The Evaluation of Lime Juice Adulteration by Comparing Cyclic Voltammetry and Electronic Tongue Methods

Gh. Bahrami[1], M. H. Aghkhani[1*], M. R. Golzarian[2], B. Deiminiat[3]

1- Department of Biosystems Engineering, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
2- Centre for Artificial Intelligence and Machine Learning, Edith Cowan University, Australia
3- Department of Chemistry, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran
(*- Corresponding Author Email: aghkhani@um.ac.ir)
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Abstract

The present study investigated the use of the cyclic voltammetric electrochemical method and the electronic tongue (e-tongue) method for detecting adulteration in lime juice. Since the measurement of citric acid content in lime juice is an accepted indicator of lime juice adulteration in laboratories, at first, attempts were made to determine its concentration using a potentiostat device and the cyclic voltammetry method, which involved various electrodes including glassy carbon, graphite, gold, and carbon nanotube and gold nanoparticle-modified glassy carbon electrodes. Different conditions were considered by testing citric acid at multiple concentrations in buffers with different pH levels. The results showed that the electrochemical behavior of citric acid was weak, so conventional electrochemical methods could not be used to check its behavior. In the second part, a portable electronic tongue system (e-tongue) was evaluated. Eight samples of adulteration levels (from 5% up to 95%) were created in lemon juice (0, 5, 10, 20, 40, 70, 95, and 100% impurity). Unsupervised models including Principal Component Analysis (PCA) and Hierarchical Clustering Analysis (HCA), and supervised models including Multilayer Perceptron (MLP) neural networks and Support Vector Machine (SVM) were used. Based on the results, the PCA fingerprint showed good discrimination between different levels of adulteration, and HCA further confirmed this. The results of the analysis of supervised methods showed that the MLP model outperformed the SVM model in predicting fraud levels with a success rate of 99.33% and high correlation coefficients ($R^2 = 0.9973$, RMSE = 0.09). These results show that the proposed system can separate different levels of adulteration in lemon juice and can be used as a taste quality control system.

Keywords: Classification algorithms, Galvanostat, Large amplitude, Potentiostat, Voltammetric pulse

Introduction

Citrus limon (HOC (CO_2H) (CH_2CO_2H)$_2$) is the fruit of the lemon tree from the genus Citrus (Penniston, Nakada, Holmes, & Assimos, 2008). Lemons are cultivated mostly for their juice. These fruits contain high amounts of citric acid, which is responsible for their sour taste (Berovic & Legisa, 2007). Natural sour lemon juice is an unfermented but fermentable product that is mechanically obtained from the endocarp of fresh, mature, and healthy Lime fruits (Citrus aurantifolia 'Persia'). Lemons have traditionally been used to treat many diseases (Penniston, Steele, & Nakada, 2007; Mohammadian, Barzegar, & Mani-Varnosfaderani, 2021) including gout, flatulence, obesity, and colds. Its consumption is also effective in controlling high blood pressure and preventing cardiovascular diseases and depression (Murphy & Mendoza, 1990; White, Aladangady, Rolton, McColl, & Beattie, 2005; Yilmaz, Batislam, Basar, Tuglu, & Erguder, 2008; Lyu, Yuan, Liu, Simon, & Wu, 2022; Rodríguez-García, Sánchez-Quesada, & Gaforio, 2019). This fruit is used in different forms, such as juice, fresh fruit, and oil (Guyon *et al.*, 2014). The leading lime producers of the world, including India, Mexico, Argentina, Brazil, and Spain, account for 16, 14.5, 10, 8, and 7 percent of its global production, respectively (Miaw *et al.*, 2018). The main components of lemon fruit are organic acids, amino acids, flavonoids, vitamins, water, and sugars (Jamil *et al.*, 2015).

Owing to lemon juice's health benefits, its consumption as a food additive is growing (Barzegar, Nabizadeh, Kamankesh, Ghasemi, & Mohammadi, 2023). Recently, different types of adulteration in lemon juice have been elucidated (Bayati, Nazari, Hassanzadazar, & Hosseini, 2020). The Association of the Juice and Nectar Industry (AIJN) of the European Union has provided a reference (AIJN, 2013). However, the economic profit, the increasing growth of consumption, and the lack of accurate methods to identify natural lemon juice have made its market vulnerable to fraud. Common adulterations in the lemon juice industry include adding citric acid and water to pure lemon juice (Jahani *et al.*, 2020; Lyu *et al.*, 2022). Lime juice adulteration is harmful economically and to people's health (the digestive system, dental health, liver function, and kidney health) as it imposes losses for authentic lime juice producers on one hand and jeopardizes people's health on the other (Kamthania, Saxena, Saxena, & Sharma, 2014; Das, Goswami, & Biswass, 2016; Wang & Jablonski, 2016; Karami, Rasekh, & Mirzaee-Ghaleh, 2020; Rasekh *et al.*, 2021). Factors affecting plant properties include environmental conditions during growth, storage conditions, and cultivar diversity. Therefore, it is difficult to identify fraud in them (Saffariha *et al.*, 2020; Saffariha, M., Jahani, A., Jahani, R., & Latif, 2021). Currently, the conventional methods for investigating food fraud are gas chromatography (GC) (Alizadeh, Pirsa, & Faraji, 2017), high-performance liquid chromatography (HPLC) (Huynh, Vo, & Kha, 2023), liquid chromatography with mass spectrometry (LC-IRMS) (Deo & Sakhale, 2022), and mass spectrometry (IRMS) (Kelly, Brodie, & Hilkert, 2018). Most previous studies to determine the main organic acids of fruit juices, using expensive (Nunes, Pauluk, Dos Anjos, Lopes, & Quináia, 2015), and time-consuming methods have required the use of experts and advanced devices (Uckoo, Jayaprakasha, Nelson, & Patil, 2011; Scherer *et al.*, 2012; Shojaee, AliAbadi, & Salamzadeh, 2020; Brendel, Schwolow, Rohn, & Weller, 2021; Khorramifar *et al.*, 2022). The development of non-destructive methods is expanding significantly. These methods are generally performed by senses such as taste, sight, and smell. (Kiani, Minaei, & Ghasemi-Varnamkhasti, 2017; Jahani *et al.*, 2022; Zorpeykar, Mirzaee-Ghaleh, Karami, Ramedani, & Wilson, 2022). This research introduces a fast, cheap, and online method that can be performed by non-specialists without destroying the sample. So far, there have been no reports published on the use of the tasting machine method to detect lemon juice adulteration. Therefore, the objectives of this study are: (1) designing an intelligent technique based on an electronic tasting system along with multivariate methods, and (2) evaluating the system to detect adulteration in counterfeit lemon juice samples based on its taste characteristics.

Electrochemical method

Lime juice value is assessed by the concentration of citric acid and the titratable acidity. Presently, one can easily adulterate fake lime juice by mixing citric acid, sugar, and water (Berovic & Legisa, 2007). The measurement of organic acids has been reported in different materials, such as urine samples, soil, honey, and water (Chen *et al.*, 2020; Yin, Li, & Shen, 2020; Bougrini *et al.*, 2016). So far, various methods have been used to determine adulteration in lime juice, such as assessing impurity in citrus juice by measuring the concentrations of citric and isocitric acids and calculating their ratio (Kvasnička, Voldřich, Pyš, & Vinš, 2002). Another proposed method uses liquid chromatography-mass spectrometry (LC-MS) to detect lime juice adulteration with water (Wang & Jablonski, 2016). A method also uses mass spectrometry to detect lime juice adulteration (Guyon *et al.*, 2016).

Electronic tongue

This research used the electronic tongue (e-tongue) method and the electrochemical method. E-tongue is a robust but inexpensive method that does not need complicated laboratory tools. The method does not consider the nature of the compounds in the material, but it provides a digital fingerprint for each solution or nutrient (Ghasemi-Varnamkhasti, Mohtasebi, & Siadat, 2010). In this system, which uses electrodes made of noble metals, the signals collected by sensors are processed by a pattern recognition algorithm, resulting in the categorization or even quantification of some physicochemical characteristics of the samples (Wu, Yue, & Yuan, 2018). The e-tongue method can classify and identify solutions like coffee, milk, tea, vegetable oil, beer, and fruit juices. The technique has been very successful in detecting adulterations. For example, the system has been used to distinguish pure honey from different adulteration levels, and adulterated milk from pure milk, and to detect adulteration in vegetable oils (Bougrini *et al.*, 2016; Teye, Huang, Han, & Botchway, 2014; Benjamin & Gamrasni, 2016; Lu, Deng, Zhu, & Tian, 2015; Wu *et al.*, 2018; Khaydukova, Cetó, Kirsanov, del Valle, & Legin, 2015; Huang *et al.*, 2014; Bougrini *et al.*, 2016; Benjamin & Gamrasni, 2016; Kurnia Hartati, Bambang Widjanarko, Dewanti Widyaningsih, & Rifa'i, 2017; Labrador *et al.*, 2010; Oliveri, Baldo, Daniele, & Forina, 2009). In most cases, alumina powder is used as a standard method for cleaning the electrodes. A mechanical system for electrode polishing was designed (Olsson, Winquist, & Lundström, 2006). However, because the preparation and maintenance costs of this system are high, it is not commonly used in laboratories. Despite the good results of using electronic tongues in detecting adulterations in different materials, to the best of our knowledge, no research has ever investigated the use of e-tongues in lime juice adulteration detection. Therefore, this research explores their use for the first time.

