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**Determination of geographical origin by multi-elemental profiling combined with machine learning techniques: a study on Chinese Geographical Indication (GI) rice**

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**ABSTRACT**

The demand for geographical indication (GI) rice has increased amongst Chinese consumers, which makes the GI rice at high risk of adulteration due to the high value and limited production. This study aimed to develop a novel strategy to determine geographical origins of Chinese GI rice, which was non-targeted data analysis based on multi-elemental profiling using inductively coupled plasma mass spectrometry (ICP-MS). One hundred and thirty-one samples from six types of Chinese GI rice were analyzed, and 80 % and 20 % of the dataset were used as training set and testing set respectively. Two machine learning algorithms, support vector machines (SVM) and random forest (RF), along with feature slection (reliefF algorithm) were implemented to build classificaition models. For both SVM and RF, four elements (Al, Rb, B, and Na) only could enable the prediction of geographical origins of six GI rice with 100% accuracy. These results demonstrated that the feasibility of using ICP-MS combined with machine learning techniques as an effective strategy for authentication of GI rice in China.

KEYWORDS

rice, ICP-MS, Geographical Indication, machine learning, feature selection, chemometrics

**INTRODUCTION**

In the current world of globalization, geographical authentication of food materials has emerged as an important issue, which is crucial for both quality assurance and food safety1. The notion of geographical indication (GI) first originated from the 19th century in Europe, with the purpose of protecting industrial property rights2. Nowadays, products with GI certification generally possess given quality, reputations or other characteristics attributable to geographical origins3, thus making it possible for them to be differentiated from competitors’. According to the European Union (EU) quality schemes, three different systems have been enforced to protect the origin integrity of agroproducts and food: protected designation of origin (PDO), protected geographical indication (PGI) and traditional specialties guaranteed (TSG)4. Currently in China, three government sectors supervise and protect GIs from different aspects at the administrative level5, including the State Administration for Industry and Commerce/ the Trademark Office (SAIC/TMO), the General Administration of Quality Supervision, Inspection and Quarantine (AQSIQ), and the Ministry of Agriculture (MoA).

Rice (*Oryza sativa L.)* is among the world’s top three largest food crops and is a staple food for nearly 50% of the world population6. China is leading in the rice paddy production in the world, with 220 million metric tons in 20187.With the improvement of people’s living standard, there is a growing demand domestically for rice with traceable origins8. For example, Wuchang rice, one GI rice produced in Heilongjiang province in China’s northeast region, is known for the superior quality and unique sensory characteristics. Due to the gap between the limited production and the high market demand, the price of Wuchang rice is roughly twice that of other domestic ones, making WuChang rice vulnerable to adulteration such as partial substitution and fraudulent labeling9,10. Therefore, determination of geographical origins of rice is of great importance for protecting the rights of farmers, retailers and consumers3. Yet to our knowledge, there is by far still a lack of universally recongnized techniques for the protection Chinese GI rice.

In recent years, various analytical approaches with the aid of multivariate analysis (MVA) and machine learning (ML) techniques are in rapid development to address the issue of geographical authentication. Among all the analytical approaches, inductively coupled plasma mass spectrometry (ICP-MS) analysis has drawn more and more attentions, thanks to its capability of determining the level of both metal and non-metal elements, with the advantages of wide dynamic range, high throughput and relateviely easy requirement for sample preparation11. With the evidence showing that element composition in plants are vestly determinded by factors such as soild characteristics and agricultural practice12,13, the multi-elemental profiling based on ICP-MS analysis already been proved to be a promising information souces to determine origins of rice14–16.

