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Research Article

Lycium species and variety recognition technology based on electrochemical sensing of leaf signals

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Abstract

Identification of plant species and variety has important application value in the process of agricultural production. In this work, we try to use electrochemical fingerprinting technology to collect the electrochemical behavior of electrochemically active substances in plant leaf tissues. Twenty *Lycium* species and varieties were specifically selected to investigate the recognition ability of electrochemical fingerprinting. Two different extraction solvents and electrolytes were used to create different collection environments. The results show that different *Lycium* spp. can exhibit different electrochemical fingerprints. Different species of the same species exhibit relatively similar electrochemical fingerprints. After the second derivative processing, the electrochemical fingerprint of plants can be used for classification and recognition by different machine learning models. Partial least squares discriminant analysis (PLS-DA), k-nearest neighbor, (KNN), support vector machine (SVM), random forest (RF) and stochastic gradient boosting (SGB) were used to establish recognition model of *Lycium* spp. The results show that SGB has the best identification accuracy for electrochemical fingerprint after second derivative treatment.

Keywords: electrochemical fingerprint; goji berry; species identification; machine learning; phytochemistry

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Introduction

Goji berry is a traditional Chinese plant that can be used both in food and medicine. Goji berry resources are divided into 7 species. In addition, over the years of breeding, there are many different varieties of goji berry (Vidović *et al.*, 2022; Fatchurrahman *et al.*, 2022; Liu *et al.*, 2022). Different varieties of goji berry have their own unique advantages, nutritional composition and physiological active ingredients in the content and effect of different (Skenderidis *et al.*, 2022; Li *et al.*, 2021). For example, *Lycium ruthenicum* is rich in anthocyanins, proanthocyanidins, flavonoids, polysaccharides and other physiological components (Yun *et al.*, 2022; Tian *et al.*, 2021; Qin *et al.*, 2021). It has a significant effect on immune regulation, which is 22 times and 55 times of vitamin C and vitamin E respectively. *Lycium barbarum* (Ningqi No. 7) was developed from the Ningxia goji berry production park in 2010 (Qin *et al.*, 2022). This variety has bright red fruits, large and uniform grains and high reducing sugar content. Through the study of pharmacology and composition, it is found that its polysaccharide can regulate the immune function of the body, and has obvious inhibitory effect on human lung cancer cells and cervical cancer cells (Lv *et al.*, 2021; Zhen *et al.*, 2021; Ma *et al.*, 2023). Therefore, it is the best way to trace the origin of *Lycium barbarum* by using various analytical techniques to determine species and varieties based on their own characteristics. The rapid development of modern instrumental analysis technology and computer technology provides conditions for accurate tracing of agricultural food origin. Compared with the traditional analysis methods, the modern instrumental analysis technology has the advantages of high sensitivity, low detection limit, simple and fast, so it is widely used in the quality control process of various foods (Karimi-Maleh *et al.*, 2023).

Plant recognition based on picture and spectrum technology is the most important two strategies (Wäldchen *et al.*, 2018). Image recognition has advantages across different genera, but there are many challenges within the same genus (Wäldchen *et al.*, 2018). Near infrared spectroscopy is one of the most important spectral techniques. The near-infrared spectrum region is consistent with the combined frequency and the frequency doubling absorption region of the hydrogen-containing groups (O-H, N-H, C-H) vibration in organic molecules (Kademi *et al.*, 2019; Beć *et al.*, 2019). Most of the characteristics of hydrogen groups in organic molecules can be obtained by scanning the near infrared spectrum of the samples to be tested. Near infrared spectroscopy is convenient, rapid, efficient, accurate and reliable, and has the advantages of low cost and no damage to the sample (Bisutti *et al.*, 2019). Near infrared spectroscopy has experienced about half a century of development journey so far has become one of the most promising analysis techniques in the new century. Near infrared spectrum analysis technology generally includes two processes, namely model building and classification recognition (Beć *et al.*, 2020). The model is established by associating the near infrared spectrum with the component content or property type of plant samples to be measured by stoichiometric method, and then determining the relationship between the two. After the optimal classification recognition model is selected, the optimal classification recognition model can be established to classify and identify the varieties of samples to be tested. Gas Chromatography-Mass Spectrometry (GC-MS) is a powerful technique for the analysis of volatile compounds in plants. It can identify a large number of compounds and provide information on the molecular weight, structure, and abundance of each compound. GC-MS can be used to analyze the essential oils and other volatile compounds of plants, which can be used for species identification (Rohloff, 2015). High-Performance Liquid Chromatography (HPLC) is a powerful technique for the separation and quantification of various chemical compounds in plants. HPLC can be used to analyze various plant pigments, flavonoids, and other chemical compounds, which can be used for plant identification. Nuclear Magnetic Resonance Spectroscopy (NMR) is a powerful technique that can be used to identify the structure of organic molecules in plant samples. It can provide information on the molecular structure, the number of protons and carbon atoms, and the connectivity of atoms in molecules. NMR can be used for the identification of different plant species and the analysis of plant metabolites (Leiss *et al.*, 2011).

