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2D Pattern Recognition of White Spirit Based on the Electrochemical Profile Recorded by Screen-Printed Electrode

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The rapid identification of liquor is valuable for both research and food safety purposes. However, rapid and accurate identification has been difficult because most of the current analysis methods are lab-based. In this work, we established an electroanalytical technique to detect the distribution of electrochemically active compounds in liquor. Because the chemical composition of different varieties is largely controlled by the fermentation process, this method has considerable potential for liquor identification. Six brands of nongxiang-flavor liquors were collected to confirm the feasibility of the proposed methodology. PCA and 2D pattern recognition were used for identification. The results indicate that the electrochemical profiles of the six brands can be distinguished using their voltammetric data.

Keywords: Electrochemical profile, White spirit; Electroactive compounds; Food authentication; Wuliangye

1. INTRODUCTION

White spirit is a traditional liquor unique to China. In the long process of its development, it has formed a unique craft and style, which are famous in the world of distilled liquor [1–3]. Among the numerous liquor types, nongxiang-flavor liquor is an excellent representative of China's traditional brewing culture and craftsmanship, and its sensory flavor has been widely recognized by consumers. White spirit brewing is a process in which microorganisms make use of raw grain materials to decompose and metabolize liquor under appropriate conditions [4–8]. The quality and style of white spirit are closely related to the type and quantity of functional microorganisms in the environment. The flavor of nongxiang-flavor liquor depends on the type and content of trace substances. However, nongxiang-flavor liquor has different qualities and tastes attributable to different materials, production conditions, production techniques and blending. At present, research on nongxiang-flavor liquor has mainly focused on the impacts of microorganisms and brewing technology indicators on white spirit quality [9–11].

The development of modern analytical techniques has greatly promoted the analysis of volatile compounds, organic acids and other flavor substances in liquor, including GC-MS, HPLC and capillary electrophoresis [12–16]. The flavor substances in wine can be divided according to their different chemical properties into alcohols, aldehydes, acids, esters, ketones, sulfides, acetal compounds, pyrazines, furans, aromatic compounds and other compounds. The amounts of various flavor compounds in baijiu ranges from a few nanograms to several hundred milligrams per liter, making accurate quantification difficult [17,18]. Instrumental analysis technology provides an effective analysis method for the study of flavor compounds in traditional fermented food, with high sensitivity, good repeatability, and robust information with fast analysis speed. The emergence of solid-phase microextraction (SPME) has further improved the detection accuracy of volatile compounds in complex substrates such as liquor [19–26]. SPME technology is a rapid analysis technology that integrates sample pretreatment and adsorption extraction into one, requires a small amount of sample and short analysis time and is well suited for the detection and analysis of trace components in liquor during its production process and for monitoring. At present, SPME combined with GC-MS has been successfully applied to the analysis of volatile components in liquor and other beverages. These methods often exhibit excellent accuracy and reliability. However, they can suffer from drawbacks such as high instrument costs, complex preprocessing and a long-term analysis process. Thus, a new approach considers liquor identification from the perspective of electrochemistry. Electrochemical recognition is different from traditional analytical chemistry instruments [27–33]. Specifically, an electrochemical system is concerned not with the concentration of a particular compound but with the overall intensity of the signal generated by the interaction of different components of the system. The method focuses on detecting the overall feature of differences between samples.

In this work, we investigated the feasibility of the electrochemical method for identifying nongxiang-flavor liquor. *Luzhoutequ, Wuliangye, Jiannanchun, Tuopai Gujingongjiu* and *Songheliangye* were selected. A disposable screen-printed electrode was used for recording the electrochemical profile of each liquor. Two different electrolytes were used for a better representation of the electrochemical profile. The liquor identification could then be achieved with a pattern recognition model.

2. EXPERIMENTAL

All reagents, including KH_2PO_4, Na_2HPO_4, acetic acid and sodium acetate, were purchased from Macklin Co. Ltd. and used without purification. Screen-printed electrodes (SPEs) were purchased from Nanjing Youyun Technology Co. Ltd. *Luzhoutequ, Wuliangye, Jiannanchun, Tuopai Gujingongjiu* and *Songheliangye* were purchased from a local supermarket. The working electrode, reference electrode and counter electrode were carbon paste, Ag/AgCl and carbon paste, respectively. Phosphate buffer solution (PBS) was prepared by mixing stock solutions of 0.1 M disodium hydrogen phosphate and sodium dihydrogen phosphate. Acetate buffer solution (ABS) was prepared with 0.1 M sodium acetate and acetic acid.

Electrode surface modification was carried out at room temperature. Typically, 5 µL of liquor sample was dip-coated on the working electrode of the SPE and dried naturally. The electrochemical profile of each liquor was carried out using a CHI760 electrochemical workstation with the SPE integrated into a three-electrode system. The electrolyte is a 0.1 M PBS (pH 7.0). Differential pulse voltammetry (DPV) was used for electrochemical recording. The scan range was −0.2–1.2 V. The pulse amplitude was 50 mV. The pulse width was 0.05 s. The pulse period was 0.5 s.

