Study on cyclic voltammetric electrochemical fingerprint method for origin traceability of rice

Min Sha 1, a,*, Zhilong Tang 1, b, Ding Zhang 2, c, Zhengyong Zhang 1, d, Jun Liu 1, e

1 School of Management Science and Engineering, Nanjing University of Finance and Economics, 3 Wenyuan Road, Nanjing, 210023, China
2 School of Chemical Engineering, Nanjing University of Science and Technology, 200 Xiaolinwei Road, Nanjing 210094, China.

a, *Corresponding author e-mail:minsha@nufe.edu.cn, b 716914460@qq.com, c 414679192@qq.com, d 95644190@qq.com, e 70881784@qq.com

Abstract. The origin traceability of geographical indication rice has always been highly concerned, this paper focused on the perspective of non-equilibrium samples, and the feasibility of using the cyclic voltammetric electrochemical fingerprint of rice to identify the origin of rice was studied. Jiansanjiang, Fangzheng and Xiangshui rice, whose production places were close, were selected, and the fingerprint collection method and data preprocessing method were explored respectively. Fingerprints with rich information, significant features were obtained by fast detection. Principal component analysis combined with support vector machine analysis were used to establish the discrimination model, the recognition accuracy of the prediction set was over 90.0%, showing a good application prospect. The electrochemical fingerprint of the non-equilibrium sample measured by real-time tracking of the reaction process could not only reflect the differences in the types and contents of the sample components, but also show the differences in the chemical properties of the components, providing a new idea for easy and accurate identification of food.

Keywords: Rice, origin traceability, cyclic voltammetry, electrochemical fingerprint, support vector machine.

1. Introduction
Rice is the main food of the Chinese people, providing nutrients and trace elements for the human body [1], and its quality is closely related to its geographical origin. Geographical indication rice is very popular among consumers. However, its low output has led to a serious phenomenon of manufacturing and selling fake goods. The status quo of rice that is difficult to identify needs to be improved urgently.

The methods for identifying geographical indication rice at home and abroad mainly include electronic information coding technology (such as bar code technology, radio frequency identification technology), biological and chemical technology (such as DNA traceability technology [2], mineral elements [3], isotopes [4], volatile components [5], etc.), intelligent sensory bionic technology [6] and chemical component cluster characterization technologies (such as Raman spectroscopy [7], proton...
nuclear magnetic resonance spectroscopy [8], mass spectroscopy [9] and other fingerprinting techniques, etc.

The above-mentioned researches are all based on detecting the physical and chemical signals of steady-state or equilibrium rice, focusing on characterizing the types and content of rice components, and cannot reflect the differences in the chemical properties of rice components. Cyclic voltammetry is based on the electrochemical workstation tracking the redox reaction in real time to obtain the kinetic signal, which can be used to determine the compound and its concentration. According to this principle, each point in the cyclic voltammetric diagram is considered to contain important information of the analyte, which can reflect the differences in the chemical properties of rice components, thereby establishing a new technology for identifying and evaluating samples. At present, cyclic voltammetry has been used for the quality analysis of wine and tea products, and there are few studies on the traceability of the geographical origin of rice.

Rice has complex sources, various varieties and complex composition. It is lack of scientific rigor to judge the type, authenticity or quality of rice by only relying on one or several component indicators, and the results are unreliable. Therefore, it is necessary to establish fingerprint technology from different angles, and obtain more fingerprint information through a variety of complementary methods, construct a relatively complete rice fingerprint system, and provide scientific and reasonable technical choices and data support for the research on the traceability of rice origin. In this work, cyclic voltammetry method was used to identify three kinds of geographical indication rice from Heilongjiang Province. Suitable sample pretreatment methods and instrument acquisition conditions were explored. The feasibility of cyclic voltammetric electrochemical fingerprints for the traceability of the origin of rice by combining with pattern recognition methods were investigated.