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Materials and Methods

Sample preparation

The common fraud in the lemon juice industry is adding water, lemon pulp, and wheat straw (Mohammadian, Ziaiifar, Mirzaee-Ghaleh, Kashaninejad, & Karami, 2023; Jahani *et al.*, 2020). In the first stage, fresh yellow Shiraz lemons were bought from the Hamadan market in Iran. With a juicer, pure lemon juice was obtained. To obtain samples that could be tested, the obtained lemon juice was passed through a strainer, and the lemon pulp was separated. The resulting pure lemon juice was transferred to the laboratory. Wheat straw was soaked in hot water for 24 hours until its color turned yellow and became very similar to the color of lemon juice. Water, lemon pulp, and citric acid were added to the obtained solution to replicate the taste of pure lemon juice. The sample obtained was regarded as a completely fake sample. To detect different levels of adulteration in lime juice, eight different levels of natural and adulterated juice mixtures were prepared including 100% natural juice and juices adulterated by 0%, 5%, 10%, 20%, 40%, 70%, 95%, and 100% (Figure 1). After mixing the samples with a mixer, three 30-mL samples were separated from each. Twenty-four obtained solutions were stored in a dark and dry place to avoid any chemical or physical reactions.

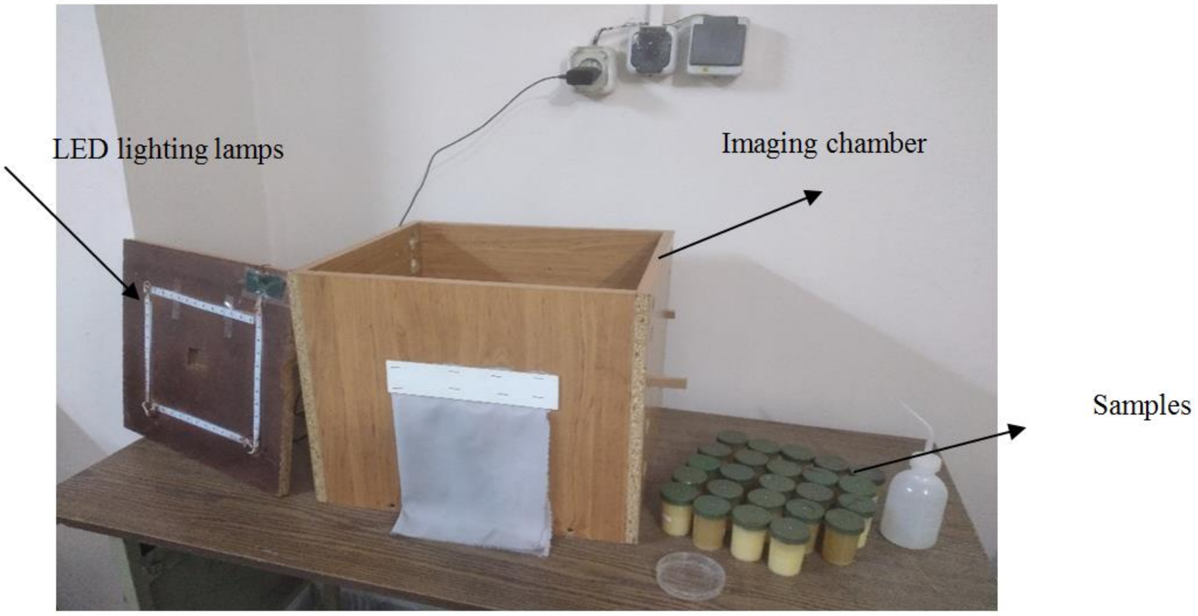

Fig.1. Imaging chamber

Electrochemical method

A potentiostat (DropSens, Metrohm, Spain) and an Autolab (PGSTAT302N; Metrohm-Autolab, Spain) controlled by a PC using the Nova 1.8 software were used for measurements (Chira, Bucur, & Radu, 2017). The structure of the electrodes was composed of the working electrode (five electrode types, including glassy carbon, carbon nanotube-modified glassy carbon, gold nanoparticle-modified glassy carbon, gold, and graphite), the reference electrode (Ag/AgCl), and a platinum electrode as the counter electrode (Figure 2). Analysis was performed at different concentrations and in different buffers, including acetate and sulfate buffers. The electrode modifiers, which included gold nanoparticles and carbon nanotubes, were purchased at laboratory purity from Sigma Company. Double-ionized water was used to dilute different solutions and wash the electrodes.

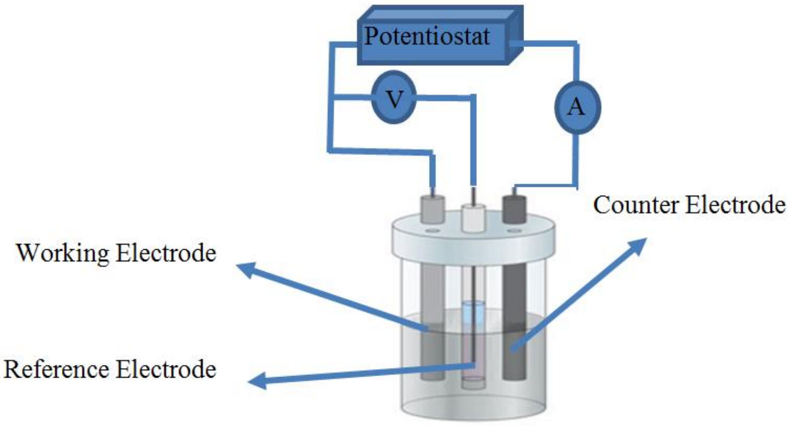

Fig.2. Structure of the voltammetric method

To modify the electrodes with gold nanoparticles, they were first polished with

Al_2O_3 particles with a diameter of 0.3 µm to ensure the purity of their surface and the lack of any pollutants, including impure particles. They were then put in an HNO_3 solution and deionized water, after which they were transferred into an ultrasonic device to fully clean their surfaces with ultrasonic waves. After that, to activate the electrode surfaces, they were put in 0.1 µM sulfuric acid solution, and the cyclic voltammetry method (20 cycles from -0.6 to 2 V at a scanning rate of 100 mVs^{-1}) was applied (Lin, Ni, & Kokot, 2013). The resulting electrodes were free of any impurities and were fully active. To set gold nanoparticles, first, a solution was made of 7.5 cc of $HAcCl_4$ 250 ppm, 3 cc of KCl 0.1 M, and 5 cc of HCl 2 M. The solution was set on the electrodes by the cyclic voltammetry method (in the range from -0.4 to 1.4 V with a scanning rate of 50 mVs^{-1} for 20 cycles). Then, the electrodes were washed with double distilled water and put at room temperature so that the gold nanoparticles dried and covered the glassy carbon electrode surface.

Another method to modify the electrode is to use carbon nanotube particles. The surface of the glassy carbon electrode was cleaned through the steps described in the previous section. Then, the carbon nanotube solution was obtained by the following steps. First, 1 mg of carbon nanotube (MWCNT) was dissolved in 5 mL of the solution (double deionized water (DDW), ethanol, and dodecyl sulfate with a ratio of 3:1:1), and the solution was exposed to ultrasonic waves in an ultrasonic device for 30 minutes to become homogeneous. Next, 5 µL of the solution was dripped on the electrode, and it was left at room temperature for the solvent to evaporate. Finally, the carbon nanotube-modified electrode was obtained and named MWCNT/GCE (Masikini, Ghica, Baker, Iwuoha, & Brett, 2019). Citric acid at analytical purity grade was purchased from the Sigma-Aldrich Company (Sigma-Aldrich, St. Luis, MO) and was prepared at different concentrations using deionized water and different buffers. The buffers used included phosphate buffer (pH = 7) at a concentration of 0.1 M L^{-1} and acetate buffer (pH = 7) at a concentration of 0.1 M L^{-1}. Then, the electrode was tested in four steps: (i) non-modified glassy carbon electrode; (ii) gold nanoparticle-modified glassy carbon electrode; (iii) carbon nanotube-modified glassy carbon electrode; and (iv) carbon nanotube and gold nanoparticle-modified glassy carbon electrode (Masikini *et al.*, 2019).

Cyclic voltammetry was adjusted at a range of 0 to 1.5 V with a scanning rate of 100 mVs^{-1} and a one-time cycle. In this procedure, when anodic or cathodic flow peaks were observed, a calibration curve was drawn to obtain the concentration of the unknown material. In the voltage-current diagram, the height of the current intensity peak expresses the substance concentration value. By testing several different concentrations and measuring the resulting peak current, the relationship between concentration and current can be obtained. By changing various variables in the experiment, such as electrolyte type, scanning speed, amount of acidity, and concentration time, the optimal conditions of the experiment can be obtained (Xin *et al.*, 2020).

