

Authenticating Rice by Elemental Profiling Using ICP-MS and Statistical Modeling

Identifying the geographical origin of rice using Agilent 7900 ICP-MS and Agilent Mass Profiler Professional software



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Introduction

China is the world's largest producer of rice, which is a vital staple food for almost half of the world's population. Given the huge global demand for rice, varieties with higher value are a target for food fraudsters, who routinely adulterate or mislabel expensive food items for financial gain. The price of rice in China depends greatly on where the plants are grown, so producers use Geographical Indication (GI) to differentiate their products in the marketplace. Rice that is promoted based on its region of origin is more vulnerable to adulteration whenever demand for the product is higher than the amount that can be produced. Methods of food fraud include partial or total substitution of authentic product with lower grade product and inaccurate product labeling. Determining the geographical origin of rice is important for farmers, retailers, and consumers, as each group can be impacted financially by food fraud (1). Verifying food origin and authenticity is also crucial for food safety and regulatory compliance. If food suppliers, manufacturers, or retailers are found supplying contaminated or incorrectly labeled products, they may be fined or prosecuted.

The elemental composition of food crops and processed foods varies with respect to plant variety, soil type, environmental factors, and agricultural practices including irrigation and use of fertilizers. The analysis of a wide range of elements in foods is often performed using ICP-MS, following microwave-assisted acid digestion of samples (2–4). The results provide an “elemental fingerprint” of each sample that is representative of the sample’s geographic origin. This information is useful for authentication studies and has been applied to foods including tea and spices (5, 6).

Agilent ICP-MS systems include the Octopole Reaction System (ORS⁴) collision/reaction cell (CRC) that is optimized for removal of all common polyatomic overlaps using helium (He) collision mode. Helium mode allows the same cell settings to be used for many elements, providing high-quality data sets needed for food-authenticity studies. Agilent ICP-MS data is compatible with Agilent Mass Profiler Professional (MPP), a chemometrics software package that is designed to process large sets of MS data. MPP includes a choice of data classification methods, enabling analysts to build robust models to predict attributes (such as sample origin) of unknown samples.

In this study, trace-element data for 90 rice samples of known geographical origins was acquired using an Agilent 7900 ICP-MS. The large sample data set was then processed using MPP software. Once it was determined that the origin of each sample could be differentiated using Principal Component Analysis (PCA), the MPP software was used to build prediction models using different class prediction algorithms. The prediction models were then used to identify the origins of 24 ‘unknown’ test samples.

Experimental

Instrumentation

An Agilent 7900 ICP-MS equipped with an Agilent SPS 4 autosampler was used for all measurements. The 7900 includes a glass concentric nebulizer, quartz double-pass spray chamber, 2.5 mm injector quartz torch, Ni interface cones, and ORS⁴ cell. The instrument was controlled using Agilent ICP-MS MassHunter software, and data analysis was performed using MPP software. The functions described are available in MPP version 15.0 or later. The typical instrument operating parameters are given in Table 1.

Table 1. Agilent 7900 ICP-MS operating conditions.

Parameter	Setting
RF Power (W)	1550
Sampling Depth (mm)	8
Nebulizer Gas (L/min)	1.16
Lens Tune	Autotune
He Flow Rate (mL/min)	5
KED (V)	5

Calibration standards

Calibration standards for 24 elements were prepared from Agilent standard solutions using 5% (v/v) nitric acid (HNO₃) solution for dilution. The standards included: multi-element calibration standard-2A (part number 8500-6940), multi-element calibration standard-4 (p/n 8500-6942), and environmental calibration standard (p/n 5183-4688). The rhodium (Rh) internal standard (ISTD) solution was prepared from a single element Rh standard (p/n 5190-8509). The calibration standards, except mercury (Hg), were premixed no longer than 24 hours before analysis. Hg was added into the mixture just before use.

Reagents, reference material, and samples

Nitric acid (69%) was bought from Merck Millipore (Darmstadt, Germany). De-ionized water (DIW, 18.2 MΩ·cm) was obtained from a Milli-Q system (Millipore, MA, USA).

