



Rapid Identification of Orange Juice Adulteration Using Voltammetric Profiling and Machine Learning

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Abstract: In this study, the differential pulse voltammetry with a gold electrode and machine learning was employed to detect adulteration in orange juices. The method assessed both natural and commercial juices, along with their mixtures containing known proportions of natural juice. Initially, an unsupervised machine learning algorithm, Cluster Analysis (CA), was used to highlight differences, demonstrating the ability to distinguish between natural and flavored orange juices. Subsequently, supervised machine learning methods, including Interval Partial Least Squares – Linear Discriminant Analysis (iPLS-LDA) and Interval Partial Least Squares – Random Forest (iPLS-RF), were applied for classification purposes. The RF model achieved up to 95% classification accuracy, greatly exceeding 67.5% of iPLS-LDA. This enables reliable detection of orange juice adulteration. The RF model struggled to accurately distinguish between the “Natural” and “Mixed” categories, particularly for samples containing a medium proportion of natural orange juice (around 45–50%). The integration of voltammetric fingerprints with machine learning enabled a fast, cost-effective classification method for on-site analysis with portable sensors. This approach proved more efficient than other complex analytical techniques.

Keywords: Voltammetry; machine learning; orange juice adulteration; Interval Partial Least Squares (iPLS); Linear Discriminant Analysis (LDA);

1. Introduction

In the food industry, classifying and controlling the quality of agricultural products, especially fruits, is essential for maintaining standards and protecting consumer health. Among these fruits, oranges are highly valued for their rich content of vitamins, fiber, and antioxidants. However, due to their popularity, fresh orange juice products are frequently targeted for adulteration with chemical flavorings [1], leading to counterfeit products [2, 3] and commercial fraud involving natural orange juice. Recent advancements in machine learning have greatly improved the processing of large datasets, enabling rapid chemical analysis of various products. This has enhanced adulteration detection and geographical indication verification with high sensitivity and accuracy. As a result, machine learning has become a valuable tool in safeguarding food integrity and ensuring the authenticity of agricultural and food products [4, 5]. To

identify and classify analytical samples, two basic approaches are commonly employed: i) Targeted analysis: This method utilizes datasets containing the concentrations of specific compounds in multiple samples. Certain compounds serve as markers to assess authenticity and classify products. For example, polyphenol content can help detect adulteration in different types of orange juice [6, 7], while flavonoid content can distinguish grapefruit juice from other citrus juices [8] or differentiate concentrated and non-concentrated orange juices with high accuracy [9]. However, targeted analysis requires high analytical costs, as managing multiple parameters across samples demands significant resources. ii) Non-targeted analysis: This approach considers the entire dataset, including all measured signals, without identifying specific compounds. Spectroscopic techniques such as UV-VIS, NIR [11], Raman [10], and NMR [12] have been effective in providing data for statistical analysis, enabling the detection and quantification of adulteration in orange and grapefruit juices [10].

Machine learning algorithms enhance classification accuracy in non-targeted analysis. Unsupervised methods like Principal Component Analysis (PCA), Support Vector Machines (SVM), Data-Driven SIMCA, and soft-PLS-DA outperform traditional PLS-DA. Additionally, algorithms such as logistic regression, PCA, SVM, and Artificial Neural Networks (ANN) have proven effective in handling high-dimensional and complex analytical data. These techniques not only aid in identifying characteristic chemical markers but also optimize classification through highly accurate predictive models [14, 15]. Recently, the integration of multivariate analysis (chemometrics) with electrochemical analysis has offered outstanding advantages, including high sensitivity and selectivity, rapid analysis time, effective data processing, and the ability to eliminate background noise [16]. This approach enables not only the simultaneous quantification of organic acids [17] or polyphenols [18] in fruit juices (e.g., orange, lemon, and others) but also the classification of fruits with an accuracy exceeding 90% [18].

This study aims to assess the feasibility of the voltammetric method with a gold electrode for analyzing natural and commercial orange juice. It focuses on different orange varieties and mixtures of natural and commercial juices. Data from voltammograms (current intensity vs. potential matrices) are combined with machine learning to develop models for classifying natural and commercial orange juice and predicting adulteration ratios. This approach not only enhances the accuracy of food fraud detection but also opens up broader application potential in the food industry.