E-tongue method

Hardware fabrication

The measurement instruments were prepared with a potentiostat designed by the Dadepardazan Gostaran Niru Company (Iran) (Figures 3 and 4), a signal processing block, a mixed-signal circuit, an analog-to-digital converter (ADC), a digital-to-analog converter (DAC), and a voltammetric unit. The processing core consisted of a transceiver and an ATmega32 microcontroller. The analog unit was responsible for adapting and amplifying DAC and ADC signals. The voltammetric unit was responsible for applying a voltage to the working electrode and collecting the current intensity from the counter electrode. Since the voltammetry unit signals are unstable (Masot *et al.*, 2010), stabilizing units were added to the circuit. These circuits include capacitors that are connected in parallel with the feedback loop of operational amplifiers. This device allows pulsed voltammetry measurements in which potential and pulse length can be configured for each specific application. In this research, 50-ms pulses were applied.

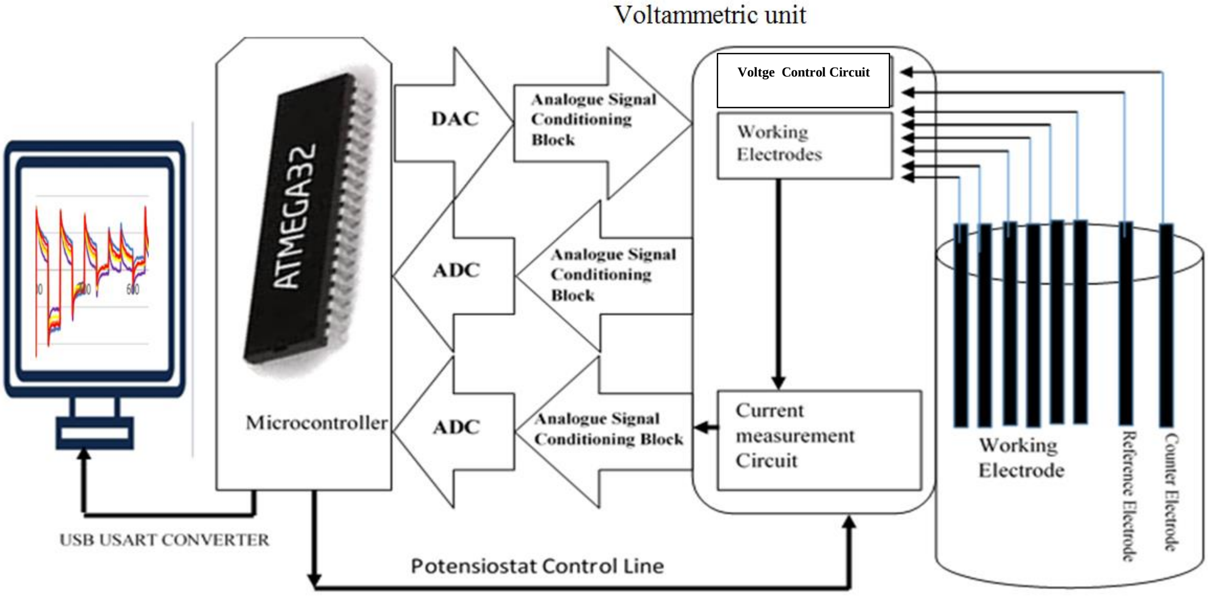

Fig.3. Schematic of Electronic Tongue

The voltage was distributed as cascade voltammetry at an incremental (decremental) rate of 200 mV between +1 and -1 V (to prevent water hydrolysis), and the potential was adjusted to zero after each increment (Figure 6). The experiments were conducted with five electrodes made of noble metals, including gold, silver, platinum, titanium, and palladium. The electrodes were put inside a steel case for their protection. The platinum and silver/silver chloride electrodes were used as working and reference electrodes (Figure 5). A Windows-based software package was employed to control the device, apply large-amplitude pulsed voltage (LAPV), and collect current intensity responses (Winquist, Wide, & Lundström, 1997). The sampling rate was set at 250 samples per second, and each pulse was applied for 2 seconds (a total of 500 samples). Twenty-four samples, obtained in the sample preparation section, were prepared. Since five electrodes were used in each experiment, a 500 × 240 matrix (8 combinations of pure and adulterated juice, 3 samples from each, 2 repetitions, 5 electrodes, and 500 data points from each electrode) was obtained as the data matrix. After each experiment, its effect was eliminated by polishing the electrodes 20 times with alumina powder with a particle diameter of 0.3 µm.

Features extraction

A conventional method to reduce the dimensions of the features extracted by the e-tongue system is local (Winquist *et al.*, 1997). Since pulse time-voltage is divided into several independent zones (Figure 6), and the e-tongue system’s signal response is distributed at the bottom and top of the time axis, it is possible to calculate the statistical data of each signal zone separately and organize it based on a comparison of the main signals. Five statistical features, including mean, standard deviation, amplitude, maximum, and median, were calculated for each step of the signal.

Fig.4. Electronic tongue hardware

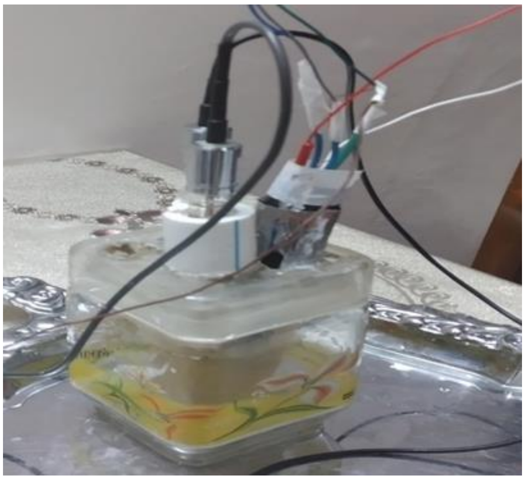

Fig.5. Electrodes including gold, platinum, palladium, titanium, and silver as working electrode, Ag/AgCl electrode as reference, and platinum electrode as counter electrode

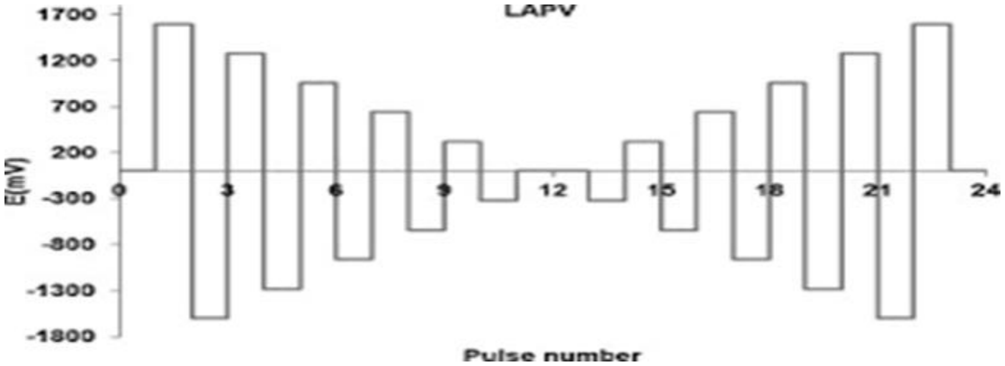

Fig.6. LAPV pulses generated by electronic Tongue

Data analysis

The data pattern was detected using unsupervised methods, including hierarchical cluster analysis (HCA) and principal component analysis (PCA), and supervised methods, including multilayer perceptron (MLP) and support vector machine (SVM).

MLP and SVM were used to predict the concentration of the natural juice, and PCA and HCA were employed to discover whether there were significant differences between natural and adulterated samples. These methods are described in detail in the literature (Scott, James, & Ali, 2006).

PCA is used to convert a series of correlated variables into a set of uncorrelated linear variables, or the so-called principal components. The components obtained have the least correlation with one another. The effect of each component can be precisely determined using a score plot (Wold, Esbensen, & Geladi, 1987).

HCA clustering method is one of the unsupervised methods. The dendrogram of this clustering can be built top-down or bottom-up. In divisive methods (top-down), all data are first put into a single cluster. Then, the classification is done hierarchically (Gutierrez-Osuna, 2002). In the agglomerative method (bottom-up), each observation starts in its own cluster, and is merged with others as the process progresses. As such, larger clusters that constitute related objects are created (Gualdron *et al.*, 2007). The present research used the agglomerative clustering method.

SVM is an algorithm for statistical learning based on keeping empirical risk at a fixed level while minimizing the confidence interval (Schölkopf, Smola, & Bach, 2002). The main idea of the algorithm for separating input patterns into two classes is to use several hyperplanes. This algorithm was first used to classify two linear datasets, but its expanded form can be used for multi-class problems and nonlinear data (Haddi *et al.*, 2011). Hyperplanes are unique and are obtained during the training step to obtain the highest margin. The details of classification by SVM are given (Yin *et al.*, 2020). This research studied the multi-class classification problem.