A Rice Flour Standard Reference Material (SRM) 1568b was bought from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). 90 rice samples of known origins (GI designated products) were collected directly from rice processing factories in five provinces in China: 15 samples from each province, except for 30 samples of two varieties collected from Liaoning. The locations included: Wuchang, Heilongjiang; Panjin, Liaoning (two lots of sample of different types); Sheyang, Jiangsu; Jingshan, Hubei; and Guigang, Guangxi. The samples were labeled as HLJ, LN-1, LN-2, JS, HB, and GX, respectively.

Sample preparation

All lab materials including digestion vessels were soaked in a 30% (v/v) HNO₃ solution for 24 h and rinsed three times with DIW before use. Approximately 0.5 g of rice sample was weighed into a polytetrafluoroethylene (PTFE) digestion vessel and mixed with 6 mL HNO₃. The sample was predigested by placing the vessel in a fume hood overnight. It was then transferred into the microwave oven (Anton Paar, Austria) for acid digestion using the heating program shown in Table 2.

After digestion, the solution was cooled to room temperature and diluted to 50 mL with DIW. The NIST-1568b SRM was analyzed with every batch of rice samples to verify the digestion method.

Table 2. Typical microwave digestion parameters.

Stage	Time (min)	Temperature (°C)
Ramp	15	Ambient to 180
Hold	20	180
Cool	Approx. 45	180 to ambient

Results and discussion

Detection limits (DLs) and background equivalent concentrations (BECs)

The typical 7900 ICP-MS DLs and BECs calculated from the ICP-MS MassHunter calibrations for the 24 elements are shown in Table 3.

Table 3. DLs and BECs of the 24 elements measured in this study.

Element	DL (µg/L)	BEC (µg/L)	Element	DL (µg/L)	BEC (µg/L)
11 B	0.33	0.064	60 Ni	0.012	0.047
23 Na	0.091	1.03	63 Cu	0.0011	0.016
24 Mg	0.092	0.075	66 Zn	0.018	0.18
27 Al	0.37	0.47	75 As	0.0020	0.0020
39 K	0.39	11.90	78 Se	0.048	0.019
44 Ca	0.030	0.137	85 Rb	0.00001	0.0009
47 Ti	0.0064	0.0021	88 Sr	0.0015	0.0006
51 V	0.0015	0.0007	95 Mo	0.0011	0.0019
52 Cr	0.0021	0.014	111 Cd	0.0039	0.0049
55 Mn	0.0037	0.0042	137 Ba	0.0033	0.0069
56 Fe	0.022	0.13	201 Hg	0.0001	0.0009
59 Co	0.0005	0.0062	208 Pb	0.0097	0.12

SRM recoveries

The NIST-1568b rice flour SRM was analyzed two or three times during each batch run to verify the accuracy and precision of the method. The results from four separate batch runs are shown in Table 4. The mean concentrations were in good agreement with the certified concentrations (80–120% recoveries) for all elements apart from Hg. The low recovery for Hg was probably due to the instability of the low concentration of Hg in 5–8% HNO₃ (7).

Hg recovery would likely be improved significantly by adding HCl to the acid mix used for sample digestion and stabilization. HCl ensures that chemically unstable elements such as Hg are retained in solution. Any Cl-based polyatomic overlaps formed are easily controlled on Agilent ICP-MS systems using the standard He cell mode (8).

Table 4. Recovery and reproducibility data for certified elements in the NIST-1568b rice flour SRM, n=10.

Element	Certified Concentration, µg/g	Mean Measured Concentration, µg/g	Standard Deviation, µg/g	Mean Recovery, %
24 Mg	559 ± 10	540	27	97
27 Al	4.21 ± 0.34	4.11	0.15	98
39 K	1282 ± 11	1276	62	100
44 Ca	118.4 ± 3.1	114.3	8.3	97
55 Mn	19.2 ± 1.8	18.3	1.0	95
56 Fe	7.42 ± 0.44	7.30	0.30	98
59 Co*	0.0177 ± 0.0005	0.0170	0.0010	96
63 Cu	2.35 ± 0.16	2.29	0.15	98
66 Zn	19.42 ± 0.26	17.73	1.81	91
75 As	0.285 ± 0.014	0.269	0.020	94
78 Se	0.365 ± 0.029	0.351	0.026	96
85 Rb	6.198 ± 0.026	5.352	0.211	86
95 Mo	1.451 ± 0.048	1.405	0.110	97
111 Cd	0.0224 ± 0.0013	0.0197	0.0022	88
201 Hg	0.00591 ± 0.00036	0.00395	0.00127	67
208 Pb*	0.008 ± 0.003	0.009	0.001	116

*Reference value: not fully verified value but can be used interchangeably with certified value.