In recent years, we have proposed an analytical technique based on electrochemical fingerprinting (Zhou *et al.*, 2020; Wang *et al.*, 2020; Xu *et al.*, 2020; Fan *et al.*, 2021; Zheng *et al.*, 2021; Fu *et al.*, 2021; Wang *et al.*, 2021). It can collect oxidation signal of electrochemically active substances in plants. It has been used to identify different plants. However, the efficiency of identification has been a challenge. In this work, we collected the leaves of 20 species of *Lycium spp.* (species + variety) and collected their electrochemical fingerprints. The raw data of the electrochemical fingerprint were processed with second derivatives. Partial least square-discriminant analysis (PLS-DA), linear support vector classification (LinearSVC) and random forest (RF) were used to identify different species.

Materials and Methods

All *Lycium* spp. were collected June 2022 at Wolfberry resource nursery in Ningxia and identified by botanist in Ningxia Academy of Agricultural and Forestry Sciences. All information is listed in Table 1. Water and ethanol have been used as extraction solvent. The extraction process was conducted accordingly to our previous report (Zhang *et al.*, 2022). Phosphate-buffered solution (0.1 M, PBS, pH 7.0) and acetate-buffered solution (0.1 M, ABS, pH 4.5) were used as electrolyte.

All electrochemical fingerprints of the *Lycium* spp. were collected using a CHI1210 electrochemical workstation. A typical three-electrode system has been used. A glassy carbon electrode, a Pt wire and a Ag/AgCl (3 M KCl) were used as working electrode, counter electrode and reference electrode, respectively. For electrochemical fingerprints collection, 1 mL of *Lycium* spp. extract was added into 4 mL of electrolyte. Then, differential pulse voltammetry (DPV) was used for scanning in the range of -0.2 V to 1.0 V (pulse amplitude: 50 mV; pulse width: 0.05 s; pulse period: 0.5 s). The experimental data was then normalized for further analysis.

The second derivative is used for electrochemical fingerprinting of all species. Stoichiometric methods and machine learning algorithms were used to identify differences in the electrochemical fingerprint data of different species between samples. Partial least square-discriminant analysis (PLS-DA), linear support vector classification (LinearSVC) and random forest (RF) were used to identify different species. The confusion matrix is used to evaluate the effectiveness of the classification model.

Table 1. Sample information of collected *Lycium* spp.

Species/Variety	Abbreviation	Species/Variety	Abbreviation
L. barbarum (Ningqi No.1)	LN-1	*L. barbarum* (Ningqi No.2)	LN-2
L. barbarum (Ningqi No.3)	LN-3	*L. barbarum* (Ningqi No.4)	LN-4
L. barbarum (Ningqi No.5)	LN-5	*L. barbarum* (Ningqi No.6)	LN-6
L. barbarum (Ningqi No.7)	LN-7	*L. barbarum* (Ningqi No.8)	LN-8
L. barbarum (Ningqi No.9)	LN-9	*L. barbarum* (Ningqi No.10)	LN-10
L. chinense	LC	*L. ruthenicum*	LR
L. barbarum	LB	*L. truncatum*	LT
L. yunnanense	LY	*L. chinense var. potaninii*	LCP
Wild species	WS	*L. cylindricum*	LCD
L. barbarum (Ningnongqi No.4)	LNN-4	*L. barbarum* (Ningnongqi No.5)	LNN-5

Results and Discussion

Figure 1 shows the electrochemical fingerprint of all *Lycium spp.* after ethanol extraction under PBS. It can be seen that many of these *Lycium spp.* have a very distinct electrochemical oxidation peak around 0.2 V.