The solutions were classified using an MLP neural network, which is a network based on at least three layers. The input layer of the neural network was the features obtained in the feature extraction stage. The input matrix data were divided into 70% training, 15% validation, and 15% testing. In the two stages of testing and training the network, two indices of mean and standard deviation were used to evaluate the results in terms of accuracy and precision. The logsig and tansig transformation functions were used to select the transfer function of the hidden layer. The trainlm and trainbr algorithms were tested to select the optimal training algorithm. To select the optimal number of hidden layer neurons, the range of 5 to 20 neurons was evaluated through a trial-and-error approach (Abutaleb, 1991). The next layer is known as the output layer. Since eight lime juice samples were evaluated, eight neurons were put in the output layer.

$$\text{Sigmoid function} = \frac{1}{1 + e^{-\sum F_i W_{ij} + b}} \qquad (1)$$

$$\text{hyperbolique function} = \frac{1}{1 + e^{-\sum 2F_i W_{ij} + b}} - 1 \qquad (2)$$

where, F_i is the input, b is the bias, and W_{ij} is the weight of the j^{th} neuron.

Neural networks use various training functions to optimize weights and biases. The present research used the Bayesian regularization back-propagation (Trainbr) and the Levenberg Marquardt back-propagation (Trainlm).

Assessment of classification performance of SVM and MLP

The performance of the models used in this research was evaluated using the criteria of accuracy, precision, sensitivity, F1 score, mean squared error, and Cohen's kappa coefficient of reliability. In the confusion matrix, TP represents true positive patterns, TN represents true negative patterns, FP represents false positive patterns, and FN represents false negative patterns. The target and predicted values are represented by Ti and Pi, respectively (Niemeyer *et al.*, 2021).

$$accuracy(\%) = \frac{TP + TN}{TP + TN + FP + FN} \times 100 \qquad (3)$$

$$precision(\%) = \frac{TP}{TP + FP} \times 100 \qquad (4)$$

$$sensitivity(\%) = \frac{TP}{TP + FN} \times 100 \qquad (5)$$

$$F1 - Score(\%) = \frac{2 \times Sensitivity \times Precision}{Sensitivity + Precition} \times 100 \qquad (6)$$

$$Specificity(\%) = \frac{TN}{TN + FP} \times 100 \qquad (7)$$

$$AUC = \frac{1}{2} \times \left(\frac{TP}{TP + FN} + \frac{TN}{TN + FP}\right) \qquad (8)$$

$$YI = Sensitivity - (1 - Specificity) \qquad (9)$$

$$MSE = \frac{1}{n}\sum_{i=1}^{n}(T_i - P_i)^2 \qquad (10)$$

Results and Discussion

Electrochemical method

Results of the non-modified glassy carbon electrode

Figure 6 shows the cyclic voltammetry applied to the non-modified glassy carbon electrode. Reaching the potential at which the electrode reaction begins, the current increases in the form of a continuous-state voltammogram. The potential scan continues from a certain value before the maximum current to a value equivalent to the current peak, and because citric acid cannot react with the electrode, the current peak is not observed. The changes in citric acid concentration did not affect achieving the anodic or cathodic peaks in the electrochemical process. This electrochemical behavior shows that the glassy carbon electrode alone cannot detect the concentration of citric acid.

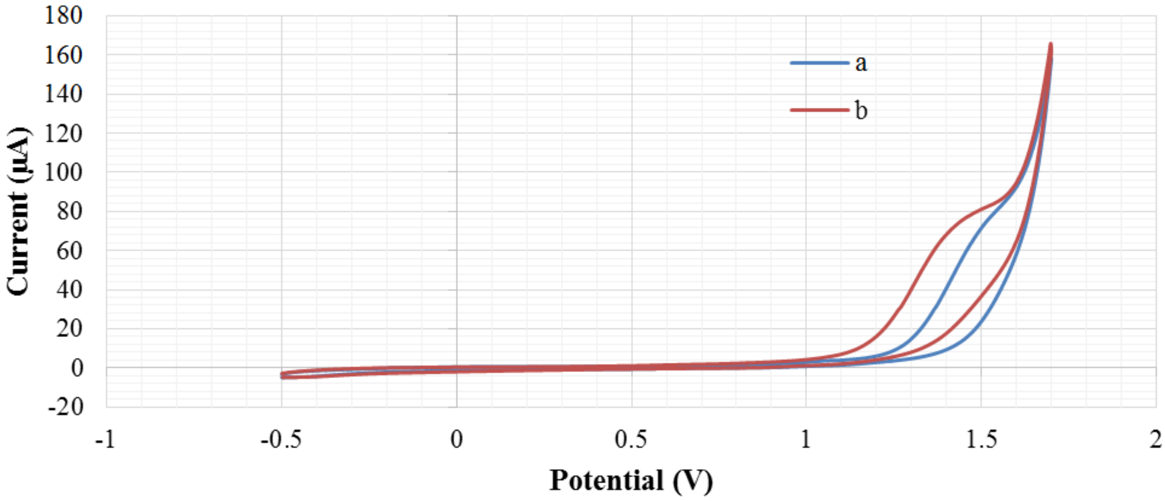

Fig.6. Glassy carbon electrode in phosphate buffer solution: (a) without the presence of citric acid, and (b) in the presence of citric acid at a scan rate of 50 mVs^{-1}

Embedding gold nanoparticles on the glassy carbon electrode

According to Figure 7, the oxidation peaks *a* and *b* are evident. The peak anodic current increased with consecutive scans, showing that the gold nanoparticles were embedded on the electrode surface. On the other hand, the cathodic peak changed with successive scans, indicating a successfully reduced reaction in the gold salt.

After the application of the gold nanoparticle coating on the electrode, the area of the voltage application surface increased, increasing the current derived from it. Figure 8 shows the reaction of the modified electrode to a 0.1 M citric acid solution. The diagram indicates that the electrode coated with gold nanoparticles had no impact on achieving anodic and/or cathodic peaks caused by citric acid concentration. As far as the scanning speed of 50 mVs^{-1}, the current intensity initially increased and then reached a plateau, but the shape of the voltammograms became wider. The broadening of the voltammogram shape as a result of increasing the scan rate increased disturbances and decreased selectivity. Therefore, according to the sensitivity and sharpness of the voltammograms, the scanning speed of 50 mVs^{-1} was chosen as the optimal condition for the next experiments.

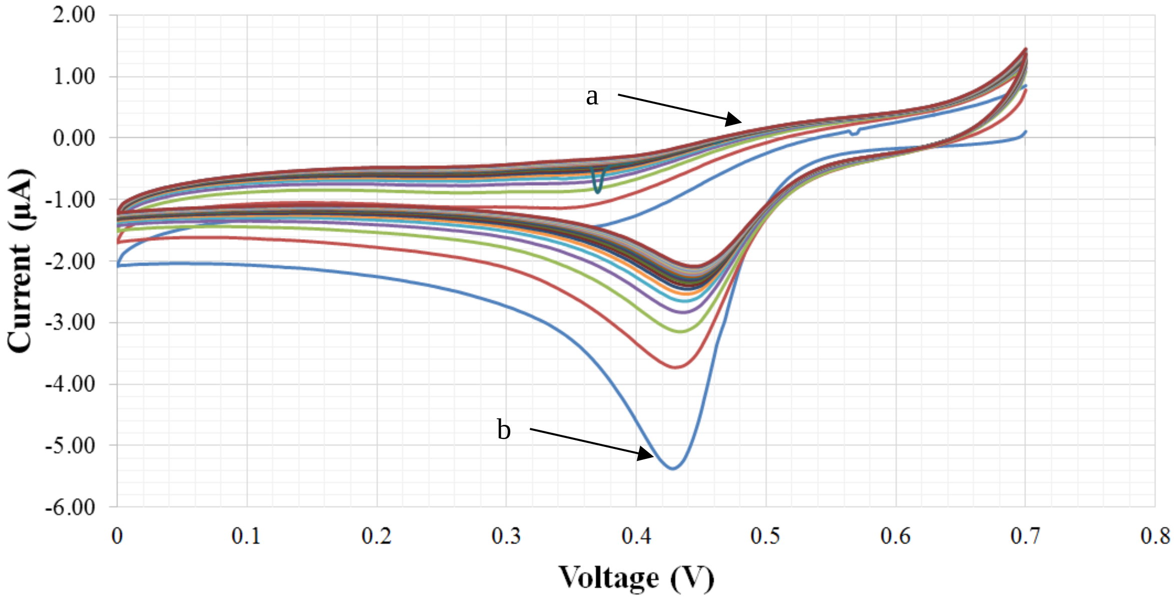

Fig.7. Applying cyclic voltammetry to deposit gold nanoparticles on a glassy carbon electrode at a speed of 50 mVs^{-1}: (a) Anodic peak, and (b) Catodic Peak