ISTD stability

Rh was used as the ISTD because it was not expected to be present in the rice samples, unlike other potential ISTD elements such as Sc and Ga. Rh standard solution (1 mg/L) was mixed with the sample using a tee connector. The analytical sequence comprising half of all the rice samples was analyzed repeatedly over 10 hours. All the ISTD recoveries were within the $\pm 20\%$ limit, as indicated by the red dotted lines shown in Figure 1. The results demonstrated that the 7900 ICP-MS has the robustness and high matrix tolerance for authenticity studies that require long-term runs of food digests.



Figure 1. ISTD recovery of sample analysis over 10 hours using the Agilent 7900 ICP-MS.

Data mining using MPP software

The multi-element data batch files (90 rice samples, 24 elements) were combined and imported into MPP chemometric software for statistical analysis. The software includes various tests such as t-test, analysis of variance (ANOVA), model building algorithms, correlation and clustering analysis, as well as extended capabilities for R algorithms. PCA (which is an unsupervised technique) was performed to see if there were significant differences between sample groups. The PCA technique evaluates the relative contribution of compounds to the separation of the groups. The three dimensional (3D)-PCA scores are shown Figure 2.

A total of 65% of the variance ratio was captured in the first three dimensions, PC1, PC2, and PC3 (Figure 2). A reasonable degree of separation of the 90 rice samples from five locations was achieved using 24 elements. The samples from HB, GX, and LN-1 were clearly separated from the other samples, while there was some overlap between samples from LN-2 and HLJ. According to the PCA loading plot generated from the MPP software, the differences were mainly driven by B, Na, Zn, and Cd for PC1, Al, Fe, Mn, and Sr for PC2, and Na, Cu, Se, and Rb for PC3. The results demonstrated the feasibility of using element concentration data to build a location prediction model, which could predict the geographical origin of unknown rice samples.

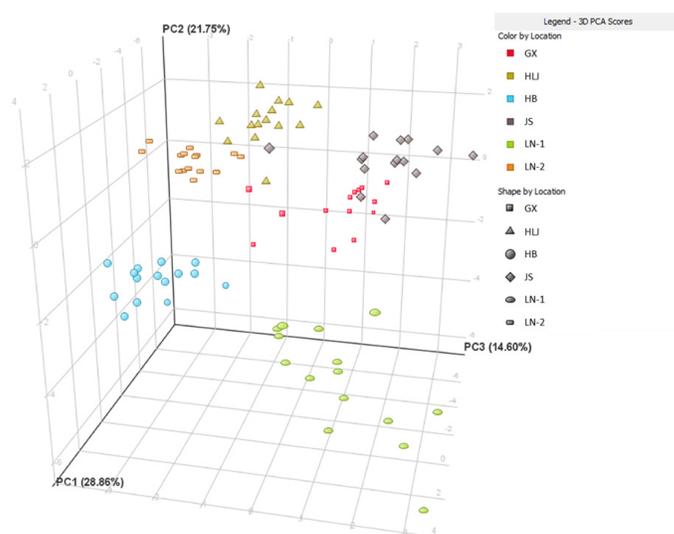


Figure 2. 3D-PCA scores of the 90 rice samples from five different locations (including two different varieties from Liaoning). The axes of the plot represent the top three components of the PCA results.

Class prediction analysis

Class prediction analysis is a useful technique that enables new, unknown samples to be assigned to a previously determined group in an unbiased fashion. It is becoming an increasingly valuable tool in quality control of complex samples, such as wine and beer (9).

MPP includes several class prediction algorithms (Figure 3). The choice of algorithms includes soft independent modeling of class analogy (SIMCA), random forest, linear discriminant analysis (LDA), partial least squares discriminant analysis (PLSDA), decision tree, support vector machine (SVM), naive bayes, and neural network. A classification model can be built using any of these class prediction algorithms.