This may be the electrochemical oxidation caused by different kinds of flavones in *Lycium* spp. (Montesano *et al.*, 2019; Liu *et al.*, 2019). The intensity of this peak varies among *Lycium* spp. For example, this electrochemical oxidation peak of most *L. barbarum* (Ningqi series) has a distinct current except for LN-1 and LN-10. In contrast, the current of this electrochemical oxidation peak of *L. barbarum* as a primitive species is not prominent. This is most likely due to the selection of nutritionally valuable individuals by *L. barbarum* during the development of the variety. It also proves that the variety development of *Lycium barbarum* is indispensable for the resource utilization of this plant (Li *et al.*, 2019; Qiao *et al.*, 2022; Chen *et al.*, 2017). LN-1 in *L. barbarum* (Ningqi series) has only a weak oxidation band at 0.2 V. This is unique fingerprint information for this variety. Another conclusion that can be drawn from Figure 1 is that different species of the same species have relatively similar electrochemical fingerprint profiles. This phenomenon has been observed not only in the Ningqi series of *L. barbarum*, but also confirmed in the Ningnongqi series. In addition, the fingerprints of different species are quite different. For example, *L. yunnanense* has a very distinct oxidation peak around 0.45V. *L. chinense* var. *potaninii* has a series of oxidation peaks between 0.2 and 0.6 V. *L. truncatum* had a weak oxidation peak at 0.85 V. However, a similar electrochemical fingerprint is also shown in Figure 1. For example, LN-9 and WS share similar electrochemical behavior, both having a distinct oxidation peak at 0.2 V. In addition, they have another wide electrochemical oxidation band at around 0.5 V. There is also a similar electrochemical behavior between LB and LN-4. They all have two distinct electrochemical oxidation peaks at 0.2 V and 0.53 V respectively. Moreover, the oxidation current of the second oxidation peak is slightly higher than that of the first oxidation peak.

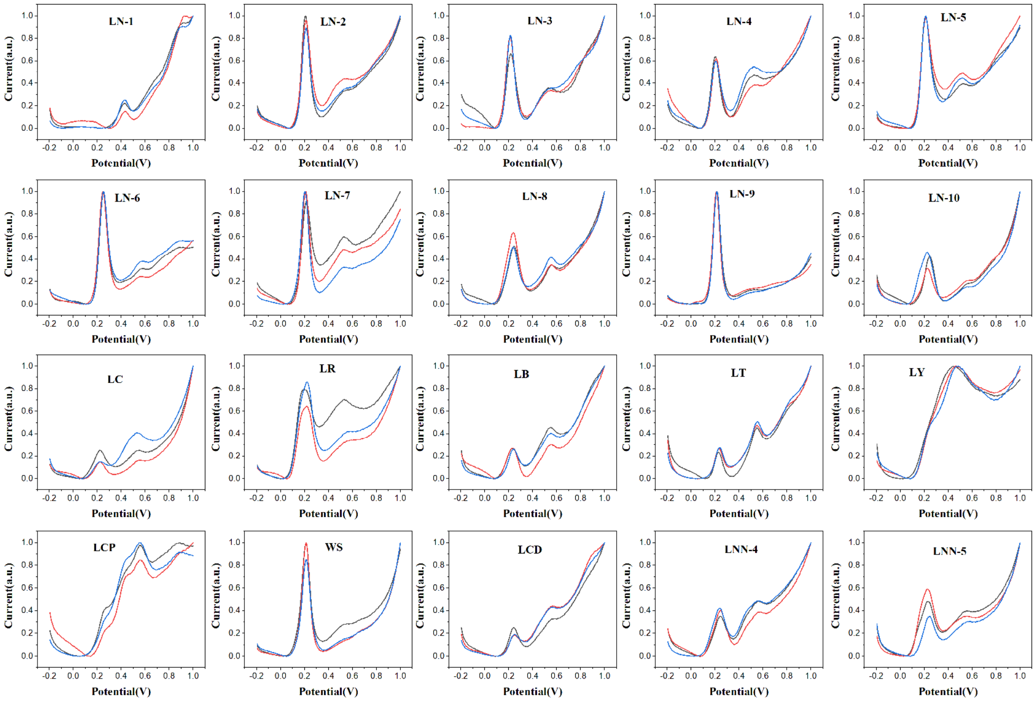


Figure 1. Electrochemical fingerprints of all *Lycium spp.* collected ethanol extraction using PBS as electrolyte