2. Experiment

2.1. Materials

The three kinds of geographical indication rice are Jiansanjiang rice from the Jiansanjiang Branch of the Heilongjiang Agricultural Reclamation Bureau, Fangzheng rice from Fangzheng County, Heilongjiang Province, and Xiangshui rice from Ningan City, Mudanjiang City, Heilongjiang Province. The rice was planted in 2019. In order to ensure the representativeness of the samples, Jiansanjiang, Fangzheng and Xiangshui rice samples were collected at 45, 37 and 39 sampling points in their planting area respectively, and about 2 kg rice sample was collected at each point. Ultrapure water was purchased from Yangzhou Zhongken Food Co., Ltd. Concentrated sulfuric acid with a content of 95.0-98.0 wt% was purchased from Sinopharm Chemical Reagent Co., Ltd. Ethanol and potassium chloride were of analytical grade and purchased from Aladdin Reagent (Shanghai) Co., Ltd.

2.2. Instruments and equipment

NA12345 rice hulling machine and NA-JCB rice milling machine were purchased from Ningbo Kemai Instrument Co., Ltd. JYS-M01 grinding mill was purchased from Joyoung Co., Ltd. CHI800D electrochemical analyzer was purchased from Shanghai Chenhua Instrument Co., Ltd. KH-250B ultrasonic instrument was purchased from Kunshan Hechuang Ultrasonic Instrument Co., Ltd. Platinum wire electrode, Ag-AgCl electrode, glassy carbon electrode, and customized jacketed glass reactors were purchased from Tianjin Lanlik Chemical Electronic High-Tech Co., Ltd.

2.3. Methods

2.3.1. Sample and solution preparation. The rice was dried, threshed, selected, hulled and milled, each rice was hulled twice and milled once. 100 g of each rice after processing were added to the grinder and pulverized for 1.5 min. The resulting rice powder was stored in a freezer, and it should be stored in a desiccator before use to equilibrate to room temperature. 3.0 wt% sulfuric acid solution and saturated potassium chloride solution were prepared with ultrapure water.
2.3.2. Electrode activation and inspection. The glassy carbon electrode was polished with 3.0 \( \mu \text{m} \) alumina suspension, and then both the glassy carbon electrode and the platinum wire electrode were ultrasonically cleaned with 3.0 wt% \( \text{H}_2\text{SO}_4 \), ethanol, and water for 1 min respectively. The Ag-AgCl electrode was washed sequentially with ethyl acetate, ethanol and deionized water.

Cyclic voltammetry was used for electrode activation, the reference electrode was a Ag-AgCl electrode, the working electrode was a glassy carbon electrode, and a platinum wire electrode was used as the counter electrode. Put the electrodes in 3.0 wt% \( \text{H}_2\text{SO}_4 \) solution, and set the test parameters of the electrochemical analyzer as follows, Init E: 0, High E: 1, Low E: 0, Final E: 1, Initial Scan Polarity: Positive, Scan Rate: 0.2, Sweep Segments: 10, Sample Interval: 0.001, Quiet Time: 2, Sensitivity: 1.e-006. Repeated the operation until the cyclic voltammetric curves overlapped well. Generally, the operation needed to be repeated 4 times.

The electrode inspection used a two-electrode system, the reference electrode was a Ag-AgCl electrode, and the working electrode was a platinum wire electrode. The open circuit voltage values of the two electrodes in a saturated potassium chloride solution were recorded at a sampling interval of 0.05 s. When the potential fluctuation within 3 min is less than 5 mV, it is judged that the electrodes are in good condition, otherwise electrode activation should be performed again.

2.3.3. Cyclic voltammetric fingerprint collection. Taking the optimized test conditions as an example, 1 g of rice flour was placed in 50 mL of ethanol and dissolved under ultrasonic oscillation for 1 h. Placed the activated electrodes in the solution to equilibrate for 8 min, set the instrument according to the electrode activation parameters mentioned above, then collected spectra. There were 20,000 data points in each spectrum.

