Study on grading of Xiaouq Baijiu based on in-situ untargeted detection of electrochemical measurements

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ABSTRACT
Traditionally, the quality grading of distilled Chinese Baijiu is achieved by artificial sensory evaluation along with gas chromatography. The subjective problems, such as poor timeliness and accuracy of grading, become the bottleneck of industry to improve product quality and efficiency. Actually, the quality of Xiaouq Baijiu is determined by the synergistic effect of all flavoring substances rather than the specific targeted molecules in Baijiu. In this study, the electrochemical measurements were used to characterize the synergistic effect of flavoring substances in-situ, which also served as an untargeted detection for grading of Baijiu. After the abundant characteristic signals of standard samples whose grades have been confirmed via many winetasters were collected, the corresponding relationship between Baijiu grades and electrochemistry signal was received by the index of multi-parameters. Then, the electrochemical recognition model of Baijiu grades was established via mathematical statistics from above characteristic signals of standard samples. Once the testing data of unknow Baijiu were imported to the recognition model, the Baijiu grade value can be obtained quickly and online from the contrast algorithm, and the average accuracy rate of is more than 80%.

INTRODUCTION

Given the amphipathy of ethanol and kinds of flavor substances such as esters,\(^1,2\) the new distilled Baijiu will be in a state of intense molecular self-assembly, whose longer time represents the shorter intermolecular spacing and is corresponding to the better quality.\(^3\) Artificial sensory evaluation is a subjective process based on human smell and taste system, and is also an untargeted evaluation of the overall synergistic effect of self-assembly in Baijiu. Inevitably, the anthropic factors and subjective differences become the vital influences restricting the accuracy of grading.\(^4\) To improve the evaluation method, the gas chromatography (GC) has been adopted to analyze one or several main esters, which is auxiliary to grade the quality of Baijiu.\(^5,6\) However, there are more than 1700 flavor compounds in Chinese Baijiu, and the content of some substances is difficult to substitute for the overall level of synergistic effect in complex system.\(^7\)

With the development of sensors and analytical technology, electronic nose and electronic tongue have been developed for Baijiu grading.\(^8\) Whereas the electronic nose can only be used to detect volatile flavor compounds,\(^9\) while electronic tongue is a target detection for substances that produce sour, salty, bitter and sweet taste in Baijiu.\(^10\) Lin et al. reported the colorimetric sensor arrays for grading of Baijiu according to the chemo dyes, which can be combined with volatile organic compounds and show the olfactory visualization as the change of concentration. Fu et al. developed the colorimetric sensor arrays based on the reactions between carbonyl flavor compound and silver
nitrate and o-phenylenediamine derivatives, serving as a targeted way to grade the quality of Baijiu. Wang at al. made use of midinfrared spectroscopy and chemometrics to acquire the age discrimination.\textsuperscript{[11]} All of these are not overall evaluations of the synergistic effect for all flavoring substances in Baijiu, so the accuracy still needs to be improved.