2.1 Reagents, Reference, and Standard Solutions

Hydrochloric acid (HCl, 37%) was procured from Sigma Aldrich (Singapore). Methanol (Merck, Germany) was utilized for preparing standard solutions, while Milli-Q water used for dilutions was obtained from a Milli-Q water system (Merck, Germany).

2.2 Sampling and sample preparation

All orange samples belonged to the *Citrus sinensis* variety and were collected from various regions of Vietnam. A total of 80 samples were sourced from 8 provinces: Hoa Binh, Ha Giang, Ben Tre, Vinh Long, Nghe An, Bac Giang, Hung Yen, and Quang Ninh. Each fruit was cut and juiced. Additionally, 50 commercial orange juice samples were collected from supermarkets in Hanoi, Vietnam. These samples included bottled or canned juices from brands such as Mirinda, Fanta, TH, Splash. For the adulteration study, two samples of pure natural orange juice were mixed with commercial orange juice at nine different adulteration levels: 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 90% natural orange juice. Each adulteration level was prepared in triplicate. A total of 54 blended samples was synthesis. Additionally, there is one sample of difference that was mixed (the other orange juice sample was mixed with a commercial sample at 10, 40, 60, and 90%) and added to the data. The sample preparation was simple, starting with centrifugation of the juices for 10 minutes at 13,500 rpm. After that, a 1:4 dilution with 0.1 M HCl was performed to reach a pH of about 1.2. Each sample was prepared in triplicate. The samples were freshly prepared and measured immediately without storage.

2.3 Data Acquisition

Electrochemical measurements were conducted using the 797 VA Computrace instrument (Metrohm, Switzerland). This instrument, controlled by VA Computrace software, consisted of a complete measurement system, including a gold working electrode, an Ag/AgCl reference electrode, and a platinum auxiliary

electrode. For each sample, a 100 μL droplet was used as the sample volume. Differential pulse voltammetry (DPV) was employed to record voltammograms under the following experimental conditions: Scan range: -0.2 V to +1.2 V (vs. Ag/AgCl); Pulse amplitude: 50 mV; Step potential: 5 mV; Scan rate: 10 mV/s. The raw data matrices (I-E) extracted from Voltammograms of samples under the conditions

2.4 Data Analysis

To perform an exploratory analysis of the data, Cluster Analysis (CA) was employed. Raw data from all samples were utilized, and the mean value of the three replicates for each sample was calculated and used in the analysis. To assess the effectiveness of the methodology, confusion matrices were generated, and performance metrics such as accuracy were determined. Accuracy was calculated as the ratio of correctly classified samples to the total number of samples, providing a clear measure of the model's classification performance.

2.5 Methods

2.5.1 Cosine similarity

$$\cos(\theta) = \frac{x \cdot y}{\|x\| \|y\|}$$

Where x and y are vectors for which the similarity is to be computed.

2.5.2 SNV

To standardize the spectral data, each spectrum $x = (x_1, x_2, \dots, x_k)$ is first centered by removing its mean, and then scaled using the standard deviation. This process results in a normalized vector $z = (z_1, z_2, \dots, z_k)$ where the data have zero mean and unit variance, facilitating comparison across different spectra.

$$z_i = \frac{x_i - \bar{x}}{\sqrt{\sum_j^k (x_j - \bar{x})^2 / k}}$$

The data is standardized using the SNV (Standard Normal Variate) method to remove variations caused by scattering effects and correct baseline shifts. After standardization, the data is further processed using the Savitzky-Golay filter with a window length of 15, a polynomial order of 3, and a second derivative calculation. This step smooths the data, reduces noise, and enhances important spectral features for subsequent analysis.

2.5.3 iPLS.