Electrochemical standardization was carried out to establish quantitative criteria of recognition, where the ratios between the current and the maximum peak current were obtained at different potentials.

3. RESULTS AND DISCUSSION

Figure 1 shows the schematic diagram of the methodology. The liquor sample was first immobilized on the electrode surface to eliminate the influence of the high ethanol content in the liquor. Then, the electrochemical profile of the liquor was recorded using differential pulse voltammetry using a positive scan. The electrochemically active compounds contribute signals during the scan, which correspond to the chemical compounds' distribution of the liquor during fermentation. During the fermentation of liquor, the long-chain carbohydrates are broken down to form different small molecules. Some of them have electrochemical activity, so they can be oxidized at lower potentials. Because the raw materials and fermentation processes of different liquors are different, the types and amounts of compounds with electrochemical activity in different liquors are different and show different electrochemical behaviors [34–38]. Thus, the electrochemical profiles could be used for the construction of a 2D density pattern, which was consequently used for liquor brand identification.

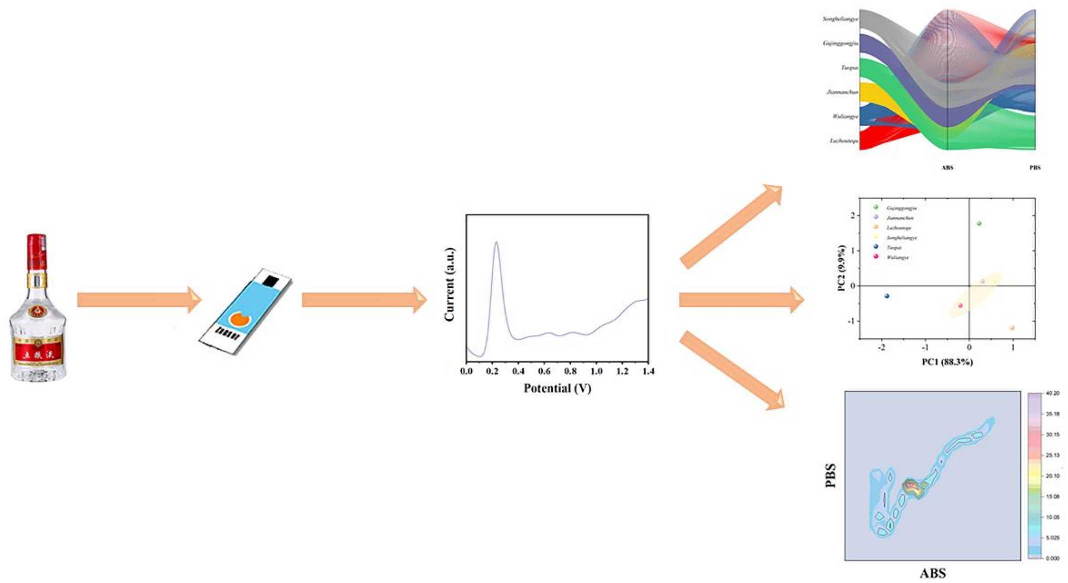

Figure 1. Scheme of recording the electrochemical profile of liquor for identification.

Figure 2 shows the DPV curves of *Luzhoutequ*, *Wuliangye*, *Jiannanchun*, *Tuopai Gujingongjiu* and *Songheliangye* recorded using PBS and ABS as electrolytes. The six brands all showed several peaks during each voltammetric scan, suggesting that electrochemically active compounds from the liquor were oxidized. Because of the complexity of the chemical components in liquor, it is difficult to distinguish each compound. However, previous studies have confirmed the electrochemical activity of ester [39,40] and alcohol compounds [39,41], which can be oxidized at low potentials. The purpose of our study is not to distinguish each compound related to the signal but to record the whole profile of all electrochemically active compounds of each sample because these compounds are controlled by fermentation processes, so differences in electrochemical profile reflect differences in liquor brand [42,43]. Alcohol compounds are produced by microorganisms acting on substances such as sugar, pectin and amino acids [44,45]. Ester compounds are the volatile components with the highest content and the most varieties in liquor and are the main substances that have the greatest influence on liquor flavor [46–48]. There are two main ways to produce ester compounds: one is through the esterification enzymes in some sweet yeasts (such as Hanson's yeast and Candida), and the other is through the slow esterification reaction. As shown in Figure 3, each sample exhibited differences using different buffer solutions. The electrochemical behavior of the molecules can be affected under different pH conditions. Therefore, combining these conditions can reveal a more comprehensive profile of the electrochemically active compounds. Moreover, Figure 2 shows the different profiles between the samples, suggesting a significant difference in electrochemically active compounds within these samples.

