Application Note

Foods

Black Pepper Origin Differentiation Using Large ICP-MS Datasets and Chemometric Tools

Elemental profiling using an Agilent 7850 ICP-MS and Agilent Mass Profiler Professional (MPP) software

Authors

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Introduction

Spice usage dates from the earliest known civilisations to the present day. Among all the spices, black pepper (*Piper nigrum* L.) is one of the most widely consumed in foods worldwide, because of its intense flavor and taste-enhancement properties. It has also been used as a preservative, insecticide, and in perfumery and beauty care, as well as in pharmaceuticals, especially in folk medicine due to its rich bioactive properties.[1] The constituents of black pepper include alkaloids, terpenes, flavones, steroids, lignans, and alkamides with the alkaloid piperine, volatile oils, and oleoresin being the major constituents contributing to its unique characteristic aroma and taste. However, these traits can be influenced by the geographical origin of pepper plants, due to factors such as soil composition, climate, and weather conditions, as well as agricultural and handling practices.[2]

Black pepper's high value and global demand are drivers for economically motivated adulteration, a fraudulent practice that can pose public health risks. Because black pepper is most often sold in ground, crushed, or mixed forms, its supply chain is difficult to monitor and manage. Also, consumer campaigns against deforestation and the European Union Regulation on Deforestation-free (EUDR) products have increased calls for greater sourcing transparency. Products with a known geographical indication can demand a higher price. Companies do not want to become implicated in false claims about the sourcing of products, and many are keen to have full visibility of their products' origin. To address this need, the European Commission has established the Protected Geographical Indication (PGI) system to protect regional specific products, thereby promoting their unique characteristics based on geographical origin and traditional competence of production. The PGI scheme has increased the demand for detailed information regarding the geographical source of food products, a trend that is likely to continue as EUDR is implemented across the EU and associated markets. Verifying the geographical origin of black pepper using elemental fingerprints is highly relevant to protecting value chain stakeholders, ensuring both quality and traceability while upholding the principles of food integrity.

Several analytical methods can be used for profiling certain elements in foods. However, inductively coupled plasma mass spectrometry (ICP-MS) is unique because of its rapid throughput, multi-element capability, ultratrace sensitivity, isotopic analysis, versatility across sample types, low sample volume requirements, compliance with regulatory requirements, and low cost.[3]

The Agilent 7850 ICP-MS is equipped with an Octopole Reaction System (ORS[4]), a collision/reaction cell (CRC) that uses helium (He) gas and Kinetic Energy Discrimination (KED) to remove polyatomic interferences on key analytes.[4] If left unchecked, these interferences can affect data accuracy in complex food products. The integration of the 7850 with Agilent ICP-MS MassHunter software streamlines data analysis. When integrated with chemometric analysis, ICP-MS demonstrates high precision in multi-elemental profiling of diverse food matrices, effectively distinguishing their geographical origin.[5]

In this study, 150 black pepper samples from five countries (Brazil, Cambodia, India, Indonesia, Vietnam) were analyzed using the 7850 ICP-MS to obtain elemental fingerprints of the samples. Agilent Mass Profiler Professional (MPP) software was used to process the ICP-MS elemental data and to construct chemometric models for distinguishing the geographical origin of the samples, as adapted from Chilaka et al.[5]

Experimental

Instrumentation

An Agilent 7850 single-quadrupole ICP-MS was used for the analysis of 38 elements in the black pepper samples. Sample introduction was performed via a peristaltic pump connected to an Agilent MicroMist nebulizer and an Agilent SPS 4 autosampler. All elements were measured using helium collision (He KED) mode, except for boron (B), which was analyzed in no gas mode. Instrument control was performed using Agilent ICP-MS MassHunter 5.1 software, and data processing was conducted using Agilent MPP software, version 15.1. Instrument operating conditions as shown in Table 1.

Table 1. Agilent 7850 ICP-MS operating conditions.