The data X (p features) is divided into n intervals, and a local PLS model is built for each interval to select the best interval. In PLS regression, similar to PCR, we aim to find components z that are linear combinations of the inputs; however, unlike PCR, PLS seeks components that not only represent the predictors x well but also serve as strong predictors of the response y , under the assumption that both X and y can be explained by a smaller set of components Z with $k < p$.

$$X = ZV^T + E$$

2.5.4 Random Forest

Random Forest is an ensemble learning method that builds multiple decision trees using bootstrap samples and random feature subsets at each split, which increases diversity and reduces correlation among trees. For regression, the final output is the average of predictions from all trees, while for classification, majority voting is used.

$$y = \frac{1}{T} \sum_{t=1}^T h_t(x)$$

where h_t is the predicted value of the t -th tree for the input x , and T is the total number of trees

3. Results and discussion

3.1 Electrochemical Fingerprint of Orange Juices

The voltammetric results of 198 orange juice samples are shown in Fig. 1 (A, B, C). The signal intensity (I) is clearly observed in the range of potential (V) from 0.8 to 1.2 V (versus Ag/AgCl electrode), with intensity of peak currents reaching a maximum between 0.04 mA and 0.6 mA. Some blended samples of natural and artificial orange juice exhibited lower peak maxima, ranging from 0.02 mA to 0.04 mA. However, most signals showed slight differences depending on the geographical origin of the samples and were quite similar to those of natural and commercial samples. This resemblance in peak shapes and minimal variations in peak heights made visual classification impractical. Even within the same category, whether natural or artificial, there were significant variations in peak maxima, which could introduce considerable noise into classification models. Therefore, signal preprocessing is essential before performing discrimination and classification.

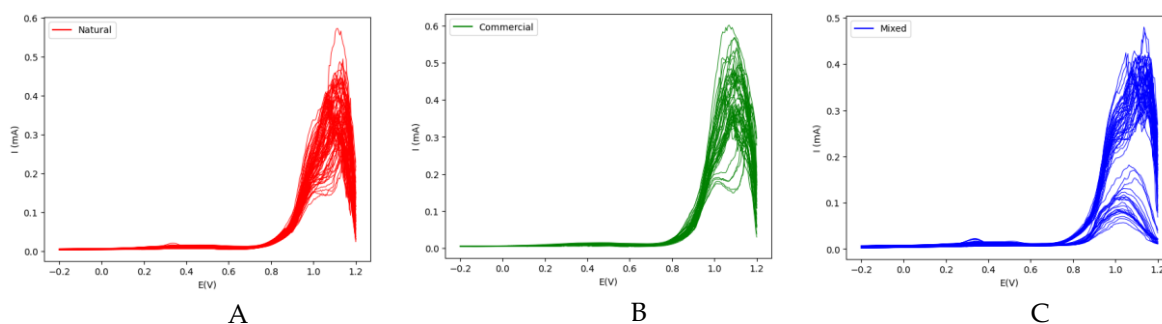


Figure 1. Voltammograms of orange juice (A- natural samples; B- commercial samples, and C- blended samples)
(sửa lại trực tung)

The Standard Normal Variate (SNV) algorithm was used to normalize the data, followed by second-order derivation and smoothing using the Savitsky-Golay algorithm. The preprocessed voltammograms are shown in Figure 2. These preprocessing algorithms significantly reduced background noise, including sample background noise and noise caused by the equipment and experimental environment. Additionally, the second-order derivation enhanced the signal differences between samples. These results contributed to the stability and objectivity of classification models, thereby improving the classification performance of the models. After preprocessing, the entire voltammetric dataset was randomly split into 158 samples (80%) for the training and 40 samples (20% remaining) for testing to perform machine learning methods. The dataset was first preprocessed using Standard Normal Variate (SNV) to correct for scattering effects and variations in sample thickness. Subsequently, the SNV-corrected spectra were smoothed and differentiated using the Savitzky-Golay filter with a 15-point window, a third-order polynomial, and calculation of the second derivative, to enhance subtle spectral features while reducing noise.