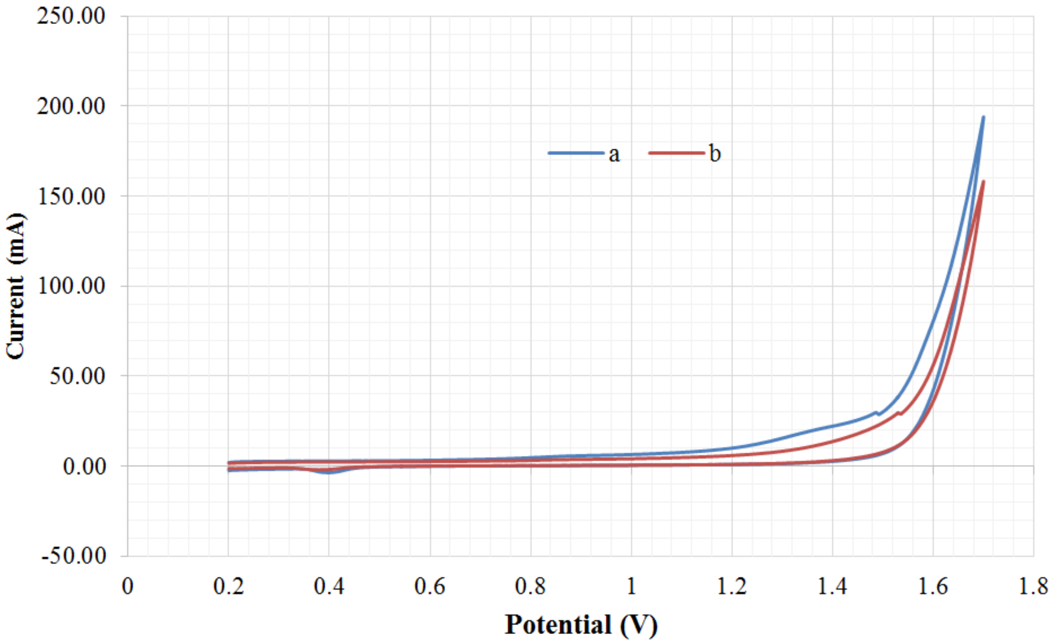

Fig.8. (a) An unmodified glassy carbon electrode, and (b) an electrode modified with gold nanoparticles in 0.1 M citric acid solution with 0.1 M phosphate buffer at pH = 5

Embedding carbon nanotubes-gold nanoparticles on the surface of a glassy carbon electrode

At this step, the glassy carbon electrode was coated with carbon nanotubes to increase its surface area. Several modifications were applied consecutively, and the results of the experiments were recorded at each step (Figure

9). With different modifications, no changes were observed in the anodic or cathodic peaks. Although it was observed in the diagram of electrode modification with carbon nanotubes and gold simultaneously (c), the peak was very small and did not significantly change with increasing citric acid concentration. This implies that the peak was caused by the synergy of the additives rather than the concentration of citric acid.

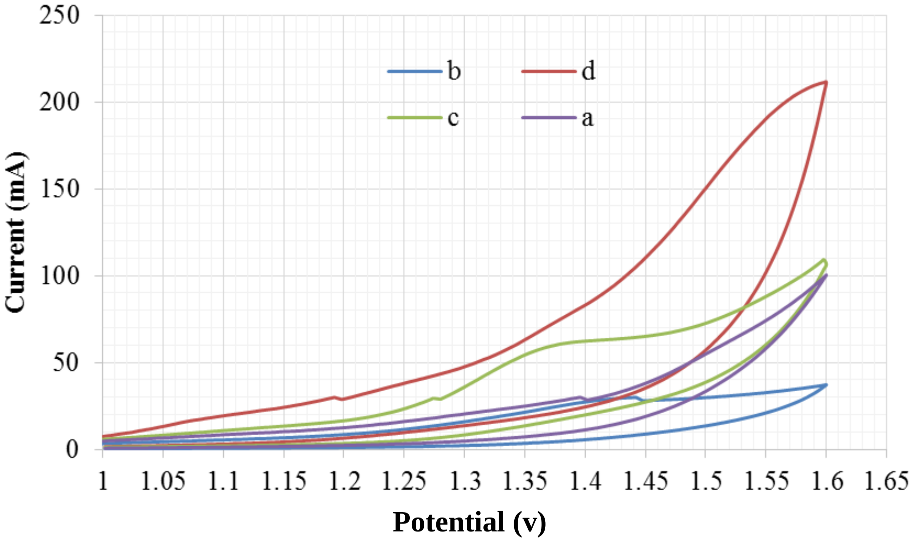

Fig.9. (a) Glassy carbon electrode in citric acid, with a concentration of 0.1 M, 0.1 M phosphate buffer, and pH 5; (b) Electrode modified with carbon nanotubes in the presence of citric acid; (c) Electrode modified with carbon nanotubes and gold nanoparticles in the presence of citric acid; and (d) Electrode modified with carbon nanotubes and gold nanoparticles without the presence of citric acid

Results of gold, graphite, and tungsten electrodes

The experiments were conducted at different concentrations and in different buffers, including acetate. Also, the experiments were conducted with other electrodes, e.g., gold, graphite, and tungsten, which yielded similar results (Figure 10).

Given the results of the experiments with different electrodes and in different buffers, since no anodic or cathodic peaks were obtained, it can be concluded that citric acid has low electrochemical activity and cannot be examined by conventional electrochemical methods.

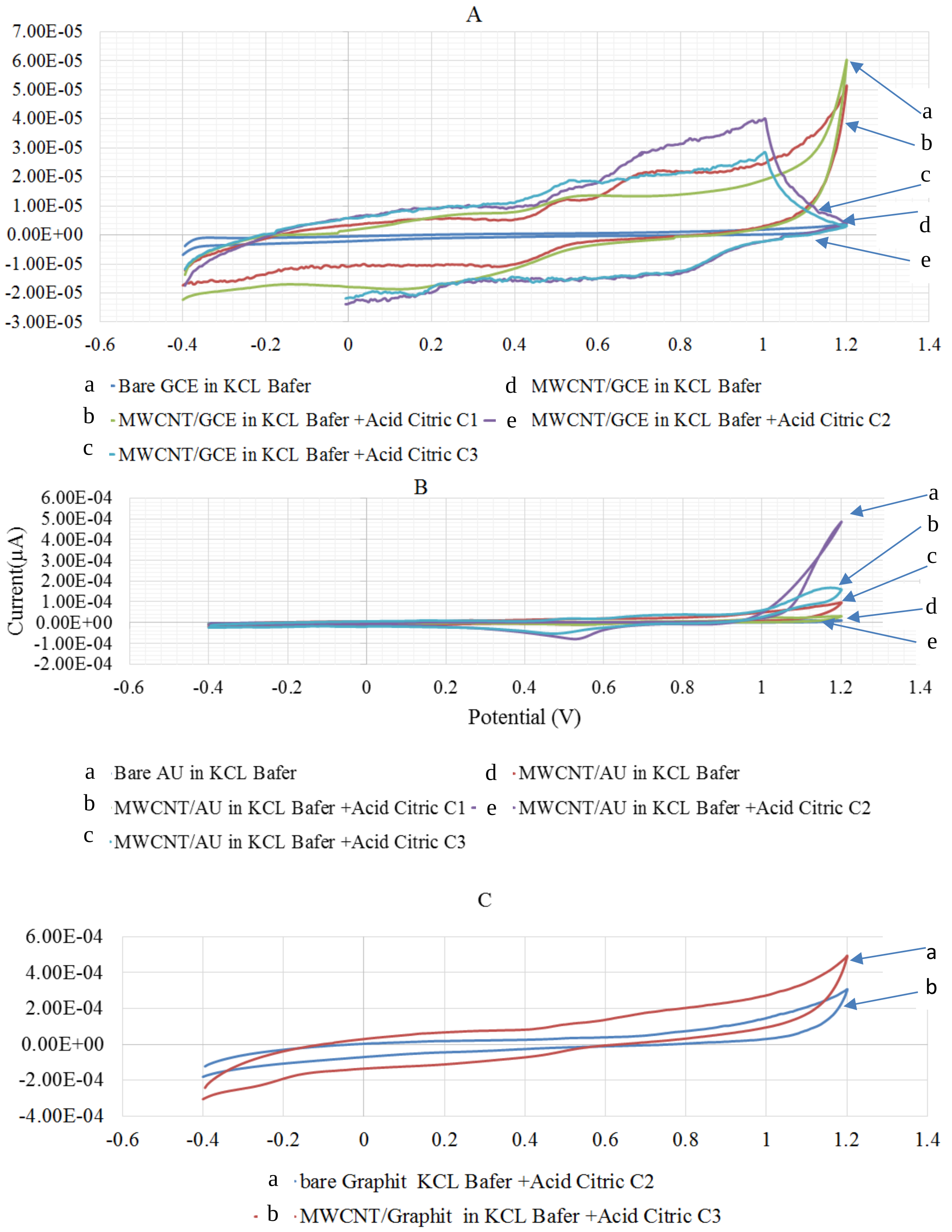

Fig.10. (A) Graphene electrode, without modification, modified with carbon nanotubes and gold nanoparticles; (B) Gold electrode in KCl buffer, modified with carbon nanotubes and gold nanoparticles; and (C) Gold electrode in phosphate buffer, modified with carbon nanotubes and gold nanoparticles

E-tongue device

The response graphs of the e-tongue device

The samples collected from the natural lime juice were tested by the e-tongue device. The software collected the signals of the current intensity obtained from the responses of five working electrodes. Figure 11 displays the response graphs of these electrodes. The maximum current intensity of the sensors' response ranged from -250 to +300 µA. On the other hand, the relative standard deviation (RSD) was 10% in different measurements, showing the system's good replicability, accuracy, and precision. As is seen in Figure 11, each electrode acted as a unique "fingerprint" for each juice sample, which shows their different responses to different solution compositions. After the measurements, 2500 data points (5 electrodes × 500 measurements) were collected by the system for each solution sample. To obtain statistical characteristics, the range of each response was divided into 20 intervals, each containing 25 data points. Then, five statistical features (mean, standard deviation, median, maximum, and amplitude) were extracted from each interval. The average of each statistical feature was calculated. As a result, the data represented by the 240 × 500 matrix (see Section Featture Extration) was converted into a 240 × 5 matrix.

