2010). As shown in Table 10, half of the samples have higher limits of Fe than the maximum permissible ones (CODEX, 1995). Lead was not violating the

permissible limits in any of the spice samples, this can be seen in Table 11 (Choi, 2011). Due to the high maximum permissible limits of Mn (200 µg/g), the metal concentrations in all the samples were far lower than these limits, Table 12 (Raouf *et al.*, 2014). Neither the levels of nickel nor those of zinc in spices were higher than the maximum allowed limits (50 µg/g), set by the safety standards established by the National Polish Ministry of Health, Tables 13 and 14 (Krejpcio *et al.*, 2007).

Heavy metals contamination in marjoram herb, under Egyptian conditions, was studied by Abd-El Rahman and Mareei (2012). Data indicate that Pb, Cd, Zn, Cu, Mn and Fe contents in the collected samples, were found at different levels. Normal levels were found in

non-packed and packed samples concerning Cu, Zn, Fe, and Mn, while levels higher than the maximum permissible limits of Cd and Pb were 90% and 37.5% in non - packed and 20% and 10% in packed samples, respectively.

Abou-Arab and Abou Donia (2000) found that, heavy metal contents in spice and medicinal plants depend on the plant species which explains for the variation in heavy metal content in different spices tested. There are many factors that play an important role in the presence of heavy metals in herbs such as, metal contents in soil and irrigation water which may be attributed to normal levels or higher contamination values due to pollution sources.

Table 5: Aluminium Concentration in µg/g in Spices

Spice	1	2	3	4	5	6	7	Average	StDev	% RSD	MPL	n>MPL
Black Pepper	184.7	42.02	36.26	414.3	128.4	153.3	137.6	156.6	117.1	74.75		5
Chili	114.3	109.6	153.0	104.5	152.7	111.4	159.1	129.2	22.52	17.43		7
Cinnamon	164.3	206.5	19.4	127.5	92.5	50.1	129.9	112.9	59.77	52.94	100*	4
Cumin	118.2	85.55	3.000	32.99	165.8	34.12	132.3	81.72	55.83	68.32		3
Curcumin	234.6	171.3	194.0	204.9	64.90	40.02	275.6	169.3	80.22	47.38		5
Total Average	130.0											

StDev: Standard Deviation. *(Yokel, 2012)

Table 6: Arsenic Concentration in µg/g in Spices

Spice	1	2	3	4	5	6	7	Average	% RSD	StDev	MPL
Curcumin	0.2200	0.6600	0.2600	0.6500	0.3800	0.4400	0.3900	0.4286	37.26	0.1597	1.4

*(Choi, 2011) **MPL**: Maximum Permissible Limit.

Table 7: Cadmium Concentration in µg/g in Spices

Spice	1	2	3	4	5	6	7	Average	StDev	% RSD	MPL	n>MPL
Black Pepper	0.0600	0.1200	0.2400	0.8300	ND	0.0100	ND	0.1800	0.2772	154.0		3
Chili	0.0500	0.0300	0.1500	0.2400	0.1800	0.0600	0.0800	0.1129	0.0724	64.20		3
Cinnamon	ND	ND	ND	ND	ND	0.0800	0.1200	0.0286	0.0464	162.5	0.1*	
Cumin	0.0800	0.0600	0.0300	0.1500	0.0700	0.1200	0.1500	0.0943	0.0430	45.66		3
Curcumin	0.2200	ND	ND	ND	ND	0.0400	ND	0.0371	0.0759	204.4		1
Total Average	0.0906											

*(Choi, 2011) ND: Not Detected n>MPL: number of samples violating MPL for the metal

Table 8: Chromium Concentration in µg/g in Spices

Spice	1	2	3	4	5	6	7	Average	StDev	% RSD	MPL	n>MPL
Black Pepper	0.4000	0.1000	0.2000	0.9000	0.3000	0.4000	0.3000	0.3714	117.1	63.90		5
Chili	0.3000	0.7000	0.4000	0.3000	0.2000	0.4000	0.4000	0.3857	22.52	37.77		6
Cinnamon	0.6600	0.8000	0.1000	1.0000	0.4000	0.2000	0.2000	0.4800	59.77	66.48	1*	3
Cumin	0.3000	0.4000	ND	0.1000	0.4000	0.1000	0.2000	0.2143	55.83	67.99		3
Curcumin	0.5200	ND	0.4000	0.4000	0.1000	0.1000	1.400	0.4171	80.22	105.4		4
Total Average	0.3737											