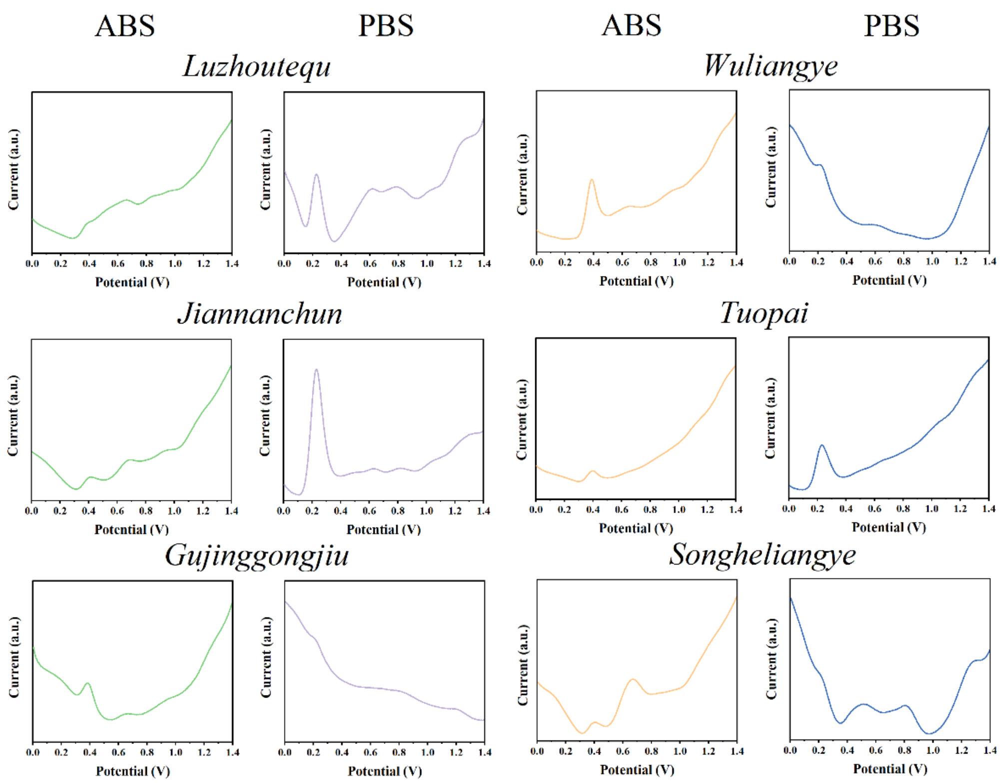

Figure 2. DPV curves of *Luzhoutequ*, *Wuliangye*, *Jiannanchun*, *Tuopai*, *Gujingongjiu* and *Songheliangye* recorded using 0.1 M ABS and 0.1 M PBS as electrolytes.

Figure 3 shows the parallel coordinate plot of the normalized current of six samples recorded using 0.1 M PBS and 0.1 M ABS as electrolytes. The parallel coordinate plot is a common method used to analyze and display multivariate data [49]. The six samples showed significantly different tendencies, suggesting that the electrochemically active compounds of the liquor can be tracked and identified. Therefore, the electrochemical profile of the liquor showed the possibility of using the electrochemical data for brand identification. In addition, electrochemical analysis is a portable technique that can be used for on-field rapid sample testing. Therefore, the development of a fast recognition method for liquor is valuable for food safety purposes.