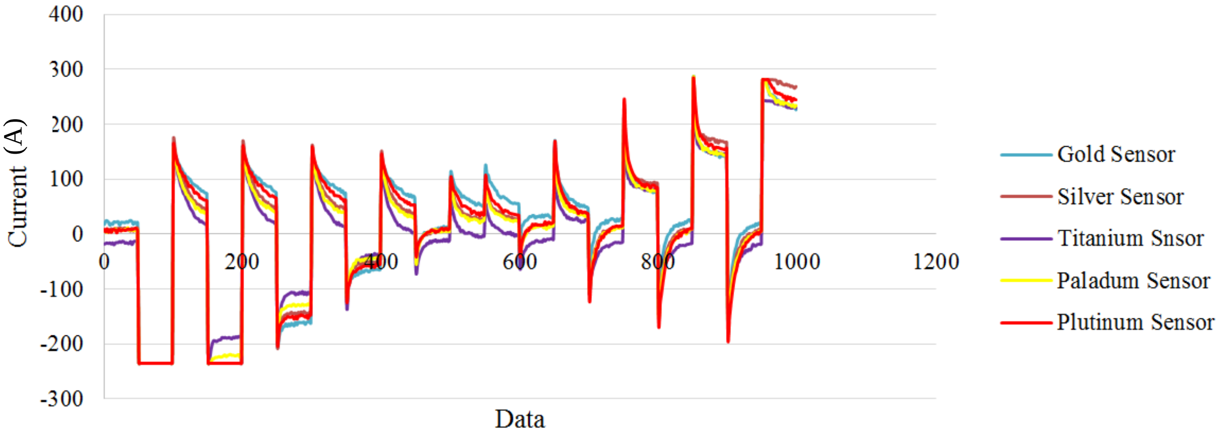

Fig.11. The response graphs of the electrodes

The results of PCA

In the PCA method, if the cumulative variance is >85%, the method can reliably reflect the features of the main data (Martınez *et al.*, 2005). The score diagram (using the statistical features) was used to determine if there were differences between pure and adulterated lime juice. Figure 12 demonstrates that together, the first and second principal components accounted for 97% of the data variance, with the first component alone accounting for 90% and the second contributing an additional 7%. In other words, most information can be explained by these two PCs. On the other hand, the difference was more significant between the samples along the first component. These results show that as the adulteration level in the juice increases, the variance between the samples also increases, allowing the algorithm to effectively distinguish the samples. The distribution of each sample was acceptable, indicating the reliability of the features extracted from each sample.

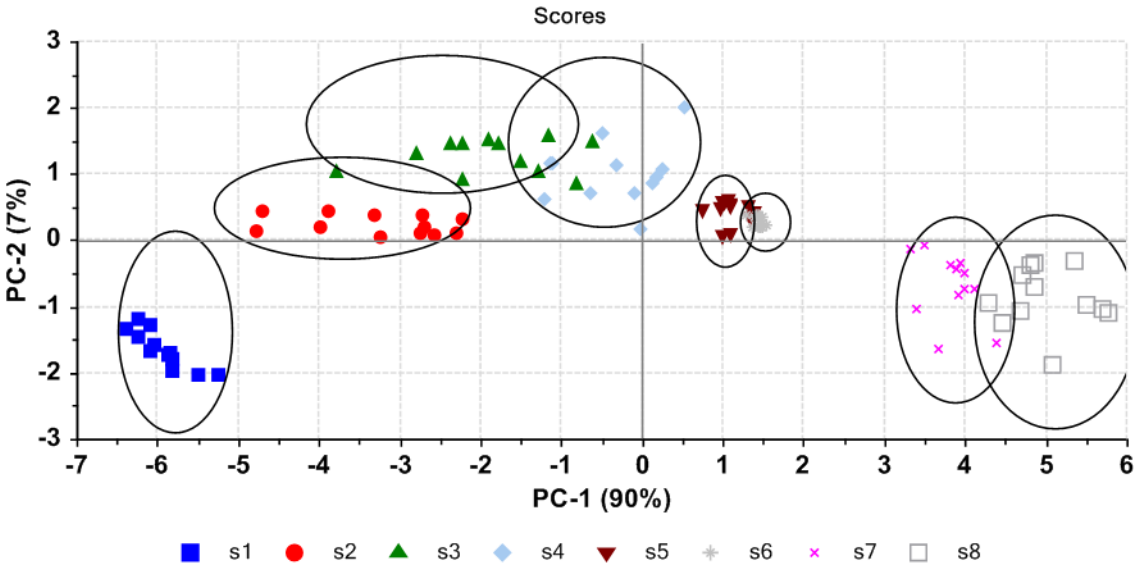

Fig.12. Score Plot of 8 samples (S1: No impurity; S2: 5% impurity; S3: 10% impurity; S4: 20% impurity; S5: 40% impurity; S6: 70% impurity; S7: 95% impurity; and S8: 100% impurity)

The loading plots (Figure 13) show that the palladium electrode detected the greatest difference between the samples. The gold, titanium, silver, and platinum electrodes achieved rankings for their distinguishing performance, respectively. If electrodes are intended to be economical, platinum and silver electrodes can be ignored, and gold, palladium, and titanium electrodes can be used reliably as working electrodes. In this research, we adhered to the initial electrode compositions and continued with five electrodes.

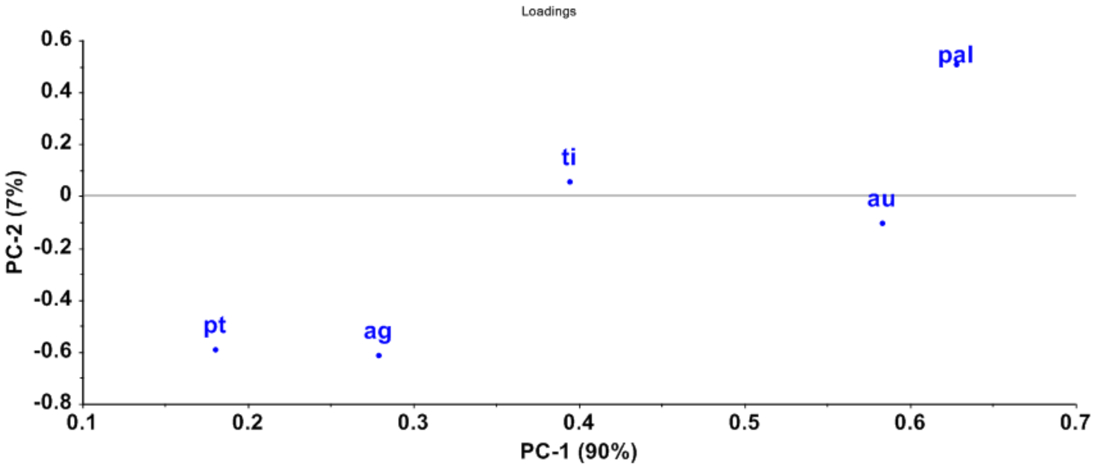

Fig.13. Loading plot, related to the electrodes of the electronic tongue system (pt = Platinum, ag = Silver, ti = Titanium, au = Gold, and pal = Palladium)

The results of agglomerative HCA

The HCA algorithm was applied to the data (Figure 14). As is seen, the pure and adulterated samples differed in the extracted features, whereas some were more similar than others (e.g., samples 3 and 4, along with samples 5 and

6).