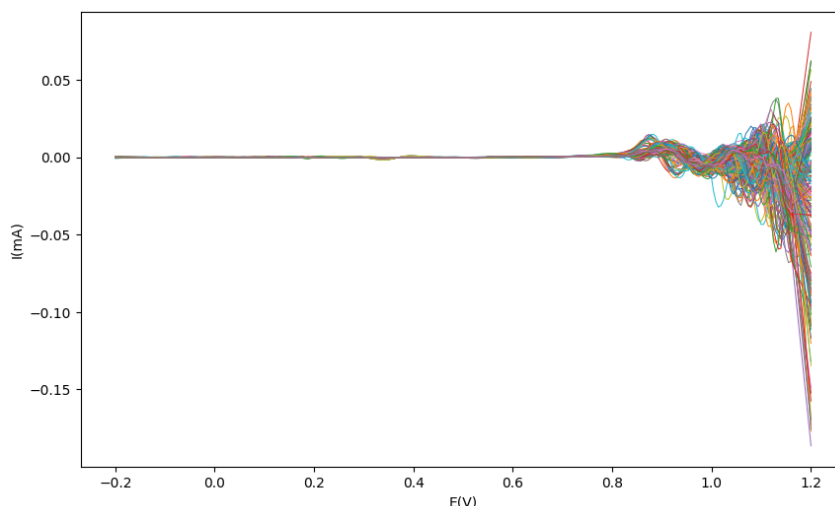


Figure 2. Voltammograms of orange juice after normalization using the SNV algorithm, second-order derivation, and smoothing with the Savitzky-Golay algorithm.

3.2. Identification and authentication of orange juice

Figure 3 illustrates the results of hierarchical clustering analysis (HCA) performed on the entire dataset of preprocessing with SNV using cosine similarity. Cosine similarity was selected as the distance metric for hierarchical clustering because it emphasizes the similarity of signal patterns rather than absolute intensity. In voltammetric data, current intensity may vary due to sample preparation, dilution, or instrumental noise, while the overall shape of the voltammogram remains characteristic of the sample type. The dendrogram shows that, even with unsupervised methods, it is possible to distinguish three data clusters: natural orange juice, commercial orange juice, and mixed orange juice (Table 1).