Figure 2 shows electrochemical fingerprints of all *Lycium spp.* collected water extraction using ABS as electrolyte. Different solvents can extract different substances from plant tissues. In addition, different electrolyte environments allow different electrochemically active substances to participate in electrochemical reactions. Thus, the same species/variety exhibits different electrochemical fingerprints in Figures 1 and 2. The addition of an electrochemical fingerprint can help to effectively identify different plants. In addition, it also improves the abundance of electrochemical signals, which helps to find out the differences in the electrochemically active substances of different samples. As can be seen from Figure 2, even the different varieties in *L. barbarum* (Ningqi series) exhibit different electrochemical behaviours. LN-1 still shows different electrochemical behavior with various variety. The remaining 9 varieties can be divided into two categories. LN-2, LN-5, LN-6, LN-7, LN-8, and LN-10 all exhibit a distinct oxidation peak near 0.4 V. The remaining varieties have a relatively weak oxidation peak at this potential. LC, as the primary species, showed only weak oxidation peak at this potential. Combined with the fingerprint information in Figures 1 and 2, the different varieties in *L. barbarum* (Ningqi series) can be identified to a certain extent. Both varieties of *L. barbarum* (Ningnongqi series) have a distinct electrochemical oxidation peak at 0.4 V, but LNN-5 has another distinct oxidation band at 0.7 V attachment. However, the electrochemical fingerprints taken under these conditions also showed some similarities. For example, LR and LCD have similar intensities at 0.4 V peaks. In addition, they have no other characteristics that can be used for quick identification. The electrochemical fingerprints of LN-7 and LB are also very similar and difficult to identify directly. Therefore, signal processing of electrochemical fingerprint is very necessary for sample identification. All species/variety in Figures 1 and 2 were fingerprinted from three separate samples and showed excellent stability. Therefore, post-processing of the original fingerprint data has the potential to be used for rapid identification.