Beyond the advance of modern analytical appoarches, another cornerstone for the success of geographical origination, is to ensure the large volumn of data generated can be properly processed and interpreted (Ref 1). As been summarized in a recent review17, MVA such as principal component analysis (PCA) , and linear discriminate analysis (LDA) is by far the dominant method for data processing, due to their simplicity and success in spotting hidden trend embedded in the dataset. However, with the increased complexity and the volumn of data generated, more advanced pattern recognition models are in urgent demand (FZ ref 2). In the past decade, ML has demonstrated its capability to process complex problems, particularly in the domain of ecology (FZ ref 3), medicine (FZ ref 4.1 and 4.2), astronomy (FZ ref 5), and bioinformatics (FZ ref 6), while only a few attempts have been made in the area of food authentication18. Notably, some of the most widely adopted techniques such as support vector machines (SVM) and random forest (RF) have been reported to outperform traditional MVA19, and lead to predition models with increased reliability and robustness.15,20

The aim of the present study was to develop a novel ML-based workflow for the determination of geographical origins of six types of Chinese GI rice. SVM and RF were utilized to uncover the hidden information from the elemental profiling obtained by ICP-MS, and thereby construct reliable predition models. Furthermore, feature selection was also applied, with the aim of identifying key biomarkers that contribute the most to the difference between GI rices.

**MATERIALS AND METHODS**

*Rice samples*

In this study, a total of one hundred and thirty-one Chinese GI rice samples were directly collected from credible rice processing factories, from five provinces in China (Heilongjiang, Liaoning, Jiangsu, Hubei and Guangxi). For simplicity’s sake, we named those samples as WC, PJ-1, PJ-2, SY, JS, and GG. An overview of the geographical information of samples was shown in Fig. 1.

*Reagents and standards*

Nitric acid (69%, part# 100441) was purchased from Merck Millipore (Darmstadt, Germany). Deionized water (18.3MΩ cm) was obtained from a Milli-Q system (Millipore, MA, USA). Multi-element calibration standard 2A (part# 8500-6940), 4 (part# 8500-6942), Environmental calibration standard (part# 5183-4688), 45Sc standard (part# 5190-8578), and 103Rh (10 mg· L-1, part# 8500-6945)were purchased from Agilent Technologies (Santa Clara, CA, USA). One certified reference material (CRM) of rice flour (1568b) was purchased from the National Institute of Standards and Technology (Gaithersburg, MD, USA).

*ICP-MS analysis*

First of all, a 30% (v/v) nitric solution was prepared by diluting nitric acid with dionized water . Before use, the Teflon digestion vessels were soaked in nitric solution for 24h and then rinsed with deionized water for three times, thus to avoid cross contamination. For pre-digestion procedure, 0.5 g of rice grains was directly digested using 6mL of nitric acid in a digestion vessel, in duplicate. The vessel was placed in a fume hood overnight and then transferred to the microwave oven (Anton Paar, Austria). The digestion temperature of 180 °C was gradually reached in 15 min, and held for 20 min. Following the digestion, all solutions were cooled to room temperature and diluted to 50mL, with dionized water, in metal-free plastic tubes.

An Agilent 7900 ICP-MS (Agilent technologies, Santa Clara, CA, USA) was utilized for multi-elemental profiling. The instrumental setting and operative conditions were adopted from a published method21 with some modifications: radio frequency power of 1550 W, radio frequency matching of 1.85 V and carrier gas flow rate of 1.05 L·min-1. The concentrations of 30 elements (10B, 23Na, 24Mg, 27Al, 39K, 43Ca, 45Sc, 48Ti, 51V, 52Cr, 55Mn, 56Fe, 59Co, 60Ni, 65Cu, 66Zn, 70Ga, 73Ge, 75As, 78Se, 85Rb, 86Sr, 93Nb, 98Mo, 107Ag, 114Cd, 133Cs, 138Ba, 201Hg, 208Pb) were measured. The internal standard solution of 103Rh and SRM was used to verify the stability and accuracy of the analyzing method.

*Statistical analysis*

One-way analysis of variance (ANOVA) coupled with Tukey’s test (*p* ≤ 0.05) was carried out on the original dataset for the comparison of elements’ levels in six GI rice. Given the possibility that there may be certain correlation between the concentrations of different elements, traditional methods of univariate data analysis was not suitable for discrimination of 12, 22. The dataset was then scaled by taking logarithmic transformation and subjected to unsupervised PCA for initial visualization. Two machine learning algorithms, RF and SVM were implemented to construct classifiers. RF was first introduced by Breiman23 and it is made of an ensemble of decision trees, which are generated from original dataset using bootstrap partition. SVM makes classifications by projecting the input vectors into a high dimensional space, and finding a hyperplane that could separate different classes24. Feature selection is a data processing technique usually used during data mining, aiming to identify relationship between one or multiple features and their dependent variable (reference 7), in this case, the type of GI rice. In our study, ReliefF25,26 was utilized to determine how much each feature contributed to the overall classification by assigning relative importance to features basing on a calculated proxy statistic26. Fig. 2 demonstrated the workflow we used for the training of classifiers and the validation of the classification models:

* + - * The entire scaled dataset generated from 131 samples was randomly splitted into a training set (n=104) and a testing set (n=27) in a stratified fashion (80:20).
      * The ReliefF algorithm was applied only to the training set. Following this, a 10-fold grid-search cross-validation27 was conducted and the mean cross-validation accuracy was reported as the metric of selecting optimal classifiers. Forward selection28 was conducted to select pre-ranked features, and stopped when the adding of features made no contribution to the improvement of the prediction accuracy. Meanwhile, all possible combinations of hyperparametes were tested.
      * The optimal classifiers generated were independently validated on the testing set.

All data analysis were carried out by R (R Core Team, 2019), RStudio (v3.5.1, Boston, MA, USA) and Python (v3.7, Python Core Team) with additional packages: dplyr (R)29, factoextra (R)30, FSelector (R)30, sklearn (Python)31, skrebate (Python)32, numpy (Python)33 and pandas (Python)34 .

**RESULTS AND DISCUSSION**

*Elemental concentrations*

As shown in Table S1, the measured concentrations of SRM agreed well with the certified values, indicating the high accuracy of ICP-MS analysis. Table 1 shows the measured concentrations of 30 elements in the six typies of Chinese GI rice. Overall, except for 208Pb, significant differences could be observed among all elements across all types of rice. However, it was not obvious which element(s)contributed the most to differentiating all types of rice.

*PCA analysis*

As shown in Fig. 3a, based on the 1st and 2nd principal component (PC), there was a clear separation among PJ-1, GG and other types. While for JS, PJ-2, SY and WC, no satisfactory separation could be achieved. The loading plot (Fig. 3b) showed that 27Al, 70Ga, 93Nb,51V, and 48Ti primarily contributed to the variations on PC1, while 23Na, 45Sc, 85Rb, 133Cs, and 114Cd contributed to both PC1 and PC2. Notably, PJ-1 and PJ-2 could be clearly separated, despite that they were from the same geological origin (Fig. 3a). A possible explaination could be that cultivar types are also contributing to the elemental composition in rice kernals14. Overall, the first and second PC cover 60.7% of the total variance. The first PC is especially pertinent of separating PJ-1 rice samples while the second PC contributes mainly in distinguishing GG rice samples. Altogether, first four PCs (PC1 to PC4) were able to explain nearly 83% of the total variances (Fig. 3c), without further class discrimination observed.

*Determination of geographical origins of six Chinise GI rice*

Sampling is fundamental to achieve reliable results from multivariate model building35, while sample scaricity along with a lack of sample representativeness are major reasons leading to unreliable classification36. In this study, rather than sampling from the market, we obtained all GI rice samples from reliable sources, which minimized the risk of modeling with “contaminated” dataset.

Fig. 4 demonstrated the key results obtained from model training. Feature selection was traditionally implemented for simplifying models and minimize the risk of overfitting on training set (reference 8). Beyond that, in chemometric studies, feature selection is capable to model the correlation between the classification result and only few explanatory variables, namely biomarkers (reference 9). As shown in Fig. 4a, based on the caculation of ReliefF algorithm, relative importance was assigned to all 30 elements, indicating how each feature (element) was contributing to the overall differentiation among six GI rice. Notably, 27Al, 85Rb, 10B, 23Na, and 86Sr were the top five elements that contributed the most to the differentiation of all six GI rice. Special care was taken here that feature selection and model optimization were only applied to the training set only, ensuring the integrity of validation process and avoid selection bias such as over-optimistic prediction (reference 10). With only one selected feature (27Al), the mean cross-validation accuracy of 48% and 63% was achieved for RF and SVM, respectively (Fig. 4b). The performance of both RF and SVM boosted significantly with more features been added. Eventually, with only four features (27Al, 85Rb, 10B, and 23Na), the accuracy of 100% was obtained by both RF and SVM with optimal hyperparameters applied (Fig. 4b).