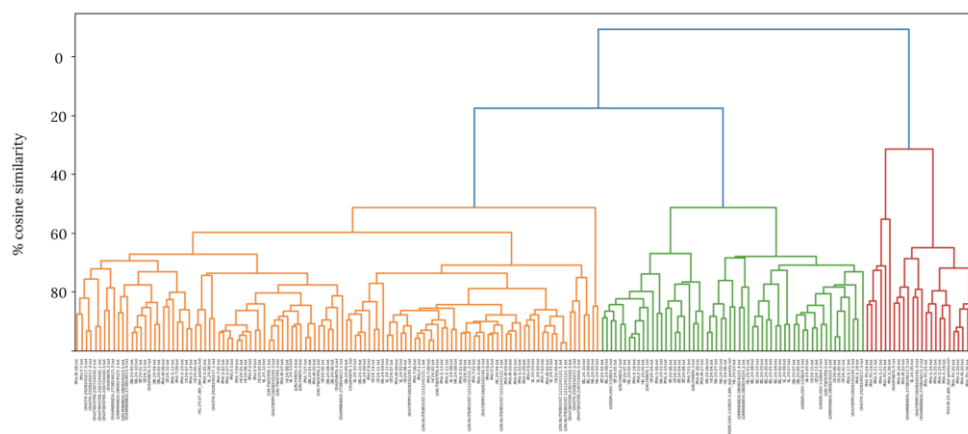


Figure 3. Hierarchical clustering using cosine similarity

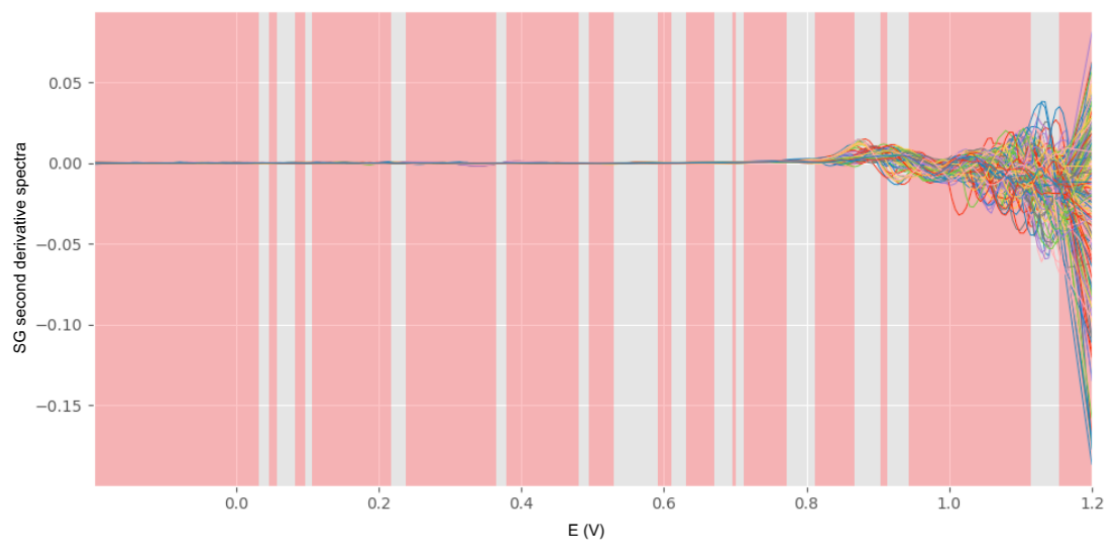
The results indicate that 80% of the mixed juice samples belong to the first group, while 62% of the natural orange juice samples appear in the second group. However, in the third cluster, the distribution of samples across the three categories does not show a clear distinction. Thus, unsupervised algorithms reveal a clear difference between the blended orange juice and natural orange juice groups, providing a foundation for using supervised machine learning models for classification.

Table 1. Percentage of each sample type in each group classified.

Sample Cluster	Mixed	Commercial	Natural
1	80%	16%	4%
2	16%	22%	62%
3	33%	27%	40%

3.3 Supervised learning for the detection of orange juice adulteration

After second-order derivation, the dataset showed a relatively large number of features (284 features obtained), requiring dimensionality reduction. Therefore, the iPLS algorithm was applied to the training dataset to reduce the number of features for building machine learning models. The iPLS algorithm selects the most relevant spectral regions with strong discriminatory power, eliminating noisy or low-information regions, which helps prevent overfitting and enhances model accuracy. The results of the features retained by the iPLS algorithm are displayed in Fig. 4. Among these, 205 features, which do not have good classification potential (highlighted in pink in the figure), were removed, leaving 79 features with the best potential for classification. The test dataset will also use these 79 features for accuracy evaluation.

**Figure 4.** Features selected by the iPLS algorithm

In model selection, factors such as linearity, accuracy, label differentiation (especially between natural and commercial juices), and performance on small datasets were considered. Typically, PLS-DA, Random Forest, Support Vector Machine (SVM), and Artificial Neural Network (ANN) with hyperparameter optimization using GridSearchCV are preferred for classification. However, both ANN and SVM initially achieved only 75% prediction accuracy, making them unsuitable for this study. Additionally, the small dataset restricted the ANN's ability to capture information, while SVM strongly misclassified between natural and commercial orange juices, further reducing its applicability. Therefore, this study only focuses on two models: iPLS-LDA and Random Forest.

3.3.1 iPLS-LDA

The iPLS algorithm was applied for dimensionality reduction using a 158×79 training dataset, consisting of 158 samples classified into three groups (natural, commercial, and blended orange juice) with 79 features extracted from the raw data. An LDA model was then built to classify these groups, but some blended orange juice samples were misclassified as either natural or commercial juice. Linear separation was not achieved due to overlap between the natural and blended orange juice groups. A deeper evaluation of the percentage of natural orange juice in the samples is shown in Fig.5, with the sample colors gradually transitioning from white to dark, representing an increasing proportion of natural orange juice. The triangle markers represent samples from the training dataset, while the circular markers represent samples from the

test dataset. The results indicate that the LDA model struggles to differentiate between natural and blended samples. This suggests that the compounds in natural orange juice may be masking the signals, making classification more challenging.