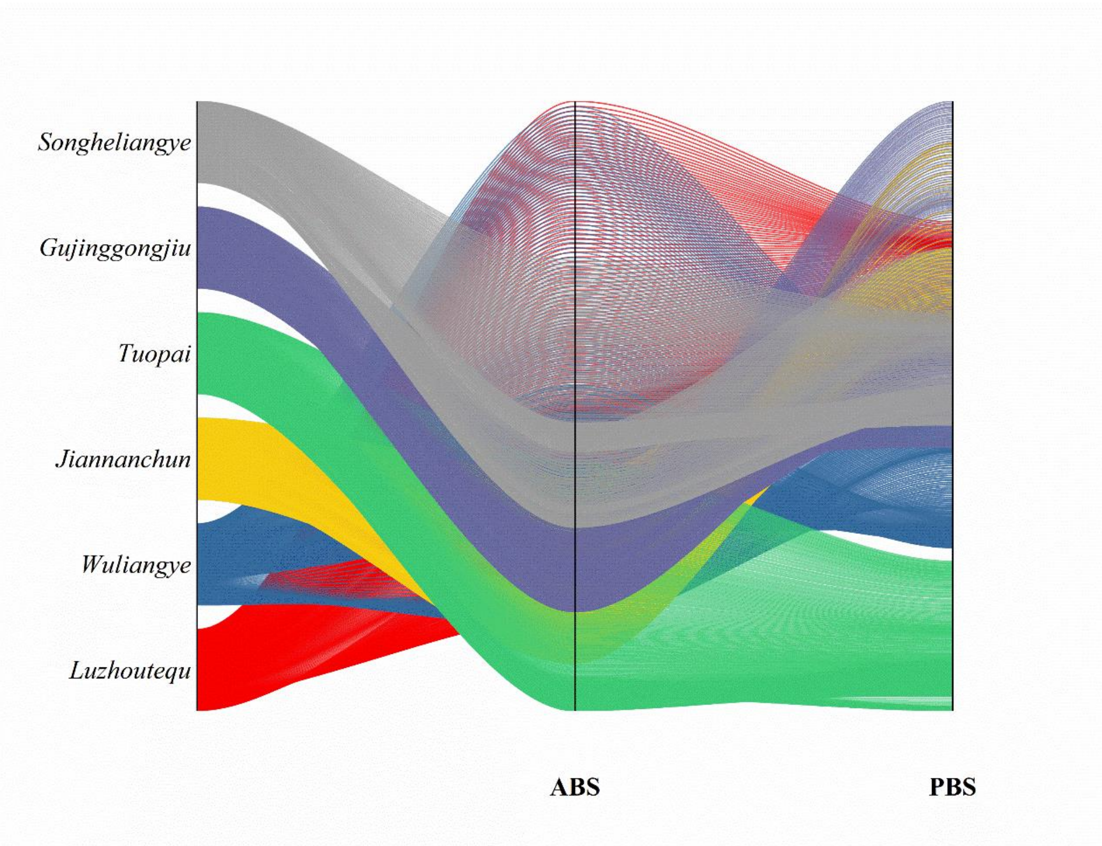

Figure 3. Parallel coordinate plot of normalized currents of *Luzhoutequ, Wuliangye, Jiannanchun, Tuopai Gujingongjiu* and *Songheliangye* recorded using 0.1 M ABS and 0.1 M PBS as electrolytes.

To further test the differences in the electrochemical profiles between the samples, principal component analysis (PCA) was carried out. PCA analysis was performed on the samples with significant differences, and the characteristic quantities of samples were compressed to reflect the relationship between samples in the low latitude space. Our previous studies indicated that the PCA of the electrochemical profile does not have a high interpretative capability [50–53]. However, in this study, the two extracted factors can reach more than 95% interpretative capability (Figure 4), indicating that there were significant differences in electrochemical profiles between the samples. During the fermentation of liquor, long-chain carbohydrates are broken down to form different small molecules. Some of these products have electrochemical activity, so they can be oxidized at lower potentials.

Because the raw materials and fermentation processes of different liquors are different, the types and amounts of compounds with electrochemical activity in different liquors are different, and the liquors show different electrochemical behaviors [34–38].

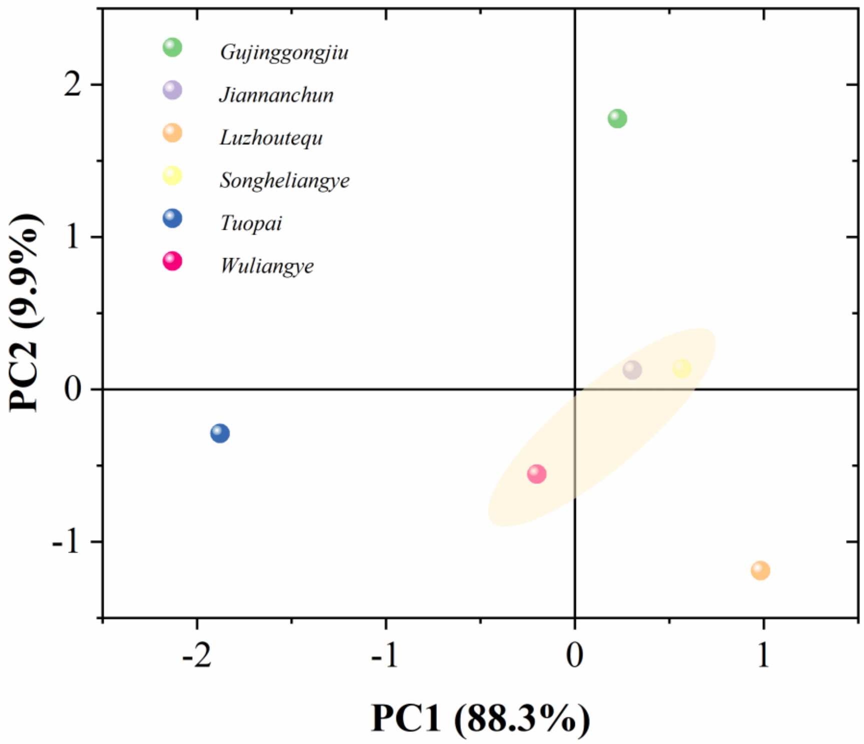

Figure 4. PCA analysis of *Luzhoutequ, Wuliangye, Jiannanchun, Tuopai Gujingongjiu* and *Songheliangye* recorded using 0.1 M ABS and 0.1 M PBS as electrolytes.