ICP-MS Parameters	No Gas Mode	Helium Mode
Plasma Mode	General purpose	
RF Power (W)	1550	
Sampling Depth (mm)	10	
Nebulizer Gas Flow (L/min)	1.07	
Spray Chamber Temperature (°C)	2.0	
Dilution Gas Flow Rate (mL/min)	0.0	
Lens Tune	Autotune	
Energy Discrimination (V)	5	
Cell Gas Flow Rate (L/min)	0.0	5.0
Number of Elements	1	37

Standard preparation

The instrument was calibrated using Agilent standards including multi-element calibration standard-1 (part number 8500-6944), 2A (p/n 8500-6940), 3 (p/n 8500-6948), 4 (p/n 8500-6942), environmental calibration standard (p/n 5183-4688), indium standard (p/n 8500-6946), and bismuth standard (p/n 8500-6936). Multi-element calibration standard mixes were prepared over a 13-point range with a maximum point of 400 ppb for calibration standards 1, 2A, 3, and 4, while the environmental calibration standard mix was prepared over an 11-point range with a maximum point of 200,000 ppb. In total, 38 elements (B, Na, Mg, Al, P, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Rb, Sr, Y, Cd, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Pb) were used for authentication of the geographical origin of the black pepper samples. Bismuth (Bi) and indium (In) were used as internal standards (ISTDs) at a concentration of 100 ppb.

The ISTDs were automatically mixed with the sample online using a T-connector. A 2% nitric acid (HNO_3) and 0.5% hydrochloric acid (HCl) solution was used to prepare all calibration standards. It was also used as the calibration blank, which was analyzed at the start of each sample batch.

Sample preparation

Thirty black pepper samples were sourced from trusted suppliers in each of five major black pepper producing countries: Brazil, Cambodia, India, Indonesia, and Vietnam, giving a total of 150 samples. Each sample was milled to a powder using a Planetary Ball Mill PM 100 (Retsch, Germany), homogenized, and stored in a resealable polyethylene bag to prevent moisture absorption and contamination. Before sample preparation and analysis, samples were stored in black, light-proof storage boxes in a dry environment at room temperature.

For extraction, 100 ± 0.5 mg of each ground black pepper sample or black pepper reference material (RM) (TBK001RM, Fapas, York, UK) was weighed in triplicate into a 50 mL metal-free polypropylene conical tube. To facilitate sample digestion, 2 mL of HNO_3 (67–69%) was added to each sample and the samples were left in the fume hood overnight. Then, 2 mL of 30% hydrogen peroxide (H_2O_2) was added to each sample.

The samples were microwave digested with a Mars 6 system (CEM, Matthews, NC, USA) using a 65 min digestion protocol. Samples were gradually heated to 95 °C over 35 min as follows: 0–5 min, samples were heated from room temperature to 54 °C; 5–20 min, samples were held at 54 °C; 20–25 min, the sample temperature increased from 54 to 65 °C; 25–35 min, samples were held at 65 °C and then held at 95 °C for another 30 min period. After cooling, the tubes were filled to 30 g with 0.5% HCl solution using a VWR SE622 balance (VWR, Leuven, Germany) and analyzed by ICP-MS.

The average concentration of each element in each sample was calculated from the triplicate measurements; data analysis was then performed on these average values using the MPP chemometrics software.

Results and discussion

Limits of detection and quantification

Limits of detection (LOD) and limits of quantification (LOQ) of the ICP-MS method for 38 elements were obtained from 10 measurements of the calibration blank. They were calculated according to International Union of Pure and Applied Chemistry (IUPAC) guidelines using the following equations: 3.3 (SD/S) and 10 (SD/S), respectively (Table 2). SD is the standard deviation of 10 independent blank samples and S is the slope of the calibration curve.[6]

The linearity of the method was assessed by the coefficient of determination (R) of the calibration curve. The R value for all elements exceeded 0.999. Representative calibration curves across the mass range are shown in Figure 1.

