Determination of the Geographic Origin of Spices Using Elemental Analysis after Microwave Digestion

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Introduction

Competitive advantage within the food industry has lead to fraudulent activities like intentional counterfeiting, substitution, adulteration, or mislabeling/ misrepresentation of ingredients within products. Many of these tricks go unnoticed by consumers and regulating government agencies due in part to the lack of standardized methods for identification. This highly specialized problem results in inferior products entering the marketplace and skewing price points for legitimate goods. One good example of this is Madagascar vanilla, price per kilo has vaulted from $47 in 2012/13 to $385 in 2016/17. This leads to potential counterfeiting and substitution from bad actors/criminals in the food industry. For origin-driven products like spices, food fraud has highlighted the need for ways to defend claims of geographic origin. This study uses three different technologies; microwave digestion, ICP-OES, and ICP-MS, to determine if spice origin from different countries could be accurately distinguished. Spice samples were sourced from a large US importer of spices that can verify the country of origin. The results of this study demonstrate the validity and applicability of the analytical approaches used.

Experimental

Samples. Over 50 spices from around the world were used in this study. All acquired from a Business-to-Business spice company in the US.

Sample Preparation. All spice samples were digested in a MARS 6 with MARSXpress Plus Vessels.

Experimental

Figure 1: MARSXpress Plus vessels (left). Spices and the vessels (middle), group photo (right).

"Plant Material" One Touch Method
MARSXpress Plus Vessels

Stages	1
Power	290 - 1800
Ramp Time	20:00
Hold Time	10:00
Temperature	200 °C

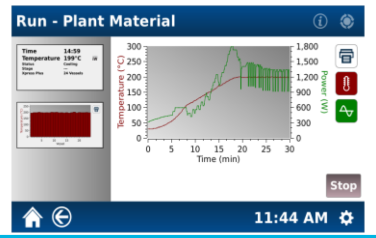

Table 1: Microwave Program Specs. with the control points for the Plant Material program that we used for all digestions (left), Screen shot from a run showing all 24 vessels are at the same temperature, and that the batch reached and held the programmed 200 °C temperature for the required 10 minutes (right).

Digested samples were then prepared for elemental analysis:

- Samples were roughly 100x dilution (0.5g into 50mL).
- Final concentration was approximately 20% acid (samples were digested in 9mL HNO3 and 1mL HCl, for a final acid concentration 18%HNO3 and 2%HCl).

Instrument parameters. An Agilent 7900 ICP-MS with the Ultra High Matrix Introduction (UHMI) system was used. The sample introduction system consisting of a micromist concentric nebulizer, quartz spray chamber, quartz torch with 2.5 mm id injector, and platinum cones.

The 5110 ICP-OES sample introduction system consisted of a SeaSpray nebulizer, double-Pass cyclonic spray chamber, and a 1.8 mm id injector torch.

Instrument operating conditions are listed in Table 2.

Table 2. ICP-MS/ICP-OES instrument operating conditions

ICP-MS Parameter	Setting	ICP-OES Parameter	Setting
RF power (W)	1600	RF power (kW)	1.20 kW
Sampling depth (mm)	10	Auxiliary gas flow (L/min)	1.0 L/min
Carrier gas flow (L/min)	0.68	Plasma gas flow (L/min)	12.0 L/min
Dilution (HMI) gas flow (L/min)	0.27	Nebulizer gas flow (L/min)	0.7 L/min
Helium cell gas flow (mL/min)	4.3	Viewing mode	Axial/Radial
Hydrogen cell gas flow (mL/min)	5	Replicate Read Time (s)	30 sec/5 sec
Energy discrimination (V)	4	Viewing height (mm)	12 mm
		Switching Valve	AVS7

Reference Standards. Various SRMs purchased from National Institute of Standards and Technology (NIST) were analyzed in this study to verify the sample preparation digestion process. NIST 1573a Tomato Leaves, NIST 1515 Apple Leaves, and NIST 1575 Pine Needles.

Samples and Sample Preparation. 50+ spices were obtained from a spice company that does B2B sales. They sourced all of the spices, and therefore know the true country of origin. This is very important when doing authentication studies. The samples were digested following the same procedure as for NIST SRM samples. About 0.5 g of each sample was weighted into 50 mL microwave vessels.

Results and Discussion

To verify the digestion process of the spice samples, four SRMs were analyzed by ICP-MS and ICP-OES. The mean results shown in Table 3 were in good agreement with the certified concentrations, where provided.