*(Choi, 2011)

Table 9: Copper Concentration in µg/g in Spices

Spice	1	2	3	4	5	6	7	Average	StDev	% RSD	MPL
Black Pepper	2.199	2.040	2.290	1.027	1.903	1.415	1.303	1.740	0.4527	26.03	50*
Chili	1.715	1.941	1.982	1.803	1.397	1.237	1.545	1.660	0.2584	15.57	
Cinnamon	1.252	0.7070	1.181	1.290	1.679	0.1370	0.6830	0.990	0.4739	47.87	
Cumin	1.831	0.7400	1.759	1.778	0.881	1.642	1.617	1.464	0.4208	28.74	
Curcumin	1.410	0.7850	1.127	1.319	1.427	0.6690	1.194	1.133	0.2772	24.47	
Total Average	1.397										

*(Nkansah and Amoako, 2010)

Table 10: Iron Concentration in µg/g in Spices

Spice	1	2	3	4	5	6	7	Average	StDev	% RSD	MPL	n>MPL
Black Pepper	178.5	58.97	74.90	599.6	111.8	166.0	108.9	185.5	173.8	93.68	100*	5
Chili	109.0	95.74	123.4	80.67	95.17	101.4	140.9	106.6	18.57	17.42		4
Cinnamon	255.9	315.6	33.46	376.1	133.7	65.58	118.7	185.6	121.2	65.32		5
Cumin	119.2	65.30	58.42	386.6	312.2	166.6	442.3	221.5	145.8	65.80		5
Curcumin	102.8	21.60	178.0	27.80	116.2	66.40	-	85.5	54.1	63.33		3
Total Average	159.0											

*(CODEX, 1995)

Table 11: Lead Concentration in µg/g in Spices

Spice	1	2	3	4	5	6	7	Average	StDev	% RSD	MPL
Black Pepper	0.6500	0.9000	0.6500	0.7700	0.2900	0.6700	0.6600	0.6557	0.1719	26.21	*60
Chili	0.6400	0.8200	1.240	2.2300	2.2000	0.7200	0.7300	1.226	0.6511	53.12	
Cinnamon	0.2800	0.3000	0.2800	0.3300	0.3300	0.6600	0.5800	0.3943	0.1456	36.92	
Cumin	0.5900	0.6200	0.2000	0.0200	0.5600	0.9800	2.0100	0.7114	0.6029	84.74	
Curcumin	0.4700	0.2300	0.3500	0.2000	0.3700	0.8100	0.5000	0.4186	0.1901	45.41	
Total Average	0.6811										

*(Choi, 2011)

Table 12: Manganese Concentration in µg/g in Spices

Spice	1	2	3	4	5	6	7	Average	StDev	% RSD	MPL
Black Pepper	9.200	6.300	3.500	75.13	118.74	40.83	6.000	37.10	41.31	111.4	200*
Chili	68.35	6.700	6.600	5.100	120.10	7.700	77.00	41.65	43.19	103.7	
Cinnamon	65.47	68.76	7.610	47.90	34.32	6.400	7.400	33.98	25.52	75.09	
Cumin	66.69	12.60	0.1000	81.58	49.70	68.05	86.32	52.15	31.12	59.67	
Curcumin	43.31	19.10	32.50	38.92	4.00	84.65	59.77	40.32	24.45	60.64	
Total Average	41.04										

*(Raouf *et al.*, 2014)

Table 13: Nickel Concentration in µg/g in Spices

Table 10: Mean Concentration in $\mu\text{g/g}$ in spices											
Spice	1	2	3	4	5	6	7	Average	StDev	% RSD	MPL
Black Pepper	ND	0.4000	0.1000	0.2000	0.3000	0.3000	0.4000	0.2429	0.1400	57.64	50*
Chili	0.4000	0.3000	0.4000	0.4000	0.1000	0.5000	0.4000	0.3571	0.1178	32.98	
Cinnamon	1.030	1.1000	0.3300	0.8000	1.0000	ND	0.2000	0.6371	0.4172	65.47	
Cumin	0.4000	0.4000	ND	ND	0.4000	ND	0.1000	0.1829	0.1914	104.7	
Curcumin	0.0600	ND	ND	ND	0.1000	ND	0.9000	0.1514	0.3078	203.2	
Total Average				0.3143							

*(Krejpcio *et al.*, 2007).