Electrochemistry is the science of studying charged interfaces and interfacial reactions of different types of conductors, and has been developed for more than 300 years.\textsuperscript{[12,13]} Electrochemical impedance spectroscopy (EIS) and potentiodynamic polarization are the sophisticated testing method based on the principle of electrochemistry. From the EIS, a section of AC disturbance signal is imposed to the electrode, and the interface state can be analyzed according to the collected response signal such as impedance, phase angle, and capacitance at different frequency. It characterizes the reaction processes of interface in electrode/solution by obtaining the kinetic parameters and frequency response, and further evaluates the characteristics of the electrode or solution with the significant advantages such as high sensitivity,\textsuperscript{[14]} wide measurement range,\textsuperscript{[15]} low interference to the system,\textsuperscript{[16]} green and safe.\textsuperscript{[17]} Potentiodynamic polarization indicates the relationship between electrode potential and polarization current, which also manifests the kinetic processes and thermodynamic results of electrochemical reactions. The above measurements have been widely used in the fields of metal corrosion detection,\textsuperscript{[18]} battery performance evaluation,\textsuperscript{[19]} environmental ion detection and so on.\textsuperscript{[20]} The differences owing to the various flavor compounds and ester assembly structures of Baijiu in different grades will result in diverse electrochemical response in the interface accordingly. The causal response is an untargeted evaluation of the overall synergy effect of Baijiu, but it can indicate the change of Baijiu quality in situ. For instances, when the content of acids in Baijiu increases, the solution resistance will drop sharply owing to the rise of conductivity; when the content of esters increases or decreases, the composition and structure of the interface electric double layer will be changed, resulting in the change of interfacial dielectric constant.\textsuperscript{[20]} Therefore, once the electrode is applied to an electrical signal, the feedback signal can be obtained through the electrochemical response of the interface, whose will be distinctions in different grades of Baijiu. Thus, the total ester content and synergistic effect of Baijiu can be accurately distinguished, and Baijiu can be graded according to its quality authentically.

In this study, the schematic diagram of principle and processes for grading of Xiaoqu Baijiu was shown in Figure 1. According to the various electrochemical response at interface caused by different flavor substances, about 200 batches of Xiaoqu Baijiu samples whose grades have been confirmed via many winetasters were measured using EIS and potentiodynamic polarization firstly. Then, the response electrochemistry parameters at different frequencies were collected to set up a database of standard samples and were adopted to analyze the theoretical identification with esters content. After that, the mathematical statistics including deconvolution and principal component analysis (PCA) was applied to explore the corresponding relationship between the grade of Baijiu and above parameters. Finally, the recognition model of Baijiu grade was established based on electrochemistry parameters, and the unknown Baijiu samples can be graded according to the matching degree between the measured signal and the model.

**Experimental methods**

**Apparatus and Baijiu**

The Xiaoqu Baijius in different batches were obtained from Hubei Jingpai Co., Ltd. Methanol and hexane were bought from Sinopharm Chemical Reagent Co., Ltd in analytical reagents. Chemical analysis was finished by GC with a GC7890B instrument from Agilent (Santa Clara CA, USA). C18 solid-phase extraction cartridge was purchased from Thermo Fisher Scientific (China) Co., Ltd. The software MATLAB was served in mathematical statistics.
Electrochemical measurements

The typical three-electrode cell was adopted to acquire EIS using CS310 electrochemical workstation (Corrtest, China), which was composed of synthesized GUITAR electrode, Ag/AgCl electrode and platinum plane, corresponding to working electrode, reference and auxiliary electrode, respectively. GUITAR electrode was prepared via composite silicon wafer and glassy carbon, and the processes had been reported in previous publications. A sinusoidal excitation of 10 mV amplitude was utilized in EIS measurements versus OCP within the frequency range from 100 kHz to 1 Hz.

GC measurements

The esters in Xiaoqu Baijiu were mainly comprised by ethyl acetate and ethyl lactate, so the total content of these two substances represented the total ester content of Baijiu and was tested by GC. Firstly, 10 mL of the Xiaoqu Baijiu was subjected to solid-phase extraction with a C18 cartridge, which was cleansed with 12 mL of methanol (50%). Meanwhile, the sorbent was rinsed with 20 mL of water, and the absorbed compounds were de-absorbed with 20 mL of hexane, and then dried by nitrogen flow. The volume of extracts needs to be adjusted to 10 mL for GC analysis. All measurements were repeated at least three times at same condition to ensure the accuracy.