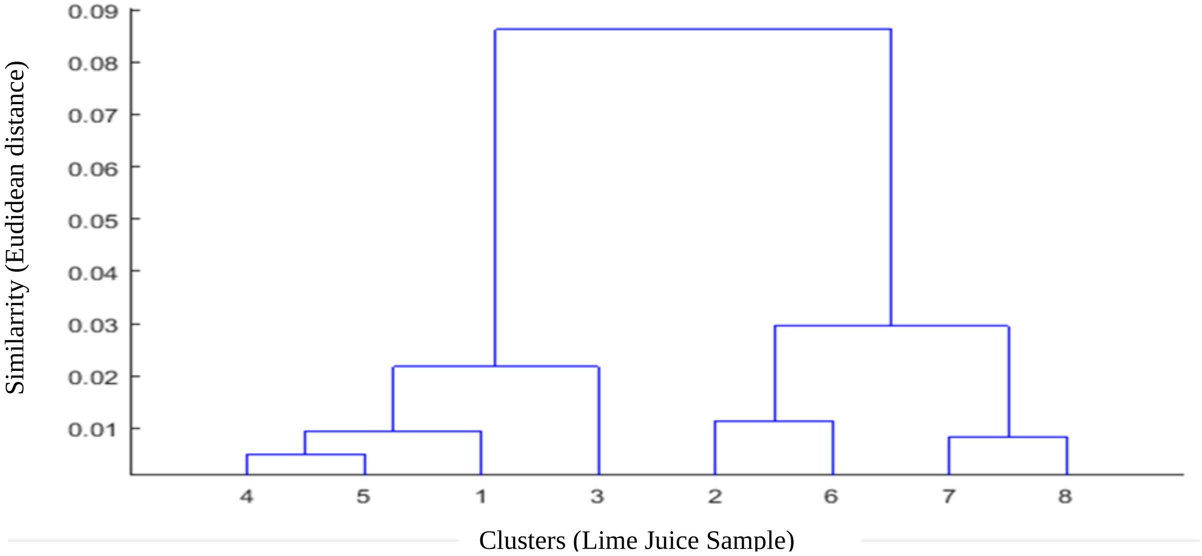

Fig.14. Hierarchical clustering diagram

From the graphs of PCA and HCA, it is clear that the results of these two methods completely agree with each other, and the recognition of the similarity of some samples is probably due to the non-linear response of the sensors.

The results of SVM

Statistical features were used as the input of the SVM model, in which 85% of the dataset was considered for training and the rest for testing. SVM is assessed by various functions, such as linear, quadratic, cubic, and Gaussian functions. After the confusion matrix was obtained, the success in classification was found to be 95.62% using the quadratic algorithm (Table 1). The results showed that the quadratic function had the best accuracy (95.8%), the lowest error (0.041), the highest sensitivity (95.8%), and the most optimal kappa coefficient (0.809) (Table 2).

Table 1- Confusion matrix of the support vector machine

Sample	Samp1	Samp2	Samp3	Sampp4	Samp5	Samp6	Samp7	Samp8	Success rate
Samp1	30	0	0	0	0	0	0	0	100%
Samp2	0	26	2	2	0	0	0	0	86%
Samp3	0	0	28	1	1	0	0	0	93%
Samp4	0	0	0	30	0	0	0	0	100%
Samp5	0	0	0	0	30	0	0	0	100%
Samp6	0	0	0	0	0	28	1	1	93%
Samp7	0	0	0	0	0	1	28	1	93%
Samp8	0	0	0	0	0	1	1	28	93%
Overall success rate									94.29٪

Table 2- Evaluation of the confusion matrix of the support vector machine

Type of SVM	Accuracy	Precision	Sensitivity	Specificity	Error	F1-score	Kappa
Quadratic	95.8%	96%	95.8%	99.4%	0.041	0.958	0.809

The results of the MLP neural network

The statistical data were fed to the MLP network as input. To generate the training, validation, and testing data, the initial data matrix with the dimensions of 240 × 5 was divided into three parts, including 15%, 70%, and 15% of the dataset, respectively. The optimal network included 15 neurons in the hidden layer, and was obtained with the logsig transformation function and the trainbr training function (Table 3). The optimal network achieved a lower detection error than other networks in both the testing and training stages.

Table 3- The results of statistical indexes for MLP classifier

Nn	TF	Trainlm				Trainbr			
		Train phase		Test Phase		Train phase		Test Phase	
		Accuracy	Precision	Accuracy	Precision	Accuracy	Precision	Accuracy	Precision
5	logsig	98.99±0.08	98.99±0.05	98.45±1.04	98.00±0.08	98.89±0.08	98.99±0.22	98.85±0.99	98.25±0.09
	tansig	98.38±0.05	98.97±0.06	98.19±0.03	98.19±1.45	98.99±0.05	98.59±0.33	99.05±1.10	98.17±1.30
7	logsig	98.96±0.14	98.93±0.29	98.56±1.05	98.10±1.93	99.03±0.18	99.15±0.07	99.13±0.28	99.05±0.07
	tansig	98.96±0.32	98.98±0.18	98.28±1.14	98.60±2.26	99.23±0.58	99.03±0.28	99.10±0.27	98.50±0.4
10	logsig	98.88±0.42	99.05±0.15	98.18±0.08	98.85±1.28	98.97±0.55	99.17±0.25	99.10±0.39	99.10±0.14
	tansig	98.76±0.55	99.03±0.19	98.04±0.24	98.07±0.45	98.99±0.26	99.15±0.22	99.12±0.18	99.15±0.07
15	logsig	99.18±0.10	99.16±0.21	99.10±0.09	98.80±2.14	100±0.00	100±0.00	99.33±0.29	99.34±0.19
	tansig	99.19±0.06	99.19±0.13	99.02±0.05	98.85±2.11	100±0.00	100±0.00	99.18±0.12	99.19±0.24
17	logsig	99.29±0.25	98.99±0.12	99.18±0.10	99.00±2.00	100±0.00	100±0.00	99.02±0.59	99.05±0.79
	tansig	99.29±0.28	99.29±0.12	99.20±0.42	99.15±0.27	100±0.00	100±0.00	99.02±0.49	99.05±0.19
13	logsig	98.98±0.10	98.96±0.21	98.48±1.02	98.95±2.05	100±0.00	100±0.00	98.50±1.00	99.00±0.11
	tansig	98.96±0.15	98.92±0.29	98.42±1.05	99.05±0.23	100±0.00	100±0.00	98.92±0.89	99.05±0.19

Then, sensitivity analysis was performed to determine the most sensitive features. In the training and testing phases, the performance of the MLP is monitored by sequentially removing each of the five features. Each feature had a significant effect on classification, provided that its removal degraded the performance of the whole network. The results show that all variables were important and effective, because excluding each feature affected the performance of the classifier (Table 4).

Table 4- The results of sensitivity analysis for MLP classifier

	Train phase		**Test Phase**	
	Accuracy	**Precision**	**Accuracy**	**Precision**
All	100±0.00	100±0.00	99.33±0.29	99.34±0.19
All- except mean	99.05±0.33	99.12±0.44	96.99±0.11	96.88±0.28
All- except Sd	99.25±1.05	99.72±0.79	97.00±2.31	97.60±3.23
All- except max	98.96±0.45	98.92±0.49	98.40±0.47	97.00±0.08
All- except amplitude	98.88±0.55	98.82±0.25	98.30±0.33	97.00±2.02
All- except median	98.88±0.32	98.45±0.20	97.65±0.97	97.70±0.45

Then, the MLP was trained with the five inputs selected in the previous step. The correlation between the actual value and the predicted value is shown in Figure 15.

The best correlation coefficient and error rate were obtained as 0.9973 and 0.09, respectively (Table 5).