Table 2. List of analytes and their selected cell mode conditions, internal standards, limit of detection (LOD), and limit of quantification (LOQ).

Element	Symbol	Mode	ISTD	LOD (ppb)	LOQ (ppb)
Boron	^{11}B	No gas	^{209}Bi	0.261	0.792
Sodium	^{23}Na	He	^{115}In	1.01	3.07
Magnesium	^{24}Mg	He	^{209}Bi	0.507	1.54
Aluminium	^{27}Al	He	^{115}In	0.722	2.19
Phosphorus	^{31}P	He	^{209}Bi	17.4	52.8
Potassium	^{39}K	He	^{115}In	11.5	34.9
Calcium	^{44}Ca	He	^{115}In	5.32	16.1
Titanium	^{47}Ti	He	^{209}Bi	0.163	0.493
Vanadium	^{51}V	He	^{209}Bi	0.013	0.040
Chromium	^{52}Cr	He	^{209}Bi	0.207	0.627
Manganese	^{55}Mn	He	^{209}Bi	0.042	0.126
Iron	^{56}Fe	He	^{209}Bi	1.40	4.25
Cobalt	^{59}Co	He	^{209}Bi	0.003	0.008
Nickel	^{60}Ni	He	^{209}Bi	0.039	0.119
Copper	^{63}Cu	He	^{209}Bi	0.033	0.099
Zinc	^{66}Zn	He	^{209}Bi	0.136	0.413
Gallium	^{71}Ga	He	^{209}Bi	0.006	0.019
Arsenic	^{75}As	He	^{209}Bi	0.015	0.046
Rubidium	^{85}Rb	He	^{115}In	0.029	0.089
Strontium	^{88}Sr	He	^{115}In	0.018	0.055
Yttrium	^{89}Y	He	^{115}In	0.003	0.010
Cadmium	^{111}Cd	He	^{209}Bi	0.004	0.012
Caesium	^{133}Cs	He	^{115}In	0.005	0.016
Barium	^{135}Ba	He	^{115}In	0.027	0.081
Lanthanum	^{139}La	He	^{115}In	0.001	0.003
Cerium	^{140}Ce	He	^{115}In	0.001	0.003
Praseodymium	^{141}Pr	He	^{115}In	0.001	0.003
Neodymium	^{146}Nd	He	^{115}In	0.002	0.005
Samarium	^{147}Sm	He	^{115}In	0.001	0.003
Europium	^{153}Eu	He	^{115}In	0.002	0.007
Gadolinium	^{157}Gd	He	^{115}In	0.002	0.007
Terbium	^{159}Tb	He	^{115}In	0.002	0.007
Dysprosium	^{163}Dy	He	^{115}In	0.002	0.005
Holmium	^{165}Ho	He	^{115}In	0.002	0.005
Erbium	^{166}Er	He	^{115}In	0.001	0.004
Thulium	^{169}Tm	He	^{115}In	0.002	0.005
Ytterbium	^{172}Yb	He	^{115}In	0.002	0.007
Lead	Pb*	He	^{209}Bi	0.002	0.006