2.3.4. Data processing. In order to remove interference and irrelevant information from the spectra data, some preprocessing should be done on the original data before data analysis. The fingerprint was denoised by wden wavelet function [10], and normalized by mapminmax function [11]. Then, the samples were identified by support vector machine (SVM). All data processing and model construction were based on Matlab 2019b.

3. Results and discussion

3.1. Optimization of fingerprint collection conditions

The effects of sample concentration, ultrasonic dissolution time, electrode equilibrium time, and scan rate on the fingerprints were investigated respectively. Experiments had found that when the sample concentration was 0.5 g/50 mL, the amount of sample needed was small, and the characteristics of the spectrum were obvious. The ultrasonic oscillation dissolution time of 1 h and 24 h had no obvious effect on the spectrum. Taking into account the requirements of quick inspection, the subsequent ultrasonic oscillation time was fixed at 1 h. When the immersion time was 8 min, the electrode could reach equilibrium with the solution, and the reproducibility of the collected spectrum was ideal. When the scan rate was 0.2 V/s, the range of current and the closed area of the curve were both large. Tested under the above-mentioned optimal conditions, the fingerprints of the three kinds of rice were obtained. The typical normalized spectra is shown in Fig. 1.
3.2. Identification of the geographical origin of rice

Two-thirds of the data of each kind of rice were selected as the training set, and one-thirds of the data as the prediction set. SVM modeling used radial basis function (RBF), optimized gamma and C parameters through grid search technology. The recognition accuracy was used to evaluate the model.

Firstly, the influence of data preprocessing such as denoising and normalization on the accuracy of the identification model was investigated. When the data was denoised, the recognition accuracy of the training set was 0%, and the recognition accuracy of the prediction set was 38.5%. When the data was normalized, the recognition accuracy of the training set was 100%, and the recognition accuracy of the prediction set was 89.7%. When the data was denoised and normalized, the recognition accuracy of the training set was 100%, and the recognition accuracy of the prediction set was 74.4%. It could be seen that normalization of data can improve the accuracy of the model. In order to reduce the dimension of the data, principal component analysis (PCA) combined with SVM were used to build a model, the recognition accuracy of the training set was 100% when built the model with the first 12 principal components, and the recognition accuracy of the prediction set was 89.7%, which was consistent with the results of using all data.

As shown in Fig. 1, the data of the last four cycles of each spectrum was almost the same, and the data of the first cycle is quite different from the data of the last four cycles. In order to reduce the dimension of the data, only the fifth cycle of data were used for the model. After the data were normalized, the recognition accuracy of the training set was 96.3%, and the recognition accuracy of the prediction set was 89.7%. PCA was further used to reduce the dimension and prevent the model from overfitting. The recognition accuracy of the training set was 96.3% when built the model with the first 9 principal components, and the recognition accuracy of the prediction set was 92.3%.

In summary, the results of models using all data and only using the fifth cycle of data were not much different. In order to improve the efficiency of the model, only the fifth cycle of data was taken, after normalization, combined with principal component analysis, the first 9 principal components was used to establish the best model.

However, the conditions of fingerprint collection in this work can be further optimized. For example, the water temperature in the ultrasonic instrument will gradually rise during the process of ultrasonic oscillation, and the temperature-controllable ultrasonic instrument could be used in the follow-up. It is expected that the best conditions will be explored further to improve the discriminative ability of the model. In a word, the method established in this work is a promising method, which is worthy of further study.

4. Conclusion

Jiansanjiang, Fangzheng and Xiangshui rice were selected as the research objects. By strictly controlling the conditions of sample preparation and fingerprint collection, the electrochemical fingerprints of rice-
ethanol mixed system with obvious characteristics were obtained, which provided experimental basis for the identification of rice from different origins. Compared with other chemical methods for identifying rice, this method only required the rice to be processed into powder, without the need for pretreatment such as separation and purification of the sample, the spectrum was obtained by analyzing the overall composition of the sample, which has two basic attributes of integrity and fuzziness. It is worth mentioning that the electrochemical fingerprint can not only reflect the difference of rice composition types and contents, but also reflect the difference of chemical properties. The identification accuracy of the method studied in this work is higher than 90.0%, which has a broad application prospect.