Direct liquor identification based on the electrochemical profile is not an efficient method. The electrochemical profiles of some of these liquors share some similar characteristics. On the other hand, pattern recognition based on multiple electrochemical datasets can overcome this problem for species identification. A 2D density plot is an effective method for data visualization. Figure 5 shows the 2D density plots of *Luzhoutequ, Wuliangye, Jiannanchun, Tuopai Gujingongjiu* and *Songheliangye* generated using the current data recorded using 0.1 M ABS and 0.1 M PBS as electrolytes. It is pertinent to note that each sample displayed a unique pattern. Therefore, identification can be easily achieved using the electrochemical behavior of the liquor recorded using two electrolytes.

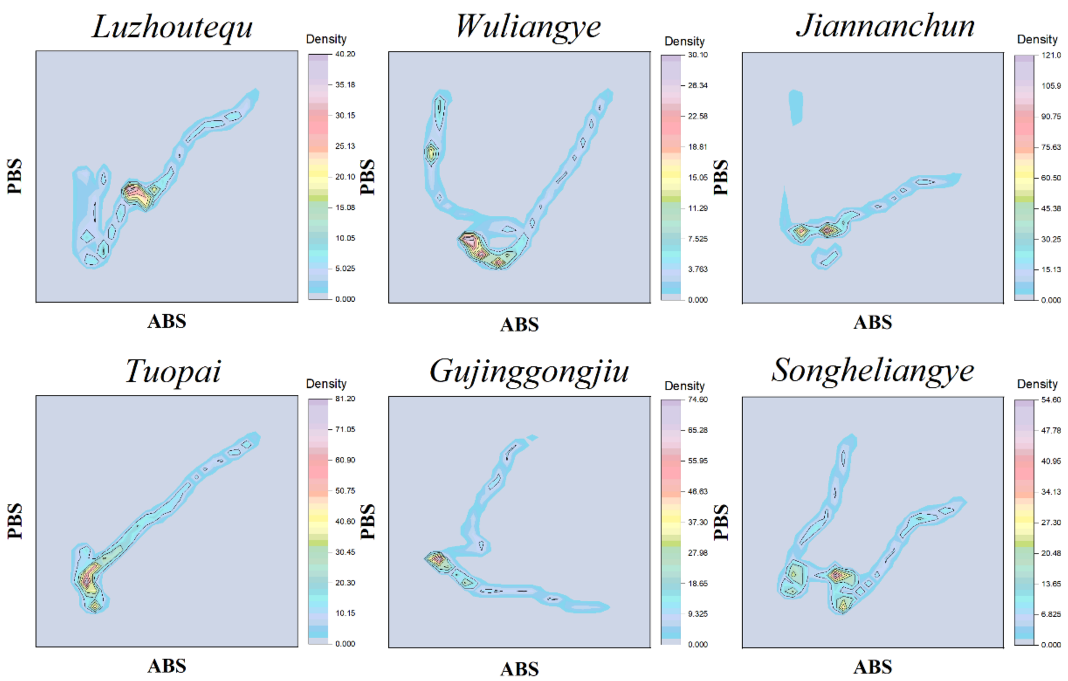

Figure 5. 2D density patterns of *Luzhoutequ*, *Wuliangye*, *Jiannanchun*, *Tuopai Gujingongjiu* and *Songheliangye* recorded using 0.1 M ABS and 0.1 M PBS as electrolytes.