** Reported as total free isotopic lead (Pb=^{206}Pb+^{207}Pb+^{208}Pb) to compensate for isotope variability.*

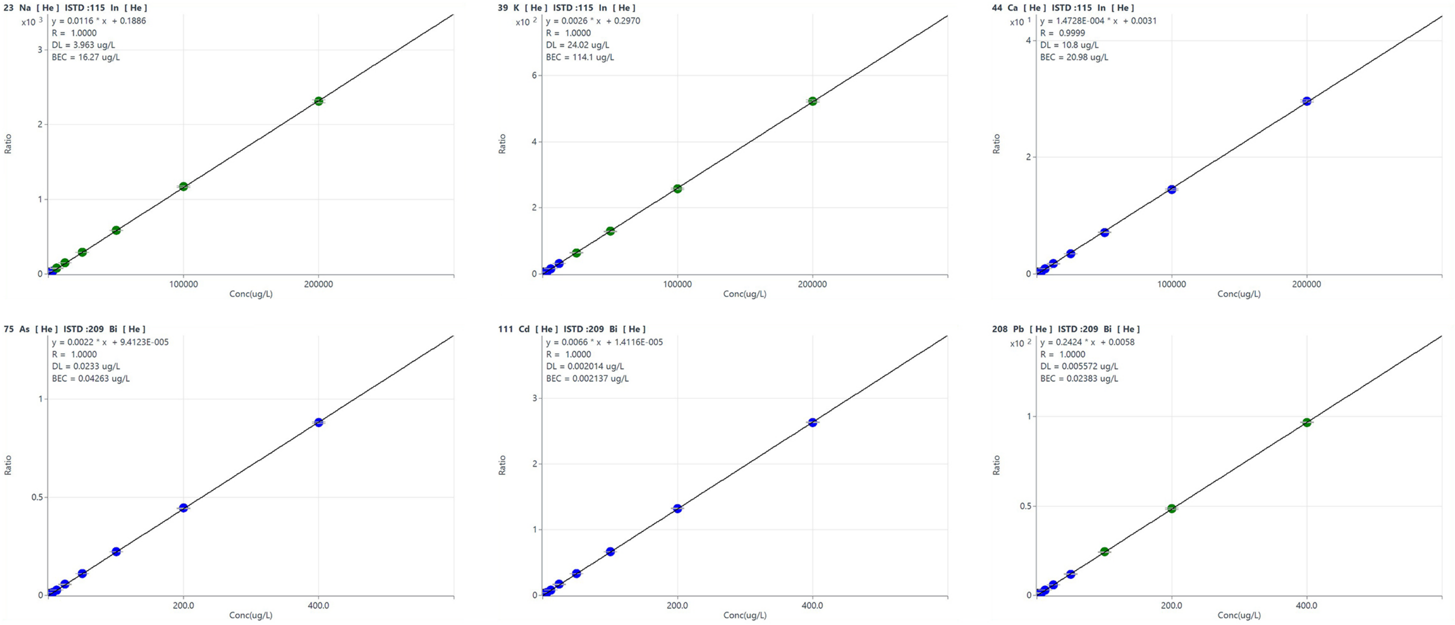

Figure 1. Representative calibration curves of Na, K, Ca, As, Cd, and Pb acquired using the ICP-MS method for black pepper. The maximum calibration point of As, Cd, and Pb was 400 ppb while the highest calibration point for Na, K, and Ca was 200,000 ppb.

Recoveries and evaluation of matrix effects

Percentage elemental recoveries were evaluated in triplicate by analyzing the TBK001RM black pepper reference material, which provided certified values for As, Cd, and Pb. The observed recoveries ranged from 102 to 119% (data not shown). In addition, a spike recovery test was conducted using the RM to assess the method's accuracy and identify any potential matrix effects. The spiked sample was analyzed in triplicate, and the corresponding recovery percentages are presented in Table 3. The recoveries of the elements ranged from 81 to 117%, indicating minimal matrix interference with the elemental measurements.

To further assess the accuracy of the elemental measurements, two replicates of the unspiked RM were analyzed at both the beginning and end of each sample batch, across five different batches on several days during the analysis of the black pepper samples. The detected concentrations of all monitored elements in the RM showed less than 10.7% variation in concentrations for all elements, highlighting the method's consistent performance over time (Table 3).

Internal standard stability

Bi and In were selected as the ISTDs for the various elements, as shown in Table 2, because they were not expected to be present in the black pepper samples. The analytical sequence, which included 30 digested black pepper samples, each prepared in triplicate, along with calibration standards, blanks, and RM control checks, was analyzed over several days. As illustrated in Figure 2, all ISTD recovery values remained within ±20% of the expected range (marked by the red dotted lines). These results indicate that the 7850 ICP-MS maintained excellent long-term stability and effectively managed varying sample matrices. Additionally, no significant matrix buildup was observed on the interface throughout the sequence.