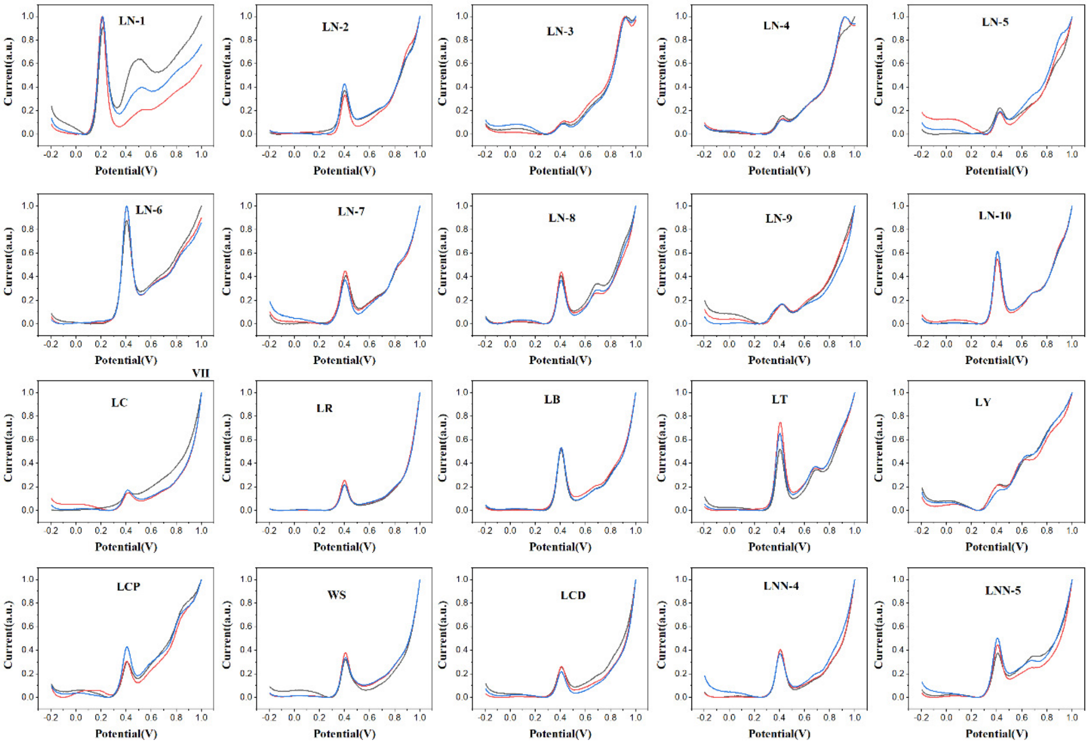


Figure 2. Electrochemical fingerprints of all *Lycium spp.* collected water extraction using ABS as electrolyte

Figure 3 and Figure 4 show the electrochemical fingerprint data of all *Lycium spp.* after the second derivative processing. It can be seen that the differences in electrochemical oxidation peaks of different samples are amplified. Then, partial least squares discriminant analysis (PLS-DA), k-nearest neighbor, (KNN), support vector machine (SVM), random forest (RF) and stochastic gradient boosting (SGB) were used to establish recognition model of *Lycium spp*. Table 1 shows the classification results of various learning methods.

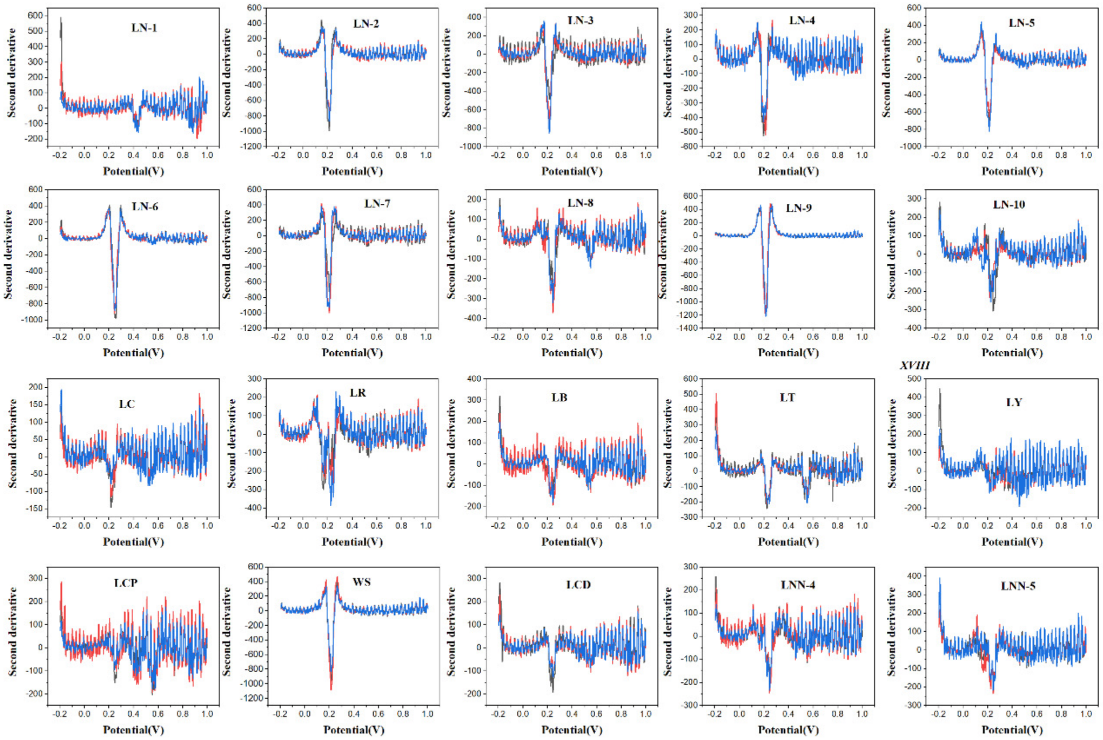


Figure 3. The second derivative curve of electrochemical fingerprints collected ethanol extraction using PBS as electrolyte