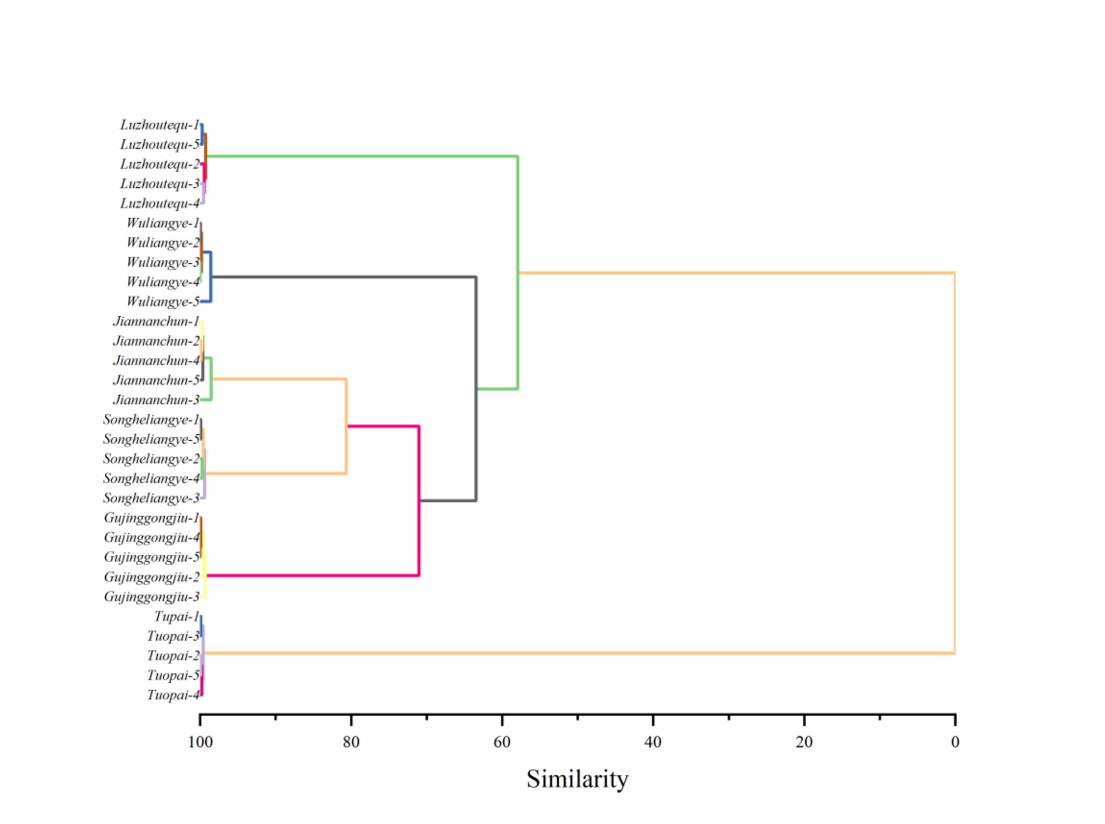

Figure 6. Dendrogram of *Luzhoutequ*, *Wuliangye*, *Jiannanchun*, *Tuopai Gujingongjiu* and *Songheliangye* based on the electrochemical profiles.

Table 1. Merit and demerit of the proposed electrochemical method compared with other analytical techniques.

Method	Advantages	Disadvantages	Reference
^{1}H-NMR Metabolomics Data	Quantification of 33 metabolites	Only for region authenticity	[54]
Microchip electrophoresis	Specific compound detection	Only for botanical origin, provenance, vintage and quality	[55]
PTR-ToF-MS	High accuracy	Only origin detection; only for low ethanol concentration	[56]
Near infrared spectroscopy	Fast	Low accuracy	[57]
Electrochemical profile-based sensor	Pattern recognition; On-filed analysis; Cheap	Cannot be used for specific compound analysis; Need database	This work

Figure 6 shows the dendrogram of five individual samples of each liquor brand. The dendrogram is divided into six clusters. Each cluster only contains one brand, suggesting that no outlier was observed in this study. Based on the above results, we proposed a simple method for nongxiang-flavor liquor identification. We believe that the voltammetric data from the liquor can be collected in a database and subsequently used for unknown brand identification. Table 1 summarizes the merits and disadvantages of the proposed electrochemical method compared with other analytical techniques.

4. CONCLUSION

In this work, we proposed an authentication method based on the electrochemical profile of white spirit. The electrochemical profiles of *Luzhoutequ*, *Wuliangye*, *Jiannanchun*, *Tuopai Gujingongjiu* and *Songheliangye* were recorded from immobilized samples using 0.1 M ABS and 0.1 M PBS as electrolytes. The recorded profile varies between the sample brands due to the presence of different contents of electrochemically active compounds. Based on the recorded electrochemical profiles, these samples can be effectively identified using a pattern recognition method. Due to the high reproducibility of the proposed methodology, this method can be used effectively for other liquor identification.