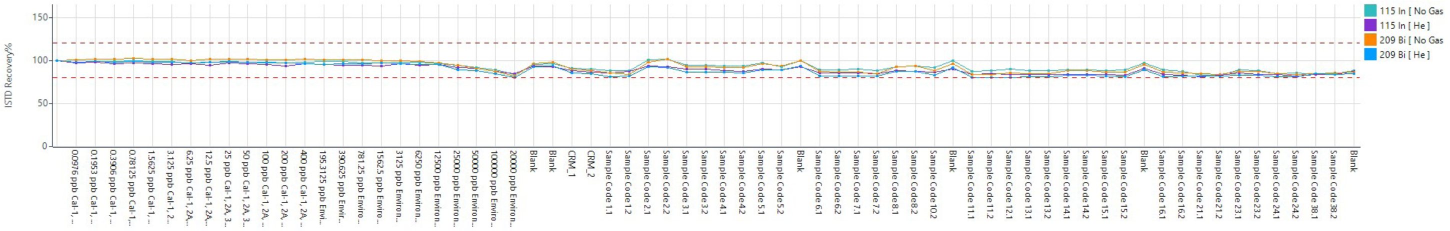

Figure 2. Recovery of internal standards (Bi and In) measured in He and no-gas modes over 11 hours (140 analyses) using the Agilent 7850 ICP-MS.

Table 3. Analyte concentration variability in the black pepper reference material across different time points and multiple days of analysis, and elemental recoveries of the spiked reference material.

Symbol	Percentage Variation (%)	Spiked Concentration (ppb)	Unspiked Sample Concentration (ppb)	Spiked Sample Concentration (ppb)	Percentage Recovery (%)
^{11}B	1.45	10,000	16,470	27,075	106
^{23}Na	3.31	1,000,000	136,421	1,118,962	98.3
^{24}Mg	1.56	1,000,000	2,184,653	3,263,568	108
^{27}Al	3.86	1,000	121396	122200	80.5
^{31}P	2.76	10,000	1,786,843	1,798,508	117
^{39}K	1.95	1,000,000	20,105,094	20,946,870	84.2
^{44}Ca	1.27	1,000,000	4,808,896	5,730,266	92.1
^{47}Ti	4.58	10,000	4,131	14,868	107
^{51}V	1.57	100	257	357	99.8
^{52}Cr	10.1	100	473	576	103
^{55}Mn	2.73	1,000	251319	252360	104
^{56}Fe	4.91	1,000,000	155864	1,114,081	95.8
^{59}Co	2.13	100	136	238	96.2
^{60}Ni	2.19	100	3554	3656	102
^{63}Cu	1.99	100	9616	9714	98.5
^{66}Zn	0.36	1,000	10751	11568	81.6
^{71}Ga	0.88	100	46.3	144	97.3
^{75}As	4.57	100	1400	1493	93.1
^{85}Rb	2.19	100	32234	32337	103
^{88}Sr	2.67	1,000	28540	29533	99.3
^{89}Y	4.29	10	119	130	111
^{111}Cd	2.30	100	725	816	91.1
^{133}Cs	2.50	100	191	285	93.9
^{135}Ba	2.51	1,000	30098	31110	101
^{137}La	3.51	10	92.8	103	105
^{140}Ce	1.08	10	137	148	113
^{141}Pr	4.03	10	17.4	27.2	98.5
^{146}Nd	2.61	10	84.0	88.2	88.2
^{147}Sm	8.04	10	17.6	27.7	100
^{153}Eu	2.69	10	6.98	16.1	91.1
^{157}Gd	8.21	10	21.3	31.1	98.2
^{159}Tb	6.45	10	2.82	11.7	88.5
^{163}Dy	8.51	10	14.4	23.2	88.1
^{165}Ho	2.99	10	2.96	11.8	88.6
^{166}Er	0.17	10	7.24	16.1	89.0
^{169}Tm	10.7	10	0.87	10.4	94.9
^{172}Yb	3.28	10	3.99	13.0	90.4
Pb*	3.85	100	1053	958	94.7