Artificial sensory evaluation

The artificial sensory evaluation was conducted by 3threenational Baijiu winetaster and six provincial Baijiu winetaster. The specific operation and requirements were consistent with Chinese standard GB/T 33404–2016. The grading results were gradually classified as: grade I, grade II and grade III.
Results and discussion

Detection of esters

The electrode interface will show differential electric double-layer owing to the different contents of esters and forming diverse molecular assembly structures in solution, resulting in significantly different of response signal to electrochemical reaction. After being measured by GC with exact esters content, some Xiaoqu Baijiu were tested using EIS and the results were shown in Figure 2. The diameter of capacitive arc decreases with the increase of esters content, indicating that the charge transfer process at the interface enhances gradually, which is related to the relative increase for the rate of charge transfer caused by organic molecules. Given the flavor substances in Baijiu are mostly small molecules like esters and aldehydes, the dielectric constants ($\varepsilon$) of above organic molecules are much smaller than that of water, so the value of interfacial capacitance ($C$) decreases obviously. Generally, the real part ($Z'$) of the impedance can be used to reflect the information of solution resistance, while the imaginary part ($Z''$) can characterize the change of capacitance. The calculation formulas of capacitance are as follows:

$$C = \frac{1}{\omega \times Z''}$$  \hspace{1cm} (1)

$$\varepsilon = \frac{4\pi K d C}{S}$$  \hspace{1cm} (2)

where $\omega$ is the angular frequency, $K$ is the static dielectric constant, $d$ is the distance between the electric double-layer, and $S$ is the relative area of the interface. The capacitance value depends on the dielectric constant of electrolyte, and the content of esters can be reflected by the size of different capacitance values according to the diverse dielectric constants of different flavor substances. When the imaginary part of the impedance reaches the maximum value, the absolute value of its ordinate is inversely proportional to the capacitance value from formula (1), that is, the larger the $Z''$ value, the smaller the capacitance value. Given the molecular structures and polarity, the smaller dielectric constant is assigned to the replacement from water to ester, which indicates the higher total ester content.

![Figure 2](image-url). The EIS of different Xiaoqu Baijiu with exact esters content measured by GC.
To better analyze the correspondence between EIS and the content of esters, the fitting data of EIS measured with different ester content are listed in Table 1. The electrochemical parameters are significantly affected by the change of ester content, while the fitting resistance (the sum of solution resistance and charge transfer resistance) decreases and the fitting capacitance value increases gradually with the increase of ester content.

The potentiodynamic polarization was adopted to investigate the relationship between the oxidation potential and current, and the curves of Xiaoqu Baijiu with exact ester content after being measured by GC were displayed in Figure 3. As the same oxidation potential, the oxidation current of samples with different ester content implied consistent difference. Specifically, the oxidation current at the same potential increased by degree with the increase of ester content, which was related to the oxidation processes of esters after adsorption at the interface. According to the electrode reaction kinetics, the oxidation current was calculated as follows:

\[
\bar{I} = nFk_a \bar{c}_A
\]

where \( \bar{I} \) is the current density of anode reaction, \( \bar{k}_a \) is the rate constant of electrochemical reaction, which is associated with reaction temperature, reaction activation energy and absolute potential of electrode. Moreover, \( n \) is the stoichiometric coefficient of electrode reaction; \( F \) is the Faraday constant; \( \bar{c}_A \) is the reductant concentration. The ester content of Xiaoqu Baijiu in different quality usually corresponds to different value of \( \bar{c}_A \), so the oxidation current at the same overpotential will be positively correlated with the ester content. After counting the oxidation potential-current of Baijiu in different batches and measured with exact ester content, the database of potentiodynamic polarization about ester content will be established, and the corresponding ester content can be matched by entering the signal data of the samples to be tested. Based on the accurate relationship between the

\[\begin{array}{|c|c|c|c|}
\hline
C_{\text{esters}} (\text{g/L}) & R (\text{Ω.cm}^2) & \text{Maximum } |Z''| (\text{Ω.cm}^2) & C_{\text{ef}} (5^{\circ}\text{Ω}^{-1}\text{.cm}^{-2}) \\
\hline
0.779 & 133740 & 41985 & 9.26 \times 10^{-10} \\
0.884 & 106010 & 35219 & 1.10 \times 10^{-9} \\
0.934 & 84327 & 28596 & 1.36 \times 10^{-9} \\
1.117 & 78408 & 27509 & 1.41 \times 10^{-9} \\
1.390 & 59973 & 25605 & 1.51 \times 10^{-9} \\
\hline
\end{array}\]