ICP-MS & ICP-OES (in Italics)	NIST 1573a Tomato Leaves				NIST 1575 Pine Needles				NIST 1515 Apples Leaves			
Element (mass and wavelength)	Certified conc	Mean Measured conc*	Recovery **	QC Criteria (80–120%) ***	Certified conc	Mean Measured conc*	Recovery **	QC Criteria (80–120%) ***	Certified conc	Mean Measured conc*	Recovery **	QC Criteria (80–120%) ***
^{23}Na	136	*111*	*82%*	PASS	63[R]	*59*	*94%*					
^{24}Mg (279.078 nm)	12000[R]	*11390*	*95%*	PASS	1060	*887*	*84%*	PASS				
^{27}Al (396.152 nm)	598	*476*	*80%*	PASS	580	*537*	*93%*	PASS				
^{39}K (769.897 nm)	27000	*28718*	*106%*	PASS	4170	*4209*	*101%*	PASS				
^{44}Ca (315.887 nm)	50500	*49844*	*99%*	PASS	2500	*2475*	*99%*	PASS				
^{51}V	0.835	0.687	82%	PASS					0.254	*0.266*	*105%*	PASS
^{52}Cr (205.560 nm)	1.99	1.70	86%	PASS								
^{55}Mn (257.610 nm)	246	240	98%	PASS	488[R]	504	103%					
^{56}Fe (239.563 nm)	368	359	97%	PASS	46	47	102%	PASS	82.7	72.2	87%	PASS
^{59}Co (238.892 nm)	0.57	0.52	91%	PASS	0.061[R]	0.065	106%					
^{60}Ni (216.555 nm)	1.59	1.44	91%	PASS	1.47[R]	1.49	101%		0.936	0.774	83%	PASS
^{63}Cu (324.754 nm)	4.7	4.0	85%	PASS	2.8	3.0	107%	PASS	5.69	4.79	84%	PASS
^{66}Zn (213.857 nm)	30.9	25.5	82%	PASS	30.9	36.7	119%	PASS	12.45	10.40	84%	PASS
^{75}As (188.980 nm)	0.112	0.119	106%	PASS	0.039[R]	0.032	83%					
^{78}Se (196.026 nm)	0.054	0.061	113%	PASS	0.099[R]	0.103	104%					
^{85}Rb									10.2	8.65	84%	PASS
^{88}Sr									25.1	23.6	94%	PASS
^{95}Mo (202.032 nm)	0.46[R]	0.39	85%						0.095	0.078	82%	PASS
^{107}Ag	0.017[R]	0.013	75%									
^{111}Cd (226.502 nm)	1.52	1.31	86%	PASS	0.233	0.227	97%	PASS	0.0132	0.0116	88%	PASS
^{137}Ba (455.403 nm)	63[R]	58	92%		6	6	100%	PASS	48.8	47.4	97%	PASS
^{201}Hg	0.034	0.037	108%	PASS					0.0432	0.0493	114%	PASS
^{208}Pb					0.167[R]	0.166	99%		0.47	0.44	94%	PASS
^{232}Th	0.12[R]	0.10	80%									
^{238}U	0.035[R]	0.023	64%									

*n=3, replicate sample digestion, each in triplicate
** FDA Elemental Analysis Manual (Section 3.4 Special Calculations) 3.4 Equation 20
*** QC Criteria FDA EAM 4.7 (80-120%) for NIST certified values
R Non-Certified Reference Value

Results and Discussion

Spike Recoveries. Spike recovery test was carried out to check the accuracy of the ICP-MS and ICP-OES methods for actual sample analysis. Four random spice samples were spiked with all elements were spiked at 20 and 60 ppb.

Recoveries were between 82% (for Ba) and 119% (for Cu). Quantitative results for the spice samples showed that the concentrations of Al, K, Ca, Mg, Na, Fe, P, S, Si, Zn and Mn, were relatively high in all spiked spice sample. Using the 7900 ICP-MS and 5110 ICP-OES direct analysis methods, excellent spike recoveries were achieved for most elements in the spiked samples. All recoveries were within ±20% as shown in Table 4. The spike results for the elements listed above were invalid as the spike levels were much too low (20 times lower) relative to the levels present in the unspiked samples, therefore NA.

Table 4. Quantitative and spike recovery results for 4 spice samples.