Acknowledgments
This research was financially supported by the Natural Science Foundation of Jiangsu Province under Grant BK20180816, the National Natural Science Foundation of China under Grant 21908109 and Grant 61602217.

References
[1] A.S.M. Saleh, P. Wang, N. Wang, L. Yang, and Z. Xiao, “Brown rice versus white rice: nutritional quality, potential health benefits, development of food products, and preservation technologies,” Compr. Rev. Food Sci. F., 18(4), 1070-1096, 2019.
[2] M.M. Voorhuijzen, J.P. van Dijk, T.W. Prins, A.M.A. van Hoef, R. Seyfarth, and E.J. Kok, “Development of a multiplex DNA-based traceability tool for crop plant materials,” Anal. Bioanal. Chem., 402, 693-701, 2012.
[3] J. Promchan, D. Günther, A. Siripinyanond, and J. Shioiwatana, “Elemental imaging and classifying rice grains by using laser ablation inductively coupled plasma mass spectrometry and linear discriminant analysis,” J. Cereal Sci., 71, 198-203, 2016.
[4] J. Wang, T. Chen, W. Zhang, Y. Zhao, S. Yang, and A. Chen, “Tracing the geographical origin of rice by stable isotopic analyses combined with chemometrics,” Food Chem., 313, 126093, 2020.
[5] D.K. Lim, C. Mo, D.K. Lee, N.P. Long, J. Lim, and S.W. Kwon, “Non-destructive profiling of volatile organic compounds using HS-SPME/GC-MS and its application for the geographical discrimination of white rice,” J. Food Drug Anal., 26(1), 260-267, 2018.
[6] M. Peris, and L. Escuder-Gilabert, “Electronic noses and tongues to assess food authenticity and adulteration,” Trends Food Sci. Tech., 58, 40-54, 2016.
[7] L. Zhu, J. Sun, G. Wu, Y. Wang, H. Zhang, L. Wang, H. Qian, and X. Qi, “Identification of rice varieties and determination of their geographical origin in China using Raman spectroscopy,” J. Cereal Sci., 82, 175-182, 2018.
[8] Y. Huo, G.M. Kamal, J. Wang, H. Liu, G. Zhang, Z. Hu, F. Anwar, H. Du, “1H NMR-based metabolomics for discrimination of rice from different geographical origins of China,” J. Cereal Sci., 76, 243-252, 2017.
[9] D.K. Lim, N.P. Long, C. Mo, Z. Dong, L. Cui, G. Kim, and S.W. Kwon, “Combination of mass spectrometry-based targeted lipidomics and supervised machine learning algorithms in detecting adulterated admixtures of white rice,” Food Res. Int., 100, 814-821, 2017.
[10] M. Sha, D. Zhang, Z.Y. Zhang, J.H. Wei, Y. Chen, M.T. Wang, and J. Liu, “Improving Raman spectroscopic identification of rice varieties by feature extraction,” J. Raman Spectrosc., 51, 702-710, 2020.
[11] H.A.H. Naji, Q.J. Xue, K. Zheng, and N.C. Lyu, “Investigating the significant individual historical factors of driving risk using hierarchical clustering analysis and quasi-Poisson regression model,” Sensors, 20(8), 2331, 2020.
[12] Y.P. LI, F.C. LI, X.H. YANG, L. Guo, F.R. Huang, Z.Q. Chen, X.D. Chen, and S.F. Zheng, “Quantitative analysis of glycated albumin in serum based on ATR-FTIR spectrum combined with SiPLS and SVM,” Spectrochim. Acta A, 201, 249-257, 2018.