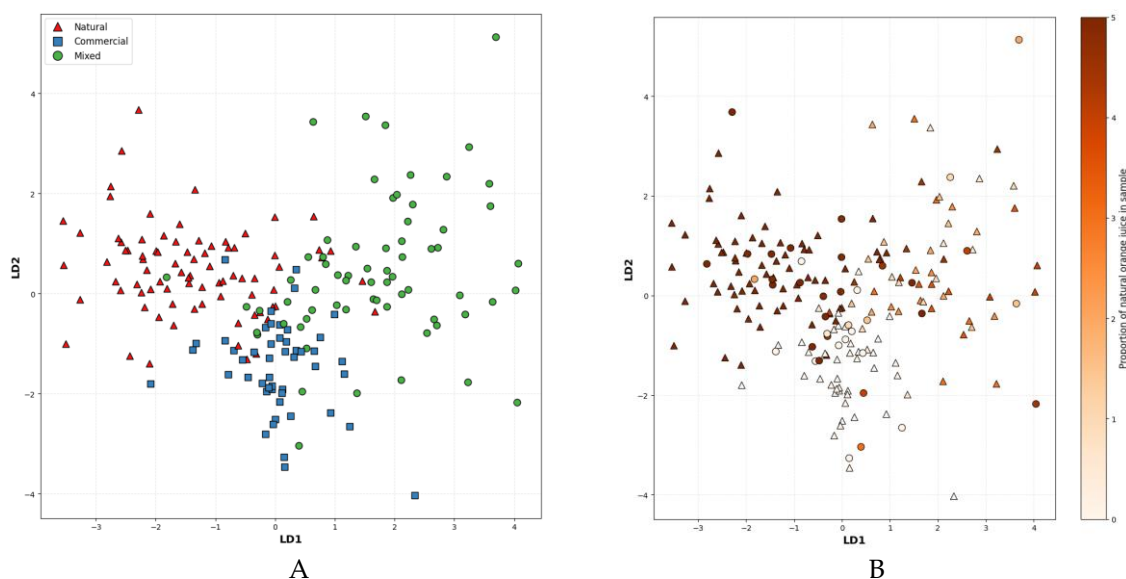


Figure 5. LDA plot of training dataset and test dataset projected onto LDA model space according to: A- corresponding labels and B- the proportion of natural orange juice in each sample.

Based on Fig. 5A, it can be observed that when the training and testing datasets are projected onto the LDA space according to their corresponding labels, there is a significant overlap between the clusters of the "Mixed" and "Commercial" labels. This explains why certain samples are misclassified into the "Mixed" group. This phenomenon reflects the characteristic similarity between commercial and mixed juice samples, especially when the proportion of natural orange juice in these products fluctuates, making it challenging for the model to distinguish between them. Additionally, Fig. 5B provides further insights as the samples are projected onto the LDA space based on the proportion of natural orange juice. It reveals that samples with natural orange juice proportions near the threshold between "Mixed" and "Natural" or "Commercial" are prone to misclassification. Moreover, this issue may stem from the insufficiently strong boundaries between clusters in the LDA space, which fail to fully capture the differences in natural orange juice proportions among the groups.

3.3.2 iPLS- RF

Using the test dataset, the iPLS-RF model achieved 95% classification accuracy, demonstrating a significant improvement over the LDA algorithm (67.5%) (Fig. 6). Based on the iPLS-RF model, only one natural sample was misclassified as a blended one, and one blended sample was predicted as a natural one. No confusion occurred between the blended and commercial groups, highlighting the superior potential of the RF algorithm and, more broadly, ensemble learning methods in classifying objects based on selected spectral features.