Element (mass and wavelength)	White Pepper Sample 58	White Pepper Sample 45	Paprika Sample 24	Basil Sample 50	ICP-MS		ICP-OES	
	Measured conc, n=3, ppb				Mean Recovery* ± 1σ	QC Criteria (80–120%) **	Mean Recovery* ± 1σ	QC Criteria (80–120%) **
^{109}Ag (328.068 nm)	2.5	6.0	5.3	11.5	106 ± 7%	Pass	107 ± 8%	Pass
Al (396.152 nm)	12863.3	35208.9	1022222.2	1290444.4	NA	NA	NA	NA
^{75}As (188.980 nm)	62.5	7.3	114.7	298.8	104 ± 2%	Pass	116 ± 10%	Pass
^{11}B (249.772 nm)	21288.9	4264.0	38800.0	18338.9	92 ± 235%	Pass	84 ± 46%	Pass
^{137}Ba (614.171 nm)	9706.9	5563.3	32433.3	31657.8	111 ± 57%	Pass	82 ± 26%	Pass
^{9}Be (313.042 nm)	1.1	2.5	34.0	19.7	100 ± 4%	Pass	109 ± 3%	Pass
Ca (317.933 nm)	4074444.4	2095333.3	25833333.3	7244444.4	NA	NA	NA	NA
^{111}Cd (214.439 nm)	451.5	43.1	31.5	37.6	100 ± 2%	Pass	105 ± 3%	Pass
^{140}Ce	14.6	52.6	1104.9	722.7	115 ± 35%	Pass		
^{59}Co (238.892 nm)	586.7	41.8	786.6	44.5	105 ± 4%	Pass	95 ± 16%	Pass
^{52}Cr (267.716 nm)	207.5	449.4	1639.6	3824.7	111 ± 14%	Pass	110 ± 9%	Pass
^{133}Cs	6.0	199.3	36.5	209.0	103 ± 1%	Pass		
^{63}Cu (327.395 nm)	9460.7	9315.1	28900.0	14090.0	119 ± 55%	Pass	99 ± 22%	Pass
^{163}Dy	1.0	4.0	90.9	42.4	112 ± 23%	Pass		
^{166}Er	0.5	1.7	48.4	21.8	114 ± 24%	Pass		
^{153}Eu	0.3	1.9	27.3	8.6	116 ± 24%	Pass		
^{56}Fe (238.204 nm)	178888.9	42313.3	1104444.4	650133.3	NA	NA	NA	NA
^{71}Ga (294.363 nm)	4.6	13.6	277.5	324.9	107 ± 3%	Pass	110 ± 7%	Pass
^{157}Gd	1.2	5.1	107.4	47.6	112 ± 23%	Pass		
Ge (209.426 nm)	332.4	4335.4	0.0	4486.2	NA	NA	NA	NA
^{178}Hf	0.5	1.2	42.9	16.9	84 ± 17%	Pass		
^{202}Hg	0.0	5.8	0.0	21.2	106 ± 2%	Pass		
^{165}Ho	0.2	0.7	18.1	8.0	114 ± 23%	Pass		
^{191}Ir (224.268 nm)	1083.6	299.4	382.0	213.8	101 ± 2%	Pass	108 ± 19%	Pass
K (766.491 nm)	36576666.7	412522.2	35601111.1	4483111.1	NA	NA	NA	NA
^{139}La	9.2	45.8	511.4	468.8	111 ± 27%	Pass		
^{7}Li (670.783 nm)	3658.3	1027.7	902.3	1206.2	106 ± 4%	Pass	117 ± 17%	Pass
^{175}Lu	0.0	0.6	7.2	1.9	114 ± 24%	Pass		
Mg (280.270 nm)	2694888.9	841077.8	7852000.0	1583555.6	NA	NA	NA	NA
55 Mn	13724.4	46072.2	89086.7	98117.8	NA	NA	NA	NA
^{95}Mo (202.032 nm)	309.8	396.3	1072.1	927.6	99 ± 2%	Pass	105 ± 4%	Pass
Na (589.592 nm)	1044666.7	15156.7	694644.4	149900.0	NA	NA	NA	NA
^{93}Nb	2.6	6.3	106.4	58.3	92 ± 9%	Pass		
^{146}Nd	7.2	27.9	578.4	299.9	105 ± 25%	Pass		
^{60}Ni	843.7	374.6	1646.4	1259.2	108 ± 11%	Pass	110 ± 9%	Pass
P (213.618 nm)	4000000.0	1757777.8	4879222.2	1117333.3	NA	NA	NA	NA
^{208}Pb (220.353 nm)	16.2	74.5	506.8	751.0	106 ± 3%	Pass	95 ± 21%	Pass
^{105}Pd	0.7	0.8	6.3	0.9	97 ± 3%	Pass	96 ± 9%	Pass
^{141}Pr	1.9	7.6	132.7	85.4	108 ± 23%	Pass		
^{85}Rb	6738.0	34775.8	15829.5	20362.2	94 ± 35%	Pass		
^{185}Re	0.7	0.0	5.0	0.2	108 ± 3%	Pass		
^{103}Rh	4.9	0.0	0.5	1.7	100 ± 3%	Pass		
S (180.669 nm)	2346777.8	842411.1	3976222.2	1065888.9	NA	NA	NA	NA
^{121}Sb (206.834 nm)	2.9	2.6	17.4	55.7	95 ± 4%	Pass	102 ± 19%	Pass
^{78}Se	303.1	14.9	47.2	83.1	106 ± 4%	Pass		
^{28}Si	54901.9	162327.7	348062.1	324615.7	NA	NA	NA	NA
^{147}Sm	1.5	5.0	120.5	55.9	102 ± 22%	Pass		
^{118}Sn (189.925 nm)	18.3	2099.0	57.6	189.0	98 ± 2%	Pass	104 ± 6%	Pass
^{88}Sr	36350.0	14511.1	214044.4	60907.8	108 ± 68%	Pass		
^{232}Th	1.8	19.1	93.8	509.7	106 ± 27%	Pass		
^{47}Ti	393.7	1603.9	59519.9	12881.6	104 ± 3%	Pass		
^{205}Tl	0.0	5.2	10.9	11.2	107 ± 2%	Pass		
^{169}Tm	0.1	0.2	6.6	2.9	115 ± 24%	Pass		
^{238}U	22.1	3807.2	37.4	0.0	107 ± 3%	Pass		
^{51}V	104.0	21.6	2723.8	852.8	114 ± 23%	Pass		
^{182}W	9.5	1.7	5.9	31.1	96 ± 10%	Pass	102 ± 10%	Pass
^{172}Yb	0.5	1.1	39.2	18.6	111 ± 23%	Pass		
^{66}Zn	21503.0	22931.7	39538.3	18788.4	NA	NA	NA	NA
^{90}Zr	16.2	29.9	1375.1	455.7	94 ± 13%	Pass	107 ± 12%	Pass

