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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 is at high risk of adulteration due to its 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 (relief 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 with 100% accuracy. These results demonstrated 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 Iearning, 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 population. China is leading in the rice paddy production in the world, with 214.4 million metric tons in 20176.With the improvement of living standard, there is a growing demand domestically for rice with traceable origins7. For example, Wuchang rice, one of GI rices produced in Heilongjiang province in China’s northeast region, is known for the superiority in terms of 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 than that of other domestic rices, making WuChang ricei vulnerable to be adutered such as partial substitution and fraudulent labeling8,9. Therefore, determination of geographical origins of rice is of great importance for protecting the rights of farmers, retailers and consumers3.

In recent years, multiple fingerprinting-based approaches have been developed to accurately distinguish rice from different origins, which generally consist of two steps of data measurement and data processing. Vibrational spectroscopy-based techniques such as Raman spectroscopy10 and near-infrared (NIR) spectroscopy11,12 have already been deployed during data measurement. Notably, methods based on multi-elemental profiling by inductively coupled plasma mass spectrometry (ICP-MS)13–15 and stable isotope ratio analysis have already shown great potential as well. In terms of data processing, a recent review16 has summarized that linear discriminate analysis (LDA) is by far the dominant statistical classifer, with the advantage of simplicity in detecting hidden information in data. Sometimes, an unsupervised Principal Component Analysis (PCA) is utilized to make data visualization. In recent years, the variety and the amount of data generated by various modern analytical instructments have been booming; more advanced pattern recognition models are essential to make good use of this gigantic and complex dataset (reference). Machine learning (ML) algorithms, specifically, random forst (RF) and support vector machine (SVM), have been showing their capabilies handling sophisticated jobs including processing microarray data (referernce), conducting astronomical object classification (reference) and predicting stock price direction (reference). In some studies, RF and SVM have outperforms traditional statistical techiniques (reference). However, these ML algorithms are still mainly under the radar in field of food authenticity, which have only been implemented by few researchers in their studies recently (reference). Fully acknowledging the competency of these ML algorithms, we decided to implement RF and SVM in this study to build classification model.

The aim of this study was to develop a novel ML-based workflow, which comprised of feature selection and model construction, for determining the geographical origins of six types of Chinese GI rice based on their multi-elemental profilings obtained by ICP-MS. As a result, the workflow not only outputs best classifiers with high prediction accuracy and robustness, but also key biomarkers which could be used to further understand the difference between GI rices.

**MATERIALS AND METHODS**

*Rice samples*

A total of one hundred and thirty-one Chinese GI rice samples were directly collected from paddy fields in five provinces (Heilongjiang, Liaoning, Jiangsu, Hubei and Guangxi). For simplicity’s sake, we named the 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)and 4 (part# 8500-6942), Environmental calibration standard (part# 5183-4688) and Scandium standard (part# 5190-8578) were purchased from Agilent Technologies (Santa Clara, CA, USA).

*ICP-MS analysis*

Before use, the Teflon digestion vessels were soaked in 30% (*v*/*v*) 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 in duplicate in digestion vessels with 6mL of concentrated HNO3 and left in fume hood overnight. The vessels were then placed in microwave oven (Anton Paar, Austria) next day. The digestion procedure was programed so that the temperature will gradually reach 180 °C in 15 min, and then kept at such temperature for 20 min. The digestion was completed when the sample solution mixtures turned into clear liquid. All solutions were cooled down at room temperature and diluted into 50mL 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 method20 with some modifications (RF power of 1550 W, RF 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 (10 mg· L-1, part# 8500-6945) was also obtained from Agilent Technologies. One certified reference material of rice flour (1568b) from the National Institute of Standards and Technology (Gaithersburg, MD, USA) was used to verify the accuracy of the analysis method.

*Statistical analysis*

One-way analysis of variance (ANOVA) coupled with Tukey HSD test was carried out on the raw data to assess the statistically significant differences (*p* ≤ 0.05) of individual element. Then raw data was scaled by taking logarithmic transformation and then subjected to unsupervised PCA for visualization.

*ML workflow*

Fig. 2 illustrated the workflow we have developed for the training of classifiers and the validation of the classification model. Two machine learning algorithms, RF and SVM were implemented to construct classifiers. For feature selection, ReliefF23,24 was utilized to determine how much each feature contributed to the overall classification.

The pre-scaled dataset containing 131 samples was splitted into a training set (n=104) and a testing set (n=27) in a randomly stratified fashion (80:20). The ReliefF algorithm was used to rank the features and applied only to the training set to avoid selection bias (e.g. over-optimistic prediction)25. Following this, a 10-fold grid-search cross-validation26 was conducted on the training set to obtain optimal classifiers based on mean cross-validation accuracy. Specifically, forward selection27 was conducted to select features that have been pre-ranked by ReliefF, which only stopped when the adding of features made no contribution to the improvement of the prediction accuracy. And meanwhle, all possible combinations of hyperparameters were tested26. Eventually, 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)28, factoextra (R)29, FSelector (R)29, sklearn (Python)30, skrebate (Python)31, numpy (Python)32 and pandas (Python)33 .