While cross-validation was applied to assess the goodness-of-fit of modeling within the training set, it is not enough to validify the classification model18. The conduction of a sencondary layer of independent validation, with the testing set, is the one and only valid paradigm37 for model assessment. The result of independent validation using the testing set is shown in table 2, where predition accuracy and kappa coefficient were shown as signs of the interrater reliability38. According to the result, both classifiers could predicit the geographical originis of all six types of GI rice with 100% accuracy. This indicated that in our study, the information from the four features (27Al, 85Rb, 10B, and 23Na) could be efficiently used to predict the geological origins. A similar approach was applied by Maione et al.15, who has identified 114Cd, 85Rb, 12Mg and, 19K as the most relevant elements for the differientiation between rice samples obtained from two geological orgins in Brazil. To get a closer look at the above mentioned elements and understand why they were playing such critical role in the differentiation, we further plotted their relative median concentration in radar plots. As shown in Fig. 5 , each GI rice demonstrated its unique elemental profiling. Interestingly for PJ-1 and PJ-2, who can be clearly separated from the PCA plots, showed significantly different elemental profiling. For example, PJ-1 had the highest level of 27Al among all six types of GI rice, whilst PJ-2 had the lowest level. Such observation agreed with previous findings that cultivar type also majorly impact on the elemental composition in rice39,40.

Beyond above four features, the elemental concentration of 114Cd also drawed our attention. In the study on Brazilian rice mentioned earlier, it was found that the level of Cd alone can be used to differentiate rice from two geological origin. The author further pointed out that it was the difference in irrigation methods that resulted in variance of 114Cd composition in two rice15. In our study, 114Cd was found in all six types of GI rice, with the concentrations all below the China national stardard of 0.2 ppm (Table 1). Particularly for GG rice, which was sampled from Guangxi province, had the highest level of 114Cd. This agreed with a national scale study, according to which the concentration of 114Cd in paddy soils from different Chinese regions varied significantly, with the higheset level found in southeast coastal regions (e.g. Hunan and Guangxi province) 41. Therefore, we further evaluated the feasility of using 114Cd as a biomarker to recognize rice from a specific region. Fig. 6 shows the differences of 114Cd concentration in the GG and none GG rice samples, which comprises all GI rice except GG. The 114Cd concentration is significantly different between GG and none GG rice samples (at significant level of *P* < 0.05). The 114Cd concentration (after log 2 scaling) of all interested samples general falls in the range of 2 to 8. Specifically, in none GG rice samples, the 114Cd concentration (after log 2 scaling) ranging from 2 to 6, while in GG rice samples the value skyrocketed to the range of 7 to 8, leaving a no overlap between GG and none GG samples. Consequently, the 114Cd concentration alone possesses strong discriminatory power to distinguish GG rice out of 6 types of GI rice samples, which correspondence with the high level of 114Cd found in the soil GG rice grew on.

Overall, it remains a challenging task to elucidate the rationale for each every top ranking elements that showed strong differentiation power. The complexity here, shall partially attribute to the sample diversity: in this study, we collected samples from all three dominate rice producing regions in China, including the northeast China plain (WC, PJ-1, and PJ-2), Yangtze River Basin (SY, JS), and southeast coastal region (GG). Such wide geological sampling scope, introduced multi-layers of complexity (e.g. soil characteristics, agricultural practices, and genotype variation), which are all closely related to the elemental profile of crops12,13. Nonetheless, as observed in the study between GG and none GG samples, planting region obviously has significant impact on the rice elemental profile.

Our study demonstrated that multi-elemental profiling using ICP-MS coupled with ML techniques, could differentiate six types of Chinese GI rice with extremely high accuracy. Particularly, we identified four elements with the most differentiation power. This opens the door for future study on the development of reliable rice classification with only a handful of elements. Further efforts would be focused on the following aspects: 1. A larger dataset consists of samples from multiple harvest years would be introduced to test the impact of potential seasonal variation. 2. considering the ultimate goal is to protect high value GI rice from potential fraudulent activities, we would introduce “positive” samples into the classification. One common solution is to dilute GI rice samples with serial does of highly “look-alikes”43 ..

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