*n=3, replicate sample digestion, each in triplicate, 4 separate spice samples. **QC Criteria FDA EAM 4.7 (80–120%) for NIST certified values. NA indicates that spike levels were too low (<5%) relative to the unspiked concentration.

Results and Discussion

Using PCA (Fig. 1-4), an un-supervised technique that displays samples based on similarities and differences in the measured variables, spice samples from different countries were separated fairly well based on country of origin and spice type .

Figure 1

- Overall significant differences between the elemental composition of the 13 different countries were found.
- 61 variables were found to discriminate significantly between the countries, and were subsequently used in the PCA (Figure 1).
- Along PCA 1, explaining 45.8% of the discrimination, Rb, Cs, Ge, Cd, Se, K, and P were among the most discriminating elements.
- Along PCA 2, explaining additional 9.6% of the variability, the REEs, were among the most discriminating elements.

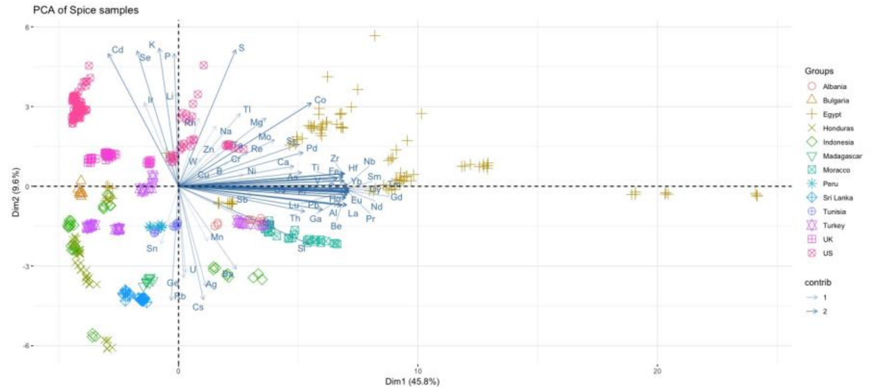

Figure 1. PCA of Spice samples based on country of origin.

Figure 2

- Differences between the elemental composition of the 21 different spices were found, shown in Figure 2.