**Table 1.** The fitting parameters of EIS measurements with different concentrations of esters.

(3)

**Figure 3.** Polarization curves of Xiaoqu Baijiu with exact esters content measured by GC.
above results of both EIS and potentiodynamic polarization and esters content, it is highly suitable to take electrochemical measurements as the evaluation index of esters content and then to grade the Xiaoqu Baijiu by quality.

**Quality grading of Baijiu**

After being measured by GC and tasted by winetasters, the EIS and potentiodynamic polarization of Xiaoqu Baijiu with known grades were carried out to obtain the standard data. Then, the features were collected according to the characteristic electrochemical parameters including real part and imaginary part of impedance, phase angle with frequency, oxidation potential with current, etc. Along with the classification of principal component analysis (PCA), characteristic electrochemical parameters were divided via the importance to electrochemical measurements, and the results are displayed in Figure 4. The Xiaoqu Baijiu in different grade indicated the main variables corresponding to diverse principle components, which manifested the obvious distinction in statistics. From the classification by principal component regions, a numerical model for Baijiu grade was established by MATLAB from corresponding features and data distributions. Specifically, the unsupervised machine learning method and neural network classification model were adopted to analyze the 200 batches of Xiaoqu Baijiu samples, and the statistical characteristic quantity is about 60.

Given the influence of alcohol by volume on the process of electrolysis and electromigration, statistics and classifications were carried out for Xiaoqu Baijiu with different alcohol content, which can be detected accurately by detector. When the electrochemical data of Baijiu without grade are input, the mathematical operation will be clustered by the recognition model according to the differences of the characteristic parameters and standard data, in which the least difference will be ranked as the Baijiu grade to be tested. The grade of 100 batches of unknown samples have been confirmed through the numerical model, and then compared with the comprehensive results of

![Figure 4. PCA score image of Xiaoqu Baijiu with different grade.](image-url)
Figure 5. (a) The accuracy of numerical model for Xiaoqu Baijiu in different alcohol content, as the red part associated to 66%, green part being of 67% and blue being of 68%. (b) Confusion matrix are presented in accordance with Figure 5a.
artificial sensory evaluation and GC measurements. The percent of accuracy and the corresponding confusion matrix are shown in the Figure 5a and 5b. With the increase volume of original alcohol, the accuracy of model identification reaches 100% for the grade II at 67%, but indicates lower value at 68%. Overall, the average accuracy rate of more than 80% confirms the high recognition degree of numerical model, presenting the probability of realistic application. Besides that, the more and more input data will continue to expand the standard database on which the calculation is based, so as to continuously improve the accuracy of the model and achieve the function of automatic upgrading.

Conclusion

Whereas the quality of Baijiu is determined by the synergistic effect of various flavoring substances, the electrochemical measurements are adopted to appraise the whole effect as an in situ and untargeted detection. The esters contents of Xiaoqu Baijiu have shown the obvious effect for the results in EIS and potentiodynamic polarization, whose the higher content presents the smaller value of the maximum imaginary part, corresponding to the greater value of interface capacitance. Moreover, the response current was positively correlated with the ester content at the same oxidation potential. The electrochemical parameters of a large number of Xiaoqu samples with known grade have been analyzed statistically to establish a mathematical model, which can automatically evaluate the Baijiu grades according to the difference between the input parameters and the standard data, and achieve classification intelligently. The average accuracy of different Baijiu grades calculated by recognition model was more than 80% compared with that of artificial sensory evaluation.

Disclosure statement

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