In the PLS-DA classification results, the specificity, sensitivity and accuracy of the training set of electrochemical fingerprints after the second derivative processing are better than the original data. However, the electrochemical fingerprint processed by second derivative in test set did not get the ideal result, and the accuracy was only 62.67%. The recognition accuracy of PLS-DA for the original electrochemical fingerprint was only 87.33%. Therefore, PLS-DA can only be applied to the identification of the original electrochemical fingerprint of *Lycium* spp.

In the KNN classification results, the specificity, sensitivity and accuracy of the training set of raw data are better than the electrochemical fingerprint after the second derivative processing. The test set shows the same trend. The accuracy of electrochemical fingerprint after second derivative treatment was only 56.67%. However, KNN does not support the identification of the original electrochemical fingerprint well. It is only 72.20% accurate with raw data. Therefore, KNN is not suitable for the identification of the original electrochemical fingerprint of *Lycium* spp. and the fingerprint treated by the second derivative.

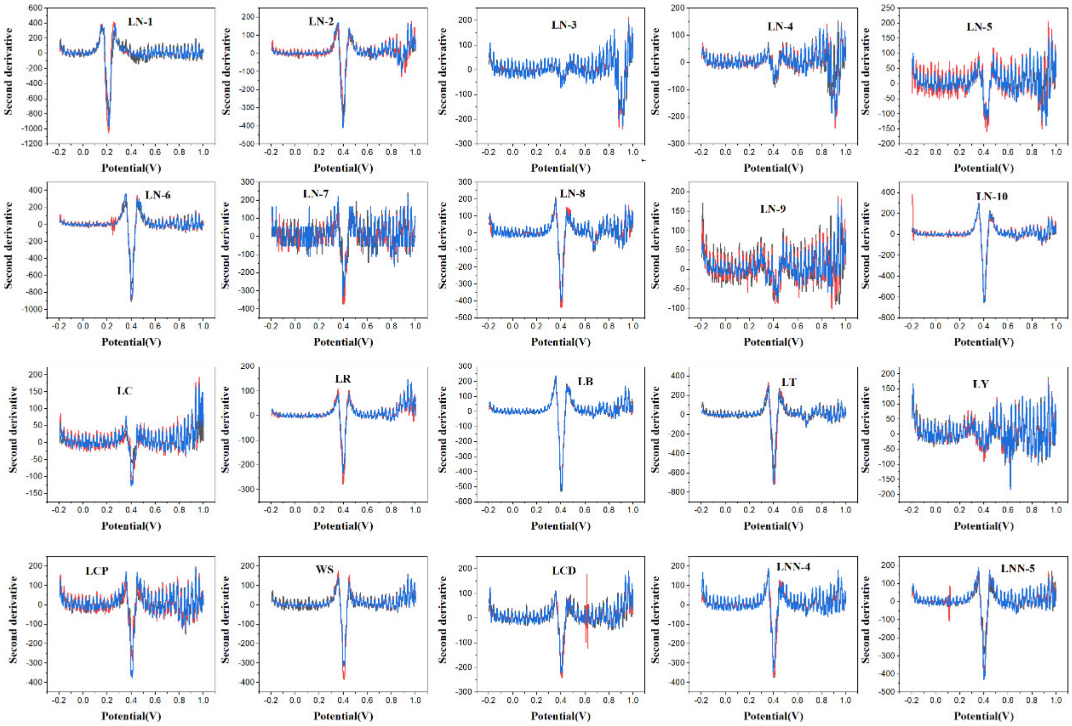


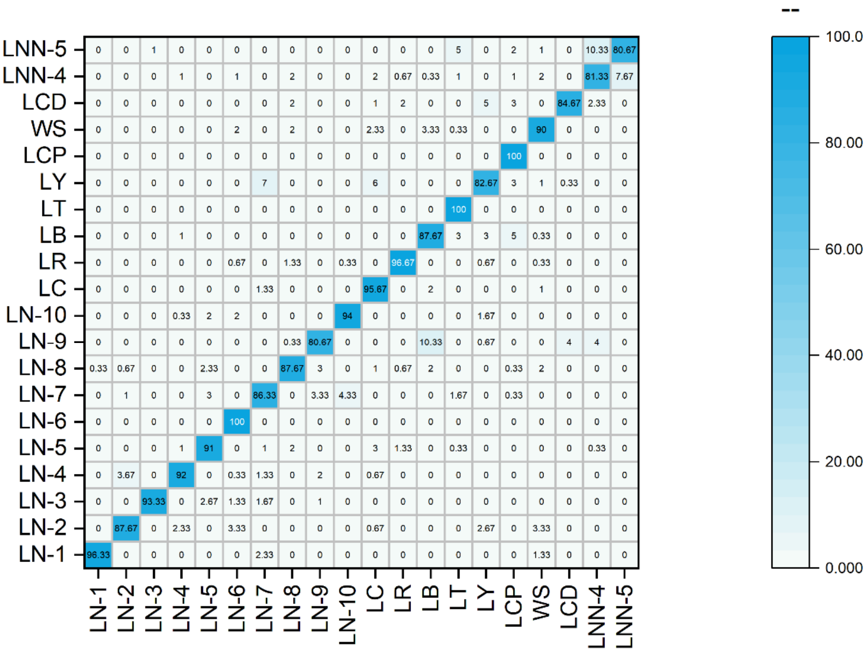
Figure 4. The second derivative curve of electrochemical fingerprints collected water extraction using ABS as electrolyte