**RESULTS AND DISCUSSION**

*Elemental concentrations in Chinese GI rice*

The measured recovery of standard reference materal (SRM) which agreed well with the certified values indicating xx (shown in Table S1). Table 1 shows the measured concentrations of 30 elements in the six typies of Chinese GI rice. Overall, except 208Pb, significant differences could be observed among all elements across all types of rice. However, based on ANOVA, no element was identified which could directly differentiate all types of rice.

*PCA analysis*

In order to get an initial overview of the entire dataset, an unsupervised PCA was conducted (95% confident ellipses included). As shown in Fig. 3a, based on the 1st and 2nd principle 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. Sepecially, PJ-1 and PJ-2 could be clearly separated, even though they were from the same geological origin, with 27Al, 70Ga, 51V, and 45Sc showed significant difference (Fig. 3a& 3b). This may because that rice discrimination is a complex issue, where not only geographical origins but cultivar types play an important role13. In general, 60.7 % of the entire variances could be explained by the first two PCs, and nearly 83% by including the 3rd and 4th PC (Fig. 3c), showing the PCA has captured significant amount of variances between species and in good shape.

*Determination of geographical origins of Chinise GI rice*

Fig. 4 and Fig. 5 show the relative importance of features and the results of cross-validation, respectively. With only one selected feature, the mean cross-validation accuracy of 48% was achieved by RF, while 63% by SVM. The performance of both RF and SVM boosted dramatcially with more features been added. Eventually, with only four features (Al, Rb, B, and Na), the accuracy of 100% was obtained by both RF and SVM along with optimal hyperparameters were applied. The result of independent validation using the testing set is shown in table 2, including accuracy and kappa coefficient which is a statistic for testing the interrater reliability34. Both classifiers could predicit the geographical originis of all types of GI rice using only four elements with an impressive 100% accuracy, considering GI rices are complex food matrix themselves collected from across the country and some of GI rices clutered closely together on PCA plot, even with thirty elements used. Moreover, the result derived from the feature selection is enlightening that only few number of elements holds the key to make the discrimination across all 6 types of GI rice. With fuilly understanding the limination of this study, we could implement the workflow on larger dataset to find the real markerThe science behind this phenomenon is still a riddle By far, it is still challenging to elucidate the rationale why these four elements are showing such strong differentiation power in this study. The complexity here, is at least partially due to the fact that we covered samples from all three dominate rice producing regions in China: the Northeast China plain (WC, PJ-1, and PJ-2), Yangtze River Basin (SY, JS), and southeast coastal region (GG). The wide geological sampling scope would potentially brought along significant diversities in factors such as soil characteristics, agricultural practices, etc, which are all closely related to the elemental profile of crops35,36

*Elemental profiling of Chinise GI rice*

The relative median concentrations of the four features were shown in Fig. 6 using radar plot. It’s obvious that each type of GI rice possessed its unique elemental profiling. Specifically, PJ-1 and PJ-2, which were from a same geographical region but with genotypic difference, showed significantly different elemental profiling. For example, PJ-1 had the highest level of Al among all the six types of GI rice, whilst PJ-2 had the lowest. The results demonstrated that the genotype also played an important role on the accumulation of metals in rice, which have been reported in muiliple stuides37,38.

*Biomaker?*

In our study, 114 Cd which is a well known carcinogenic contaminant in rice, was detectd in all six types of GI rice, although the concentrations were xx. Particaulty, the concentration of Cd in GG which was sampled from southeast costal region of China, was significantly higher than in other types. The result was consistent with the previous national scale study, which revealed that the concentration of Cd in paddy soils from different Chinese regions varied significantly, with the higheset level in southeast coastal regions (e.g. Hunan, Guangxi) 39. In a recent study conducted by Maione et al., it was reported that Cd alone could be used to differentiate rice from two Brazilian regions with satisfied accuracy14. Therefore, in our study, the feasility of using Cd as a biomarker to recognize rice from a specific region was evaluated. For better visualization, the kernel density estimation (KDE) plot40 was constructed to estimate the probability density of Cd (Fig. 7). There was a clear cutoff of xx at around 7 between GG and other types, indicating that Cd itself was sufficient to differentiate GG rice from others.

Overall, our study demonstrated that multi-elemental profiling using ICP-MS coupled with machine learning techniques, could differentiate six types of Chinese GI rice with extremely high accuracy. Particularly, we identified four elements with the most differentiation power, which opens the door to a reliable rice classification using only a handful of elements. As been pointed out by other researchers, sample scarcity along with lack of sample representativeness are major reasons leading to poor or unreliable classification41. In this study, only 131 samples from six different GI rice within one year of harvest were analyzed. Therefore, a larger dataset consists of samples from multiple harvest years shall be introduced in the future, which will increase the robustness of the classification model. In addition, considering the ultimate goal is to protect high value GI rice from potential fraudulent activities, it is of great importantce that we also introduce “positive” samples into the classification. One common solution is to dilute GI rice samples with serial does of highly “look-alikes”42 . 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 discrimination35, 43. This study is by far one of few attempts of applying machine learning techniques to process multi-elemental data, and therefore constructed classification models for rice samples. F

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