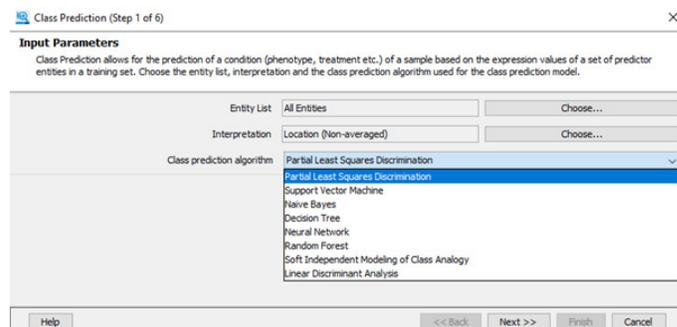


Figure 3. Agilent MPP software screen showing options for class prediction algorithms.

Building a prediction model in MPP

Eleven of the 15 rice samples from each of the five locations (two sets from Liaoning) were selected randomly to build prediction models using four different class prediction algorithms (PLSDA, SVM, LDA, and SIMCA). The remaining four rice samples from each location (24 samples in total) were used as 'unknowns' to test the accuracy of the prediction models. To build a prediction model in MPP, the analyst simply follows a six-step automated workflow, with the software automatically skipping any unnecessary steps.

Testing the prediction model

The predicted location results obtained using the PLSDA, SVM, and LDA models are shown in Table 5. The locations of all 24 samples were correctly identified using all three prediction models. A higher value (darker shading) in Table 5 indicates greater confidence in a correct match.

Table 5. Summary of the predicted location results for the 24 'unknown' rice samples.

Sample ID	Actual Location	Prediction Model					
		PLSDA		SVM		LDA	
		Predicted Location	Confidence Measure	Predicted Location	Confidence Measure	Predicted Location	Confidence Measure
GX-101	Guigang, Guangxi	Guigang, Guangxi	0.762	Guigang, Guangxi	0.742	Guigang, Guangxi	0.776
GX-102	Guigang, Guangxi	Guigang, Guangxi	0.476	Guigang, Guangxi	0.758	Guigang, Guangxi	0.658
GX-103	Guigang, Guangxi	Guigang, Guangxi	0.606	Guigang, Guangxi	0.804	Guigang, Guangxi	0.830
GX-104	Guigang, Guangxi	Guigang, Guangxi	0.769	Guigang, Guangxi	0.769	Guigang, Guangxi	0.865
HB-101	Jingshan, Hubei	Jingshan, Hubei	0.632	Jingshan, Hubei	0.774	Jingshan, Hubei	0.667
HB-102	Jingshan, Hubei	Jingshan, Hubei	0.739	Jingshan, Hubei	0.797	Jingshan, Hubei	0.760
HB-103	Jingshan, Hubei	Jingshan, Hubei	0.770	Jingshan, Hubei	0.727	Jingshan, Hubei	0.811
HB-104	Jingshan, Hubei	Jingshan, Hubei	0.531	Jingshan, Hubei	0.757	Jingshan, Hubei	0.850
HLJ-101	Wuchang, Heilongjiang	Wuchang, Heilongjiang	0.423	Wuchang, Heilongjiang	0.415	Wuchang, Heilongjiang	0.733
HLJ-102	Wuchang, Heilongjiang	Wuchang, Heilongjiang	0.651	Wuchang, Heilongjiang	0.731	Wuchang, Heilongjiang	0.901
HLJ-103	Wuchang, Heilongjiang	Wuchang, Heilongjiang	0.659	Wuchang, Heilongjiang	0.777	Wuchang, Heilongjiang	0.873
HLJ-104	Wuchang, Heilongjiang	Wuchang, Heilongjiang	0.635	Wuchang, Heilongjiang	0.724	Wuchang, Heilongjiang	0.802
JS-101	Sheyang, Jiangsu	Sheyang, Jiangsu	0.637	Sheyang, Jiangsu	0.766	Sheyang, Jiangsu	0.834
JS-102	Sheyang, Jiangsu	Sheyang, Jiangsu	0.494	Sheyang, Jiangsu	0.625	Sheyang, Jiangsu	0.866
JS-103	Sheyang, Jiangsu	Sheyang, Jiangsu	0.526	Sheyang, Jiangsu	0.735	Sheyang, Jiangsu	0.918
JS-104	Sheyang, Jiangsu	Sheyang, Jiangsu	0.562	Sheyang, Jiangsu	0.824	Sheyang, Jiangsu	0.914
LN1-101	Panjin, Liaoning-1	Panjin, Liaoning-1	0.576	Panjin, Liaoning-1	0.711	Panjin, Liaoning-1	0.881
LN1-102	Panjin, Liaoning-1	Panjin, Liaoning-1	0.738	Panjin, Liaoning-1	0.743	Panjin, Liaoning-1	0.893
LN1-103	Panjin, Liaoning-1	Panjin, Liaoning-1	0.546	Panjin, Liaoning-1	0.744	Panjin, Liaoning-1	0.450
LN1-104	Panjin, Liaoning-1	Panjin, Liaoning-1	0.594	Panjin, Liaoning-1	0.816	Panjin, Liaoning-1	0.838
LN2-101	Panjin, Liaoning-2	Panjin, Liaoning-2	0.769	Panjin, Liaoning-2	0.717	Panjin, Liaoning-2	0.832
LN2-102	Panjin, Liaoning-2	Panjin, Liaoning-2	0.593	Panjin, Liaoning-2	0.731	Panjin, Liaoning-2	0.774
LN2-103	Panjin, Liaoning-2	Panjin, Liaoning-2	0.763	Panjin, Liaoning-2	0.734	Panjin, Liaoning-2	0.863
LN2-104	Panjin, Liaoning-2	Panjin, Liaoning-2	0.535	Panjin, Liaoning-2	0.727	Panjin, Liaoning-2	0.745