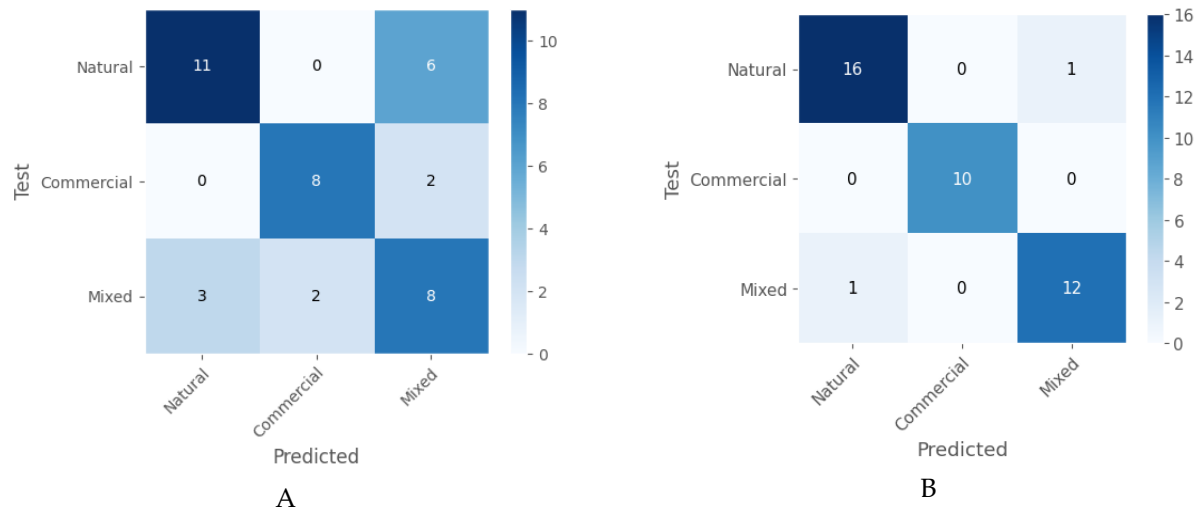


Figure 6. Confusion matrix of: A- LDA model on the test dataset, and B- Random Forest model on the test dataset

The Random Forest model also struggled to accurately distinguish between the "Natural" and "Mixed" categories, especially for samples with a medium proportion of natural orange juice (around 45-50%). This issue may stem from the inadequacy of the data features to effectively separate these two categories within the feature space.

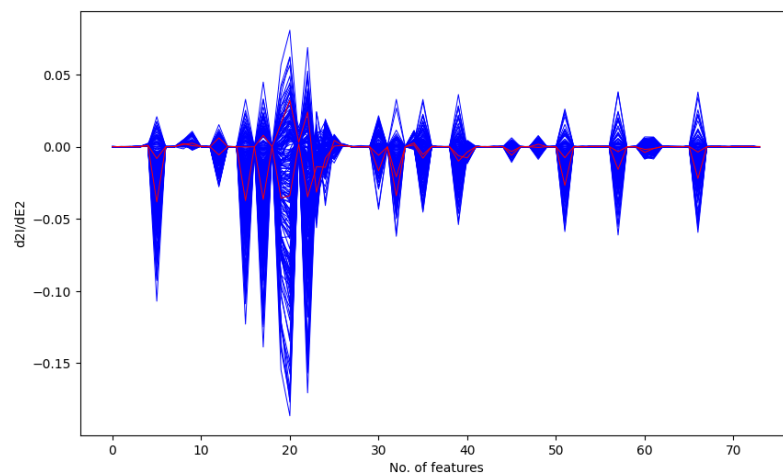


Figure 7. The second-order derivative signals vs. features of the samples classified by iPLS-LDA (red lines: misclassification samples; blue line: correct classification samples)

To check the reasons why iPLS-DA gave the low accuracy prediction, the second-order derivative signals of the misclassified samples (red lines) compared to the correctly classified samples (blue lines) were shown in Fig. 7. It can be observed that the shape of the second derivative data across features differs between correctly classified and misclassified samples. Misclassified samples exhibit abnormalities such as missing peak points and flattened or unchanged signals. These irregularities are also associated with outliers at peak or shoulder points in the voltammogram. This suggests that samples with abnormal shapes compared to the overall dataset within the same group should be identified and excluded before processing. Compared with previously reported classification results, our machine learning model achieved higher accuracy. The limitation of working on a single device is that accurate results can only be obtained for experiments conducted on that same device. However, when switching to another device, it is necessary to calibrate the measurement signals and ensure similar operating conditions to the original device to maintain accuracy.

4. Conclusion

The feasibility of using voltammetric data combined with machine learning as a screening method for determining the authenticity of juice was confirmed. Cluster analysis revealed the differences between natural orange juice, chemical-based juices, and blends of natural and chemical juices. Based on this, supervised machine learning models such as iPLS-LDA and random forest were developed for classification purposes, with the random forest model showing promising results in classification and prediction. The obtained results also highlight the usefulness of the voltammetric method using a gold electrode to assess the authenticity of orange juice. The limitations of the sample recognition method were addressed by increasing the sample size and creating stronger models. Moreover, the electrochemical approach has shown that it is capable of fast classification, low cost, and suitability for on-site analysis. This approach would enable the monitoring of raw material procurement concerning the orange variety and growing regions.

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