** Reported as total free isotopic lead (Pb=^{206}Pb+^{207}Pb+^{208}Pb) to compensate for isotope variability.*

Data analysis using Agilent MPP software

Elemental data for 38 elements in 150 black pepper samples sourced from five countries were acquired using the 7850 ICP-MS. This large data set was then compiled and imported into the MPP chemometric software for multivariate analysis. The MPP software provides a range of analytical tools for statistical analysis, clustering algorithms, correlation analysis, class prediction, and multivariate statistical techniques. These tools were used to explore data patterns, identify statistically significant differences among sample groups, and assess potential relationships between elemental composition and geographical origin.

Principal Component Analysis

To evaluate whether black pepper samples could be differentiated based on their geographical origin using elemental composition data, Principal Component Analysis (PCA) was conducted with a significance threshold of $p<0.05$. PCA is a multivariate technique that identifies patterns by transforming the original variables into principal components (PCs) and captures the maximum variance within the dataset. It also reveals the key elemental variables responsible for group discrimination.

The PCA score plots (Figure 3) show the general structure of the complete ICP-MS dataset based on the profiling of 38 elements. Samples from Brazil, India, Indonesia, and the RM formed distinct clusters. In contrast, the Cambodian and Vietnamese samples overlapped, indicating similar elemental profiles. These similarities are likely due to Cambodia and Vietnam sharing land borders, experiencing comparable climatic conditions and geology, and employing similar farming practices.

The first three PCs accounted for 77.2% of the total variance in the dataset, with PC1 (X-axis) explaining 50.6%, PC2 (Y-axis) 15.7%, and PC3 (Z-axis) 10.9%. The differentiation of black pepper samples by geographical origin, as revealed by the PCA loading values, was largely driven by Yb, Tm, Pr, Er, Nd, and Ho along the PC1, and Ca, K, Mg, Rb, Pb, and Zn along the PC2 (Figure 4).