In the SVM classification results, both the training set and the test set show good results. The specificity, sensitivity and accuracy of the training set of electrochemical fingerprint after the second derivative processing are better than the original data. The recognition rate of SVM for original data can reach 85.33%. It can achieve 91.00% accuracy on the data processed by the second derivative.

In the RF classification results, whether the electrochemical fingerprint has been treated with the second derivative has no significant effect. The recognition rate of RF towards *Lycium spp*. was lower than 90% in both the training set and the test set. This indicates that the second derivative treatment has relatively little effect on RF classification.

Table 2. Classification results of PLS-DA, KNN, SVM, RF, SGB towards electrochemical fingerprints of all *Lycium* spp

Model	Data	Training set			Test set		
		Sp (%)	Sn (%)	Ac (%)	Sp (%)	Sn (%)	Ac (%)
PLS-DA	Raw	90.67	91.33	90.00	81.33	96.33	87.67
	SD	96.33	97.67	97.67	73.33	54.33	62.67
KNN	Raw	78.33	94.00	85.57	65.65	80.00	72.67
	SD	54.33	78.33	50.00	72.33	53.00	56.67
SVM	Raw	90.67	95.67	96.20	80.67	93.67	85.33
	SD	95.33	96.67	98.00	84.00	94.33	91.00
RF	Raw	75.33	78.33	77.50	67.67	86.67	77.00
	SD	76.67	80.00	81.67	80.33	73.33	76.67
SGB	Raw	96.67	95.00	96.67	65.67	86.67	76.67
	SD	100.00	100.00	100.00	93.33	86.67	93.33