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References

1. A.V. Kolesnikov, P.A. Kozlov, I.M. Fominykh, *Butlerov Commun.*, 55 (2018) 120–126.
2. S. Wang, Y. Wang, S. Chen, Y. Li, D. Hua, Y. Jin, *J. Food Saf. Qual.*, 3 (2012) 322–325.
3. H. Wang, G. Zhang, M. Peng, Q. Zhou, J. Li, H. Xu, F. Meng, *Int. Biodeterior. Biodegrad.*, 113 (2016) 134–138.
4. M.F. Sevimli, E. Deliktaş, S. Şahinkaya, D. Güçlü, *Arab. J. Chem.*, 7 (2014) 1116–1123.
5. H. Xu, Q. Fei, H. Shan, Y. Huan, H. Mi, H. Zhang, G. Li, G. Feng, *Anal. Sci.*, 32 (2016) 183–188.
6. C. Mihaela Manta, G. Bozga, G. Bercaru, C. Sorin Bildea, *Chem. Eng. Technol.*, 34 (2011) 1739–1744.
7. A. Jun, U.W. Tschirner, Z. Tauer, *Biomass Bioenergy*, 37 (2012) 229–236.
8. V. Da Re, L. Papinutti, *Appl. Biochem. Biotechnol.*, 165 (2011) 406–415.
9. K. Ragsdale, J.R. Porter, R. Mathews, A. White, C. Gore-Felton, E.L. McGarvey, *J. Subst. Use*, 17 (2012) 323–339.
10. H. Qin, D. Huo, L. Zhang, L. Yang, S. Zhang, M. Yang, C. Shen, C. Hou, *Food Res. Int.*, 45 (2012) 45–51.
11. D.R. Negrão, M. Sain, A.L.L.L. Leão, J. Sameni, R. Jeng, J.P.F. de Jesus, R.T.R. Monteiro, *BioResources*, 10 (2015) 1553–1573.
12. G. Grindlay, J. Mora, L. Gras, M.T. de Loos-Vollebregt, *Anal. Chim. Acta*, 691 (2011) 18–32.
13. F.J. Gomez, M.F. Silva, *Anal. Bioanal. Chem.*, 408 (2016) 8643–8653.
14. T. Yamamoto, S. Wakamatsu, S. Qiao, D. Hira, T. Fujii, K. Furukawa, *Bioresour. Technol.*, 102 (2011) 2342–2347.
15. O. Makhotkina, P.A. Kilmartin, *Anal. Chim. Acta*, 668 (2010) 155–165.
16. L. Gougeon, G. Da Costa, I. Le Mao, W. Ma, P.-L. Teissedre, F. Guyon, T. Richard, *Food Anal. Methods*, 11 (2018) 3425–3434.
17. H. Campbell-Sills, V. Capozzi, A. Romano, L. Cappellin, G. Spano, M. Breniaux, P. Lucas, F. Biasioli, *Int. J. Mass Spectrom.*, 397 (2016) 42–51.
18. Z. Genisheva, C. Quintelas, D.P. Mesquita, E.C. Ferreira, J.M. Oliveira, A.L. Amaral, *Food Chem.*, 246 (2018) 172–178.
19. J. Crucello, L.F. Miron, V.H. Ferreira, H. Nan, M.O. Marques, P.S. Ritschel, M.C. Zanus, J.L. Anderson, R.J. Poppi, L.W. Hantao, *Anal. Bioanal. Chem.*, 410 (2018) 4749–4762.
20. M.A. Andrade, F.M. Lanças, *J. Chromatogr. A*, 1493 (2017) 41–48.
21. G.N. Sagandykova, M.B. Alimzhanova, Y.T. Nurzhanova, B. Kenessov, *Food Chem.*, 220 (2017) 162–167.
22. A. Aresta, P. Cotugno, F. Massari, C. Zambonin, *Food Anal. Methods*, 11 (2018) 426–431.
23. A. Ziółkowska, E. Wąsowicz, H.H. Jeleń, *Food Chem.*, 213 (2016) 714–720.
24. M. Papageorgiou, D. Lambropoulou, C. Morrison, J. Namieśnik, J. Płotka-Wasylka, *Talanta*, 183 (2018) 276–282.
25. S. Azzi-Achkouty, N. Estephan, N. Ouaini, D.N. Rutledge, *Crit. Rev. Food Sci. Nutr.*, 57 (2017) 2009–2020.
26. M. Vakinti, S.-M. Mela, E. Fernández, E. Psillakis, *J. Chromatogr. A*, 1602 (2019) 142–149.
27. M.L. Villalonga, B. Borisova, C.B. Arenas, A. Villalonga, M. Arévalo-Villena, A. Sanchez, J.M. Pingarrón, A. Briones-Perez, R. Villalonga, *Sens. Actuators B Chem.*, 279 (2019) 15–21.
28. K. Thangavelu, S. Palanisamy, S.-M. Chen, V. Velusamy, T.-W. Chen, S.K. Ramaraj, *J. Electrochem. Soc.*, 163 (2016) B726–B731.
29. M. Sakthivel, S. Ramaraj, S.-M. Chen, B. Dinesh, H.V. Ramasamy, Y. Lee, *Anal. Chim. Acta*, 1006 (2018) 22–32.
30. P. Giménez-Gómez, M. Gutiérrez-Capitán, F. Capdevila, A. Puig-Pujol, C. Fernández-Sánchez, C. Jiménez-Jorquera, *Anal. Chim. Acta*, 905 (2016) 126–133.