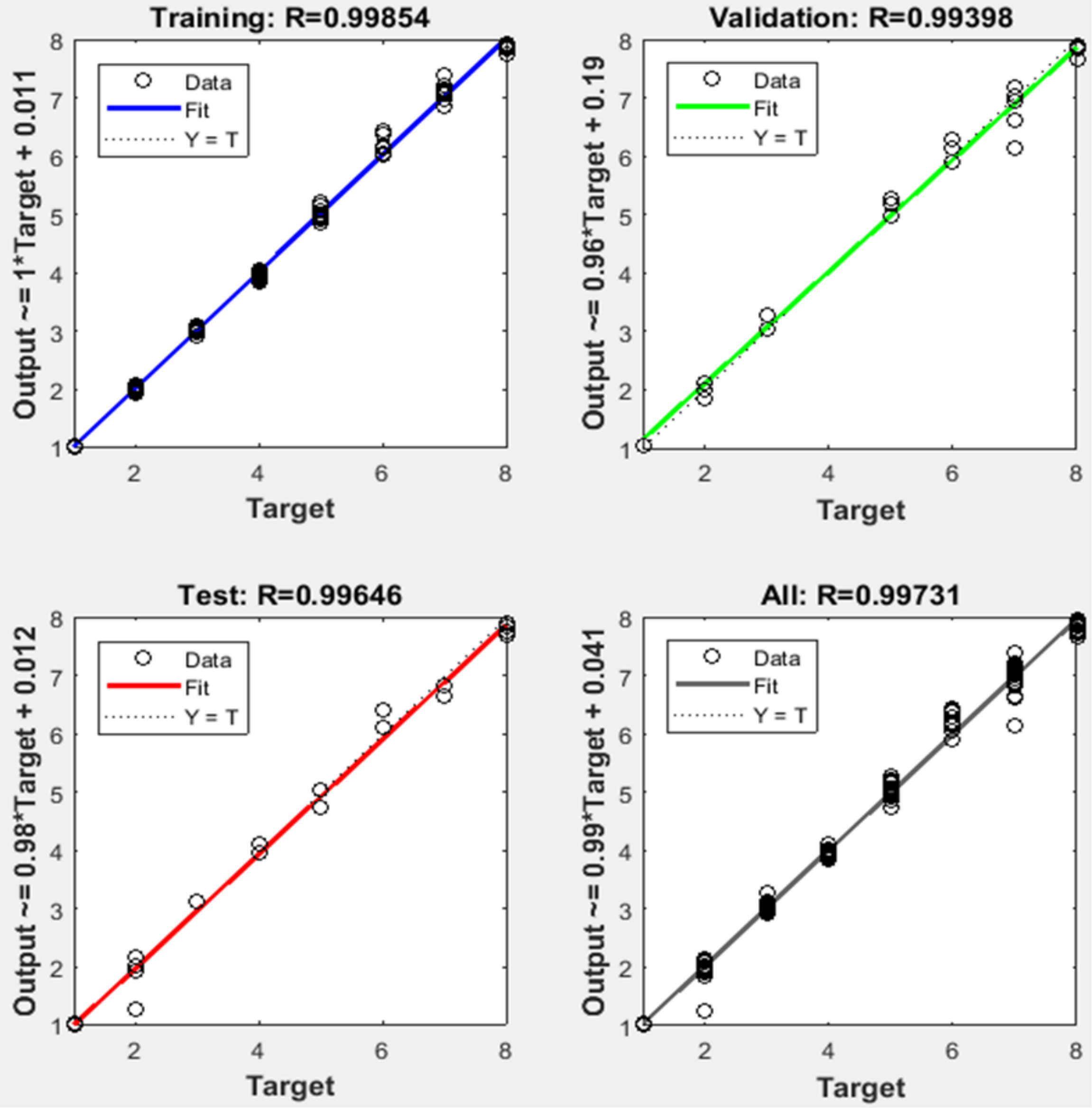

Fig.15. The results of the MLP model: estimation of lemon juice concentration values using taste sensor data (from top left, training, evaluation, testing, and total)

Table 5- The R^2 and RMSE values for different numbers of neurons in the hidden layer

Row	Number of hidden layer neurons	The R^2 value of the training data	R^2 value of validation data	R^2 value of test data	R^2 value of total data	RMSE
1	5	0.9843	0.9847	0.9844	0.9830	1.02
2	10	0.9806	0.9838	0.9874	0.9805	1.5
3	13	0.9910	0.9898	0.9868	0.9801	1.1
4	15	0.9958	0.9939	0.9964	0.9973	0.09
5	20	0.9923	0.9710	0.9650	0.9862	1.5

Then, the confusion matrix was used to obtain the classification power of the network (Table 6). The classification success rate was 99.33%. The evaluation indices of the disturbance matrix including accuracy, precision, sensitivity, specificity, error, F1-score, and kappa were obtained as 99.33%, 99.34%, 99.34%, 99.9%, 0.0069, 0.993, and

0.968, respectively (Table 7).

Table 6- Confusion matrix of the neural network

Sample	Samp1	Samp2	Samp3	Samp4	Samp5	Samp6	Samp7	Samp8	Success Rate
Samp1	30	0	0	0	0	0	0	0	100%
Samp2	0	30	0	0	0	0	0	0	100%
Samp3	0	0	30	0	0	0	0	0	100%
Samp4	0	0	0	30	0	0	0	0	100%
Samp5	0	0	0	0	30	0	0	0	100%
Samp6	0	0	0	0	0	28	1	1	94.7%
Samp7	0	0	0	0	0	0	30	0	100%
Samp8	0	0	0	0	0	0	0	30	100%
Overall success rate									99.05%

Table 7- Evaluation of the confusion matrix of the neural network

Accuracy	Precision	Sensitivity	Specificity	Error	F1-score	Kappa
99.33%	99.34%	99.34%	99.9%	0.0069	0.993	0.968

Table 2 shows the results of the SVM method and Table 7 shows the results of using the MLP model. These results reveal that the system showed better results with the MLP algorithm. By using the designed system, it was possible to estimate the taste parameters of lime juice samples in a non-destructive, fast, out-of-lab, and low-cost way compared to existing laboratory methods (HPLC and GC). Also, this method obtained better results compared to the combined data mining/NIR spectroscopy method (Shafiee & Minaei, 2018) and the method of pattern recognition techniques and FT-IR spectroscopy (Mohammadian *et al.*, 2021).

Conclusion

Two methods of electrochemical and electronic tongues were used in this research. In the first section, an electrochemical unit consisting of three working electrodes, a reference electrode, and a counter electrode, connected to a potentiostat, was designed. Its purpose was to investigate the amount of citric acid as a common indicator of lemon juice adulteration. Since citric acid is highly polar, this method was unable to detect it. Consequently, the electrochemical method proved ineffective in detecting fraud in lemon juice. The second part, based on the detection of taste components using an electronic tongue of noble metals type provided a new tool for the detection of genuine and counterfeit foods in commercial markets. These new methods had the potential to facilitate the implementation of regulatory quality assurance and to verify the quality and authenticity of food products through rapid assessment by taste. To determine the relationship between the e-tongue signal and the classification of lemon juice, PCA, HCA, SVM, and MLP classification algorithms were used and compared. The MLP method's accuracy (99.73%) was higher than others in the classification results, and it could classify lemon juice into different categories with high accuracy. Furthermore, this method is simple, quick, online, and non-destructive, and can be easily used by even non-professionals. Some recommendations are listed below:

In addition to taste features, color and smell features should also be extracted.

This system can be developed to check other liquids, such as milk, non-alcoholic beer, and vinegar.

The system should be redesigned and evaluated with other electrodes, such as iridium, gold with higher purity, and other noble elements.

Since the characteristics of lemon juice depend on the conditions of storage and breeding condition, future research should focus on testing a variety of samples under these differing conditions.

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Authors Contribution

Gh. Bahrami, as the primary author, was responsible for data collection, analysis, and interpretation. Dr. Mohammad Hossein Aghkhani and Dr. Mahmoud Golzarian contributed through overall supervision and academic guidance. Dr. Behjat Dimi provided specialized consultation for the chemistry-related sections of the manuscript. All contributors played a scientific role in the development of this article.

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مقایسه روش‌های ولتامتری چرخه‌ای و زبان الکترونیکی برای ارزیابی تقلب در آبلیمو

قاسم بهرامی[1]، محمدحسین آق خانی[1*]، محمودرضا گلزاریان[2]، بهجت دیمی نیت[3]

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چکیده

در تحقیق حاضر با استفاده از دو روش الکتروشیمیایی ولتامتریک چرخه‌ای و زبان الکترونیک، میزان تقلب در آبلیمو مورد بررسی قرار گرفت. میزان اسید سیتریک در آبلیمو به‌عنوان یک معیار مورد پذیرش در آزمایشگاه‌ها به‌منظور بررسی تقلب در آبلیمو مورد استفاده قرار می‌گیرد، ابتدا با استفاده از دستگاه پتانسیو استات و روش چرخه‌ای ولتامتری، میزان غلظت آن بررسی شد. از الکترودهای گلسی‌کربن، گرافیت، طلا، گلسی‌کربن اصلاح‌شده توسط ذرات نانو لوله‌ی کربن و نانو ذرات طلا، استفاده شد. نوع بافر و میزان pH تغییر داده شد. نتایج نشان داد که رفتار الکتروشیمیایی اسید سیتریک بسیار پایین می‌باشد و با این روش، نمی‌توان رفتار آن را بررسی کرد. در بخش دوم، سیستم زبان الکترونیکی قابل‌حمل (e-tongue) ارزیابی شد. هشت نمونه سطوح تقلب در آبلیمو (۰، ۵، ۱۰، ۲۰، ۴۰، ۷۰، ۹۵ و ۱۰۰ درصد ناخالصی) ایجاد گردید. مدل‌های بدون نظارت شامل تحلیل مؤلفه اصلی (PCA) و خوشه‌بندی سلسله‌مراتبی (HCA) و مدل‌های نظارت‌شده شامل شبکه‌های عصبی پرسپترون چندلایه (MLP) و ماشین بردار پشتیبانی (SVM) استفاده شد. بر اساس نتایج، اثر انگشت PCA تمایز خوبی بین سطوح تقلب نشان داد. این موضوع توسط HCA نیز تایید گردید. نتایج روش‌های نظارت شده نشان داد که عملکرد مدل MLP برای پیش‌بینی سطوح تقلب بهتر از مدل SVM با میزان موفقیت ۹۹/۳۳ درصد و ضرایب همبستگی بالا $R2=0.9973$ و $RMSE=0.09$ بود. نتایج نشان می‌دهد که این سیستم می‌تواند به‌عنوان یک سیستم کنترل کیفیت طعم مورد استفاده قرار گیرد.

واژه‌های کلیدی: الگوریتم‌های طبقه‌بندی، پالس ولتامتری، پتانسیواستات، دامنه بزرگ، گالوانوستات

۱- گروه مهندسی بیوسیستم، دانشکده کشاورزی، دانشگاه فردوسی مشهد، مشهد، ایران
۲- مرکز هوش مصنوعی و یادگیری ماشین، دانشگاه ادیت کوان، استرالیا
۳- گروه شیمی، دانشکده علوم، دانشگاه فردوسی مشهد، مشهد، ایران
(*- نویسنده مسئول: Email: aghkhani@um.ac.ir)

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