The results for the same test samples obtained using the SIMCA model are shown in Table 6. The SIMCA model reports the results using 'distance', where the lower the value, the more likely the sample belongs to the classified group. The SIMCA model correctly predicted all but one rice sample (HLJ-101). HLJ-101, which was predicted to be most likely from the LN-2 group, also had low confidence values in the PLSDA and SVM models (Table 5). The PCA results suggested some similarities in the elemental profiles of HLJ and LN-2 (Figure 2).

Table 6. The location results of the 24 'unknown' rice samples predicted by the SIMCA model. Green: correct prediction; Amber: incorrect prediction; Red: actual location.

Sample ID	Actual Location	Prediction Algorithm					
		SIMCA					
		Distance (Guigang, Guangxi)	Distance (Wuchang, Heilongjiang)	Distance (Jingshan, Hubei)	Distance (Sheyang, Jiangsu)	Distance (Panjin, Liaoning-1)	Distance (Panjin, Liaoning-2)
GX-101	Guigang, Guangxi	0.857	8.050	2.901	8.363	7.635	3.254
GX-102	Guigang, Guangxi	0.203	9.422	4.627	7.672	8.797	4.865
GX-103	Guigang, Guangxi	0.221	11.803	2.816	8.288	8.953	3.522
GX-104	Guigang, Guangxi	0.195	8.879	3.965	7.573	8.219	3.801
HB-101	Jingshan, Hubei	3.759	11.049	0.255	9.807	6.379	4.664
HB-102	Jingshan, Hubei	5.279	10.986	0.527	10.328	5.746	5.411
HB-103	Jingshan, Hubei	5.179	10.519	0.343	9.809	5.585	5.048
HB-104	Jingshan, Hubei	4.126	10.093	0.367	9.026	5.288	4.650
HLJ-101	Wuchang, Heilongjiang	4.806	3.262	4.684	4.202	3.043	1.841
HLJ-102	Wuchang, Heilongjiang	7.328	0.370	6.634	2.685	3.246	1.511
HLJ-103	Wuchang, Heilongjiang	8.362	0.407	7.177	2.515	3.702	1.475
HLJ-104	Wuchang, Heilongjiang	8.740	0.630	7.133	2.351	3.012	1.895
JS-101	Sheyang, Jiangsu	14.174	6.839	16.351	0.141	2.901	4.514
JS-102	Sheyang, Jiangsu	12.018	6.895	14.477	0.647	2.830	4.234
JS-103	Sheyang, Jiangsu	12.831	6.750	16.327	0.292	3.003	2.786
JS-104	Sheyang, Jiangsu	12.003	7.809	17.050	0.146	4.089	3.750
LN1-101	Panjin, Liaoning-1	15.744	10.073	10.605	3.314	0.141	5.148
LN1-102	Panjin, Liaoning-1	18.586	8.118	12.548	3.465	0.302	6.626
LN1-103	Panjin, Liaoning-1	24.607	12.810	22.225	3.157	0.892	7.842
LN1-104	Panjin, Liaoning-1	17.242	12.822	12.874	5.091	0.193	6.848
LN2-101	Panjin, Liaoning-2	11.641	2.939	8.208	2.757	2.114	0.400
LN2-102	Panjin, Liaoning-2	11.823	2.944	9.358	3.014	2.573	0.266
LN2-103	Panjin, Liaoning-2	8.010	2.334	8.650	2.498	3.692	0.379
LN2-104	Panjin, Liaoning-2	9.582	3.681	9.425	2.805	3.444	0.570