Table 14: Zinc Concentration in µg/g in Spices

Spice	1	2	3	4	5	6	7	Average	StDev	% RSD	MPL
Black Pepper	2.700	2.600	4.100	4.100	1.800	4.000	8.000	3.900	1.8685	47.91	50*
Chili	2.000	8.000	7.000	10.000	5.400	8.600	5.600	6.657	2.4277	36.47	
Cinnamon	1.020	1.400	3.610	1.200	0.000	3.000	2.800	1.861	1.1987	64.40	
Cumin	3.000	6.900	-0.510	2.600	3.400	1.200	2.200	2.684	2.1055	78.44	
Curcumin	2.880	0.5000	2.200	2.600	1.000	0.5000	0.9000	1.511	0.9421	62.33	
Total Average	3.323										

*(Krejpcio *et al.*, 2007).

CONCLUSION:

The use of microwave plasma atomic emission spectrometry for the determination of spices is superior from the point of view of lower detection limits, concentration working range, easily background and spectral interference correction, and the use of a nitrogen generator which alleviates the need of expensive and dangerous analytical grade gases. Using of microwave digestion also minimizes the time of sample preparation and the risk of direct exposure to toxic digestion acids and gases.

The results of metal determination in spice samples reveal that no general trend of contamination can be concluded. Although, some studied spice samples showed critical concentrations compared with the maximum permissible limits, these cases were limited in comparison with the total number of samples. However, there are many parameters affect the presence of metals in spices such as, metal contents in soil and irrigation water. Other criteria may be involved too such as handling, processing, transport and other environmental factors.

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تعيين المعادن في بعض التوابل المحلية بواسطة طريقة مطياف بلازما الموجات الدقيقة تلى طريقة هضم العينات بالموجات الدقيقة للعينات

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* المعمل المركزى للمبيدات بمركز البحوث الزراعية ، الدقى ، الجيزة ، مصر

الملخص العربى

تم استخدام التقنية المبتكرة مطياف الانبعاث الذري عن طريق بلازما الموجات الدقيقة – بلازما غاز النيتروجين – لتقدير عدد 10 معادن في 5 توابل (الفلل الأسود، الفلفل الحار، القرفة، الكمون، والكرم)، والتي قد سبقها هضم للعينات باستخدام طريقة الموجات الدقيقة، وقد وجد أن متوسط مجموع محتويات التوابل من الألومنيوم هو 130 ميكروجرام/جرام بنسبة عدد عينات يتعدى الحدود القصوى المسموح بها تصل إلى 68.57%، بينما لم يتم العثور على الزرنيخ فقط إلا في الكرم بمتوسط 0.4286 ميكروجرام/جرام، بحيث أن جميع عينات الكرم لديها نسب أقل من المسموح به من الزرنيخ. متوسط تركيز الكاديوم في إجمالي عينات التوابل هو 0.0906 ميكروجرام/جرام، ولم يتم الكشف عن الكاديوم في 12 عينة توابل، ولكن توجد 10 عينات بها مستويات أعلى من المسموح به من الكاديوم. أغلب العينات تحتوى على نسب أعلى من المسموح به من الكروم بمعدل تركيز إجمالي وقدره 0.3737 ميكروجرام/جرام. كل قيم النحاس في التوابل محل الدراسة ضمن النسب المسموح بها، إجمالي متوسط تركيز النحاس في العينات هو 1.397 ميكروجرام/جرام. نصف العينات تحتوى نسب من الحديد أعلى من المسموح به. متوسط مجموع تركيز الحديد في التوابل المدروسة هو 159 ميكروجرام/جرام. الرصاص الموجود بعينات التوابل لا ينتهك الحدود المسموح بها وذلك بمعدل متوسط تركيز إجمالي وقدره 0.6811 ميكروجرام/جرام. المنجنيز الموجود في جميع العينات أقل بكثير من الحدود القصوى المسموح بها وذلك بمعدل متوسط كلي وقدره 41.04 ميكروجرام/جرام. محتوى كل من النيكل والخاصين في التوابل أقل من النسب العليا المسموح بها. مجموع متوسطات كل من النيكل والزنك في التوابل هو 0.3143 و 3.323 ميكروجرام/جرام على التوالي.