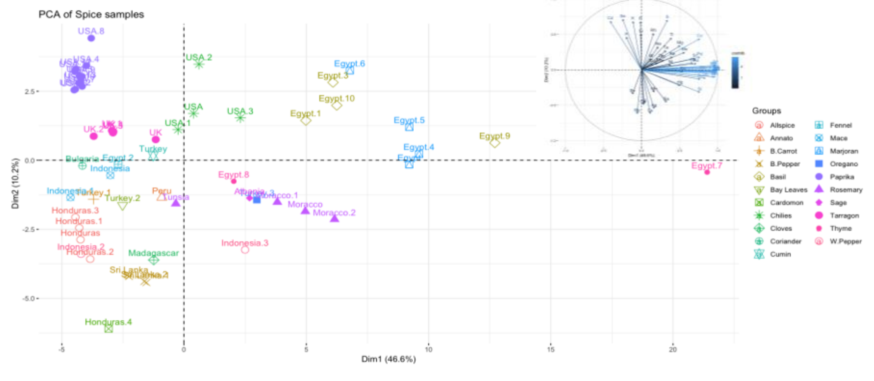

Figure 2. PCA of Spice samples based on spice type, but labeled with country of origin.

Figure 3

- PCA shows the elemental composition of different spices within one country. Separation is seen between 4 spices from Egypt.

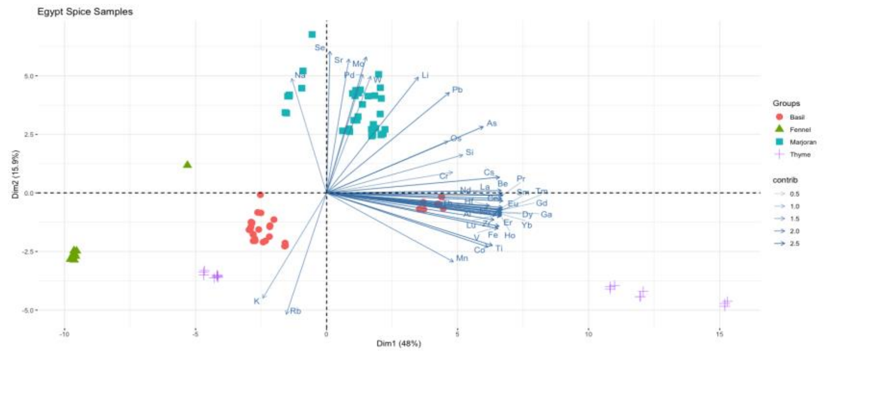

Figure 3. PCA of different spice samples (basil, fennel, marjoram, and thyme) all from Egypt.

Another question we wanted to address in this study was to see if we could see a difference between the same spice type, from different countries. Figure 4 shows a PCA of rosemary from Morocco and Tunisia. Clear separation was observed.

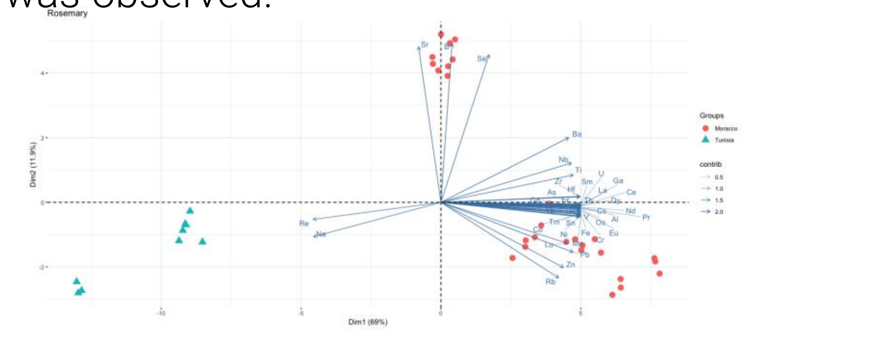

Figure 4. PCA of Rosemary from 2 different countries (Morocco and Tunisia).

Conclusions

- ICP-MS and ICP-OES can be used for the quantitative analysis of multiple elements in spice samples following acid digestion. The microwave-assisted sample preparation method resulted in excellent recovery results.
- Commercial spices from 13 different countries show discrimination between elemental fingerprints.
- While spices from different countries of origin were found to show a characteristic elemental pattern, further studies are needed to study this question, including a close monitoring of different spices from one country as well as the same spice from different countries.
- Additional samples are needed to do additional studies. Obtaining spices with known origin is challenging.