	LN-1	LN-2	LN-3	LN-4	LN-5	LN-6	LN-7	LN-8	LN-9	LN-10	LC	LR	LB	LT	LY	LCP	WS	LCD	LNN-4	LNN-5
LNN-5	0	0	1	0	0	0	0	0	0	0	0	0	0	5	0	2	1	0	10.33	80.67
LNN-4	0	0	0	1	0	1	0	2	0	0	2	0.67	0.33	1	0	1	2	0	81.33	7.67
LCD	0	0	0	0	0	0	0	2	0	0	1	2	0	0	5	3	0	84.67	2.33	0
WS	0	0	0	0	0	2	0	2	0	0	2.33	0	3.33	0.33	0	0	90	0	0	0
LCP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0
LY	0	0	0	0	0	0	7	0	0	0	6	0	0	0	82.67	3	1	0.33	0	0
LT	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0
LB	0	0	0	1	0	0	0	0	0	0	0	0	87.67	3	3	5	0.33	0	0	0
LR	0	0	0	0	0	0.67	0	1.33	0	0.33	0	96.67	0	0	0.67	0	0.33	0	0	0
LC	0	0	0	0	0	0	1.33	0	0	0	95.67	0	2	0	0	0	1	0	0	0
LN-10	0	0	0	0.33	2	2	0	0	0	94	0	0	0	0	1.67	0	0	0	0	0
LN-9	0	0	0	0	0	0	0	0.33	80.67	0	0	0	10.33	0	0.67	0	0	4	4	0
LN-8	0.33	0.67	0	0	2.33	0	0	87.67	3	0	1	0.67	2	0	0	0.33	2	0	0	0
LN-7	0	1	0	0	3	0	86.33	0	3.33	4.33	0	0	0	1.67	0	0.33	0	0	0	0
LN-6	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LN-5	0	0	0	1	91	0	1	2	0	0	3	1.33	0	0.33	0	0	0	0	0.33	0
LN-4	0	3.67	0	92	0	0.33	1.33	0	2	0	0.67	0	0	0	0	0	0	0	0	0
LN-3	0	0	93.33	0	2.67	1.33	1.67	0	1	0	0	0	0	0	0	0	0	0	0	0
LN-2	0	87.67	0	2.33	0	3.33	0	0	0	0	0.67	0	0	0	2.67	0	3.33	0	0	0
LN-1	96.33	0	0	0	0	0	2.33	0	0	0	0	0	0	0	0	0	1.33	0	0	0

Figure 5. Confusion matrix of identification of plant species using electrochemical fingerprint via SGB

In the SGB classification results, the recognition accuracy of electrochemical fingerprint was significantly improved by second derivative processing. The recognition rate of SGB was only 76.67% for the original data, but 93.33% for the electrochemical fingerprint processed by the second derivative.

Based on the above results, it can be seen that the classification result of SGB is the best. The specificity, sensitivity and accuracy of the training set are no less than 90. 00%. After the second derivative processing, the specificity and sensitivity of the test set of electrochemical fingerprint are no less than 80. 00%, and the accuracy is 93.33%. SVM classification effect is also acceptable. The specificity, sensitivity and accuracy of the training set are no less than 90. 00%. After the second derivative processing, the accuracy can reach 91.00%. The obfuscation matrix visualizes the comparison between the predicted value and the true value of the model in matrix form (Ali *et al.*, 2020). Each row in the matrix represents the predicted value of a different species/variety, and each column represents the true value of a different species/variety. In the confusion matrix of this experiment, blue indicates the accuracy of recognition. The depth of the color is proportional to the more accurate the recognition. Figure 5 shows the confusion matrix of different species/variety identified by the SGB model. It can be seen that most species/variety can be recognized. In future work, other data processing methods need to be tried to optimize the recognition efficiency. At the same time, different models can be tried. Spectral data from plant samples are widely used for species identification, but this technique is not widely used in electrochemical fingerprinting. This may be because electrochemical fingerprinting is a new fingerprint technology developed in recent years. Because the collection of electrochemical signals does not involve the separation of samples, its accuracy is limited. Our work explores the feasibility of applying common classification models to electrochemical fingerprinting.

Conclusions

This work took the electrochemical fingerprints of 20 species/varieties of *Lycium spp*. Two different extraction solvents and electrolytes were used to prepare different collection environments. Different species/varieties exhibit different electrochemical behavior. Among them, the species in the same species have relatively similar electrochemical fingerprints. These electrochemical fingerprints were processed with second derivatives and used to identify different machine learning models. The results show that SGB has the best identification accuracy for electrochemical fingerprint after second derivative treatment.

Authors' Contributions

Conceptualization, L.F. and X.W.; methodology, L.F.; software, J.Z.; validation, X.S. and J.M; formal analysis, X.S., J.M. and W.Y.; investigation, X.S., J.M. and W.Y.; resources, Y.Z. and Y.Y.; data curation, S.X. M.J. and Y.N.; writing—original draft preparation, X.S. W.Y and J.Z.; writing—review and editing, L.F. Y.Z. and Y.N.; visualization, X.W. and Y.Y.; supervision, L.F. and X.W.; project administration, L.F. and X.W. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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