Conclusion

The Agilent 7900 ICP-MS with the ORS⁴ cell operating in helium collision mode was shown to produce data with high quality, robustness, and stability, which are essential for food authenticity studies. Using a single cell gas mode for all analytes enabled the detection of 24 elements in 90 rice sample digests. Data from 66 of the 90 rice samples was used to build prediction models for the characterization of the geographical origins of the remaining 24 samples.

Agilent MPP statistical software was used to process the large data set. Data analysis using PCA showed that the elemental composition of rice was influenced by geographical origins, allowing fairly good discrimination between five geographical areas.

Four prediction models were built by following the simple steps in the MPP software. The models were trained using the ICP-MS data and tested using 'unknown' samples. The study showed that it was possible to correctly predict the origins of all the 24 'unknown' samples using multiple prediction models, rather than a single prediction model. The multiple-model approach could lead to more accurate prediction results and better understanding of the element profiles of samples.

The elemental profiling method described in this study has the potential to characterize the geographical origin of rice, and other high value foodstuffs enabling routine authenticity analysis of foods.

References

1. Dion M.A.M. Luykx, Saskia M. van Ruth, An overview of analytical methods for determining the geographical origin of food products, *Food Chemistry*, **2008**, 107, 897–911
2. Jenny Nelson, Elaine Hasty, Leanne Anderson, Macy Harris, Determination of Critical Elements in Foods in Accordance with US FDA EAM 4.7 ICP-MS Method, Agilent publication, [5994-2839EN](#)
3. Shuofei Dong, Jenny Nelson, Michiko Yamanaka, Routine Analysis of Fortified Foods using the Agilent 7800 ICP-MS, Agilent publication, [5994-0842EN](#)
4. Kazuhiro Sakai, Junichi Takahashi and Ed McCurdy, Application of the Agilent 7900 ICP-MS with Method Automation function for the routine determination of trace metallic components in food CRMs, Agilent publication, [5991-4556EN](#)
5. Jenny Nelson, Helene Hopfer, Authentication of Specialty Teas: An Application Note. *Food Qual. Safety*, **2018**, December, accessed February 2021, <https://www.foodqualityandsafety.com/article/specialty-tea-authentication/>
6. Jenny Nelson et al., Determining the metal content of the spices and identifying the country of origin, *Food Qual. Safety*, **2019**, May, accessed February 2021, <https://www.foodqualityandsafety.com/article/authentic-spices-identifying-country-origin/>
7. U.S. EPA. 2014. Method 6020B (SW-846): Inductively Coupled Plasma-Mass Spectrometry, Revision 2. Washington, DC. <https://www.epa.gov/sites/production/files/2015-12/documents/6020b.pdf>
8. Successful Low Level Mercury Analysis using Agilent ICP-MS, Agilent publication, [5990-7173EN](#)
9. Spiros A. Drivelos, Constantinos A Georgiou, Multi-element and multi-isotope-ratio analysis to determine the geographical origin of foods in the European Union, *Trends Anal. Chem*, **2012**, 40, 38–51

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Printed in the USA, September 6, 2021
5994-4043EN