31. K. Thangavelu, N. Raja, S.-M. Chen, W.-C. Liao, *J. Colloid Interface Sci.*, 501 (2017) 77–85.
32. H. Zhao, J. Xu, Q. Sheng, J. Zheng, W. Cao, T. Yue, *J. Electrochem. Soc.*, 166 (2019) H404–H411.
33. A. Karczmarczyk, A.J. Baeumner, K.-H. Feller, *Electrochimica Acta*, 243 (2017) 82–89.
34. V. Andrei, E. Sharpe, A. Vasilescu, S. Andreescu, *Talanta*, 156 (2016) 112–118.
35. L. Norocel, G. Gutt, *Aust. J. Grape Wine Res.*, 25 (2019) 161–164.
36. M.L. Villalonga, B. Borisova, C.B. Arenas, A. Villalonga, M. Arévalo-Villena, A. Sanchez, J.M. Pingarrón, A. Briones-Perez, R. Villalonga, *Sens. Actuators B Chem.*, 279 (2019) 15–21.
37. K. Thangavelu, S. Palanisamy, S.-M. Chen, V. Velusamy, T.-W. Chen, S.K. Ramaraj, *J. Electrochem. Soc.*, 163 (2016) B726–B731.
38. P. Giménez-Gómez, M. Gutiérrez-Capitán, F. Capdevila, A. Puig-Pujol, C. Fernández-Sánchez, C. Jiménez-Jorquera, *Anal. Chim. Acta*, 905 (2016) 126–133.
39. C. Capannesi, I. Palchetti, M. Mascini, A. Parenti, *Food Chem.*, 71 (2000) 553–562.
40. R. Gusmão, V. López-Puente, I. Pastoriza-Santos, J. Pérez-Juste, M.F. Proença, F. Bento, D. Geraldo, M.C. Paiva, E. González-Romero, *RSC Adv.*, 5 (2015) 5024–5031.
41. S. Ahmed, S. Tabassum, F. Shakeel, A.Y. Khan, *J. Electrochem. Soc.*, 159 (2012) F103–F109.
42. A. Doménech-Carbó, A.M. Ibars, J. Prieto-Mossi, E. Estrelles, F. Scholz, G. Cebrián-Torrejón, M. Martini, *New J. Chem.*, 39 (2015) 7421–7428.
43. I. Domínguez, A. Doménech-Carbó, *Sens. Actuators B Chem.*, 210 (2015) 491–499.
44. S. Guerrini, S. Mangani, Y. Romboli, S. Luti, L. Pazzagli, L. Granchi, *Fermentation*, 4 (2018) 26.
45. N. Dzedze, V. Van Breda, R. Hart, J. Van Wyk, *Food Control*, 105 (2019) 265–276.
46. A. De-La-Fuente-Blanco, M.-P. Sáenz-Navajas, D. Valentin, V. Ferreira, *Food Chem.*, 307 (2020) 125553.
47. K. Hu, G.-J. Jin, W.-C. Mei, T. Li, Y.-S. Tao, *Food Chem.*, 239 (2018) 495–501.
48. K. Hu, G.-J. Jin, Y.-H. Xu, S.-J. Xue, S.-J. Qiao, Y.-X. Teng, Y.-S. Tao, *Food Res. Int.*, 123 (2019) 559–566.
49. T. Opach, J.K. Rød, *Inf. Vis.*, 17 (2018) 108–127.
50. L. Fu, M. Wu, Y. Zheng, P. Zhang, C. Ye, H. Zhang, K. Wang, W. Su, F. Chen, J. Yu, *Sens. Actuators B Chem.*, 298 (2019) 126836.
51. L. Fu, Y. Zheng, P. Zhang, H. Zhang, W. Zhuang, H. Zhang, A. Wang, W. Su, J. Yu, C.-T. Lin, *Biosens. Bioelectron.*, 120 (2018) 102–107.
52. L. Fu, Y. Zheng, P. Zhang, H. Zhang, M. Wu, H. Zhang, A. Wang, W. Su, F. Chen, J. Yu, *Bioelectrochemistry* (2019).
53. Y. Lu, Y. Xu, H. Shi, P.Z.H. Zhang, L. Fu, *Int J Electrochem Sci*, 15 (2020) 758–764.
54. L. Gougeon, G. Da Costa, I. Le Mao, W. Ma, P.-L. Teissedre, F. Guyon, T. Richard, *Food Anal. Methods*, 11 (2018) 3425–3434.
55. F.J.V. Gomez, M.F. Silva, *Anal. Bioanal. Chem.*, 408 (2016) 8643–8653.
56. H. Campbell-Sills, V. Capozzi, A. Romano, L. Cappellin, G. Spano, M. Breniaux, P. Lucas, F. Biasioli, *Int. J. Mass Spectrom.*, 397–398 (2016) 42–51.
57. Z. Genisheva, C. Quintelas, D.P. Mesquita, E.C. Ferreira, J.M. Oliveira, A.L. Amaral, *Food Chem.*, 246 (2018) 172–178.