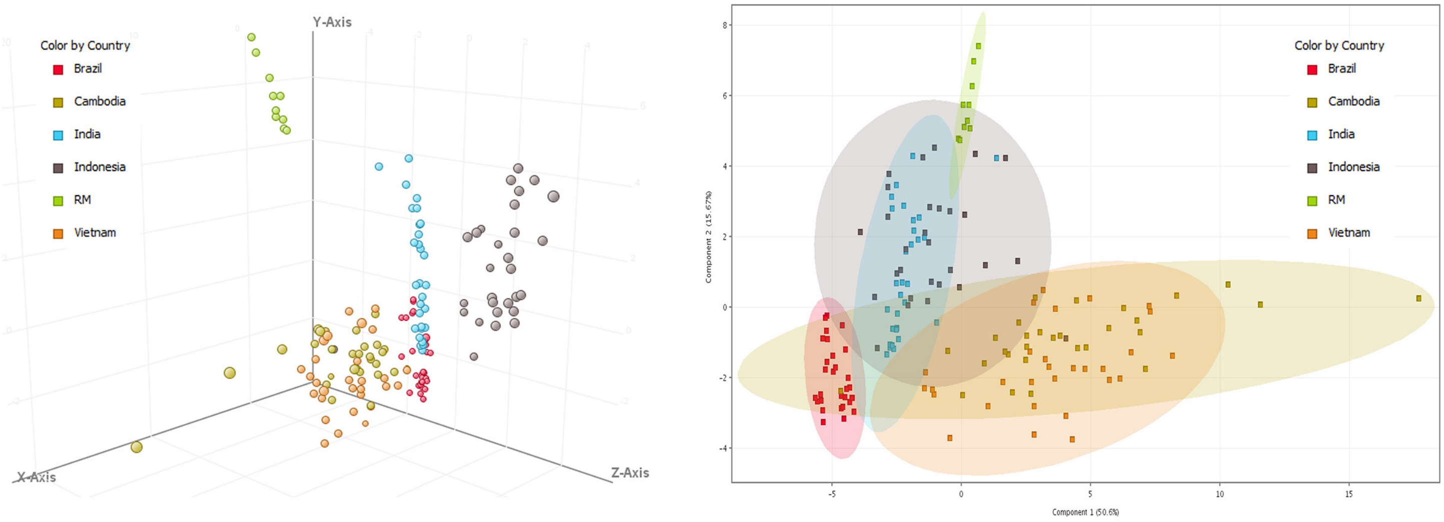

Figure 3. PCA score plots of 150 black pepper samples illustrating geographical discrimination based on elemental profiling. The 3D-PCA score plot (left) displays the first three principal components on the X (PC1), Y (PC2), and Z (PC3) axes, highlighting the variance captured by each. The 2D-PCA plot (right) shows the first two principal components (PC1 and PC2) on the X and Y axes, respectively, providing a simplified view of sample separation.

Class prediction analysis

Using the MPP software, we explored different class prediction analyses, including Linear Discriminant Analysis (LDA) and Random Forest (RF), to identify the geographical origin of black pepper samples based on their elemental composition. To build the models, the dataset comprising 150 black pepper samples was randomly split into two datasets—a training and test set—while ensuring a balanced representation of samples from the five countries. The training set comprised 80% of the black pepper samples, while the test set included the remaining 20%. The training set was used to build the models by identifying patterns that most effectively separate the samples by origin, and the test set was used to validate the model. The LDA and RF score plots were applied to the training set to visually assess sample separation and clustering. Overall, LDA and RF yielded 99.2 and 100% accuracy on the confusion matrix, respectively (data not shown). In the LDA, one Vietnamese sample was classified as Cambodian. This misclassification may have been influenced by the close geographical location of the two countries, resulting in similar elemental profiling of the samples. Figure 5 shows the LDA scores for the training set, differentiating the samples by geographical origin.

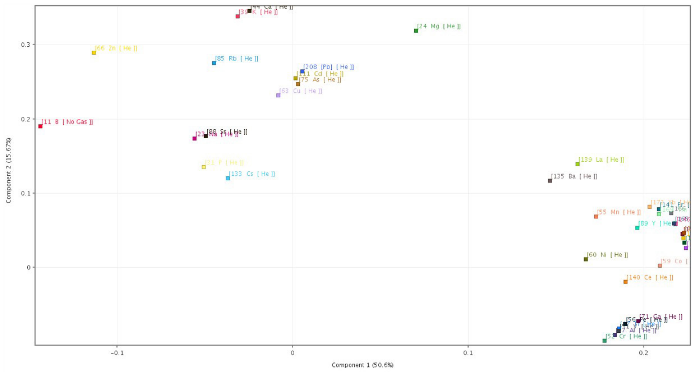

Figure 4. PCA loading plot of 150 black pepper samples illustrating geographical discrimination based on elemental profiling. The X and Y axes represent the two principal components (PC1 and PC2) that account for the greatest variance in the data.

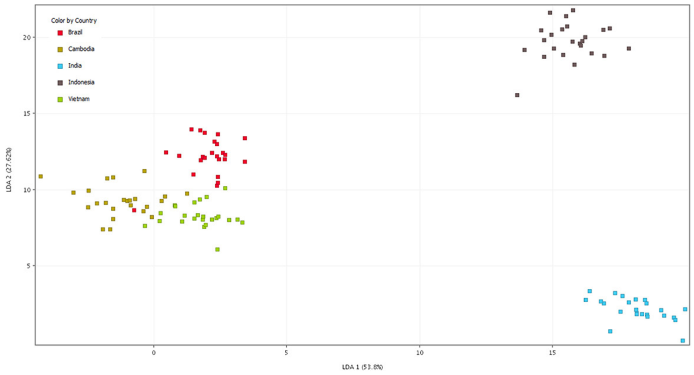

Figure 5. LDA scores plots of the training dataset comprising 80% of the black pepper samples. The X-axis: LDA 1 (53.80%) and Y-axis: LDA 2 (27.62%).

Table 4. Predicted geographical origins of the 30 black pepper test samples.

Sample Name	Actual Location	LDA Prediction Model		RF Prediction Model	
		Predicted Location	Confidence Measure	Predicted Location	Confidence Measure
Unknown B1	Brazil	Brazil	0.48	Brazil	0.96
Unknown B2	Brazil	Brazil	0.39	Brazil	0.93
Unknown B3	Brazil	Brazil	0.37	Brazil	0.98
Unknown B4	Brazil	Brazil	0.42	Brazil	0.95
Unknown B5	Brazil	Vietnam	0.24	Brazil	0.97
Unknown B6	Brazil	Brazil	0.51	Brazil	0.94
Unknown C1	Cambodia	Cambodia	0.86	Cambodia	0.86
Unknown C2	Cambodia	Cambodia	0.64	Cambodia	0.78
Unknown C3	Cambodia	Cambodia	0.58	Cambodia	0.80
Unknown C4	Cambodia	Cambodia	0.64	Cambodia	0.85
Unknown C5	Cambodia	Cambodia	0.90	Cambodia	0.85
Unknown C6	Cambodia	Cambodia	0.84	Cambodia	0.72
Unknown DI1	India	India	0.87	India	0.93
Unknown DI2	India	India	0.39	India	0.96
Unknown DI3	India	India	0.30	India	0.95
Unknown DI4	India	India	0.61	India	0.93
Unknown DI5	India	India	0.68	India	0.85
Unknown DI6	India	Vietnam	0.03	India	0.84
Unknown DO1	Indonesia	Indonesia	0.91	Indonesia	0.87
Unknown DO2	Indonesia	Indonesia	0.11	Indonesia	0.73
Unknown DO3	Indonesia	Indonesia	0.22	Indonesia	0.95
Unknown DO4	Indonesia	India	0.29	Indonesia	0.95
Unknown DO5	Indonesia	Indonesia	0.51	Indonesia	0.64
Unknown DO6	Indonesia	Indonesia	0.59	Indonesia	0.88
Unknown V1	Vietnam	Vietnam	0.06	Vietnam	0.80
Unknown V2	Vietnam	Brazil	0.36	Vietnam	0.75
Unknown V3	Vietnam	Indonesia	0.13	Vietnam	0.70
Unknown V4	Vietnam	Vietnam	0.39	Vietnam	0.76
Unknown V5	Vietnam	Brazil	0.13	Vietnam	0.72
Unknown V6	Vietnam	Vietnam	0.64	Vietnam	0.77

Conclusion

This study investigated the potential of ICP-MS elemental profiling coupled with chemometric analysis for geographical origin discrimination of black pepper samples from five countries, including Brazil, Cambodia, India, Indonesia, and Vietnam.

A total of 38 elements was measured in 150 black pepper samples using the Agilent 7850 ICP-MS. Chemometric analysis was performed on the ICP-MS elemental data using Agilent Mass Profiler Professional (MPP) software.

The PCA analysis exhibited satisfactory reliability and classification rates and demonstrated potential for the authentication of the geographical origin of black pepper. However, the separation of the Cambodian and Vietnamese samples was less distinct than the other groups, revealing potential similarities in elemental profiling between the bordering countries, which share similar climatic, geological, and agricultural conditions.

Using LDA and RF classification models on the black pepper test set, we achieved a high overall accuracy in determining the geographical origin of the samples. These findings provide empirical support for the combined use of elementomics and chemometric techniques in black pepper authentication, contributing to fraud prevention. The study also demonstrates the potential of the method for application to other food types.

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Agilent University Relations Thought Leader program and Grant ID #4628

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DE-010102

Published in the USA, January 12, 2026
5994-8741EN

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