Pattern Recognition

Portable Capsaicinoids Detecting Platform based on Multi-electrode Array and Deep Neural Network

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Abstract:	The accelerated development of communication platforms, including the Internet of Things (IoT) and fifth-generation (5G) wireless communication networks, has enabled the construction of smart chemical sensor networks for real-time monitoring of chemical safety and personal health. However, this application scenario requires a combination of various characteristics of sensors, including portability, low cost, superior sensitivity, and high intelligence. Existing pattern recognition methods discard much of the hidden chemical fingerprint information, making the application of chemical sensors to detect samples with complex compositions poor. In this study, we constructed a signal array of five metal electrodes to obtain more differentiated responses with the detection of capsaicin in stew, combined with artificial intelligence for feature extraction of complex signal data to build a convolutional neural network model, and the results showed that satisfactory predictions (RMSE=5.407) were obtained in an independent test sample. This successfully presents a strategy as well as practice for portable sensors combined with artificial intelligence algorithms for rapid quantitative detection of complex analytes.

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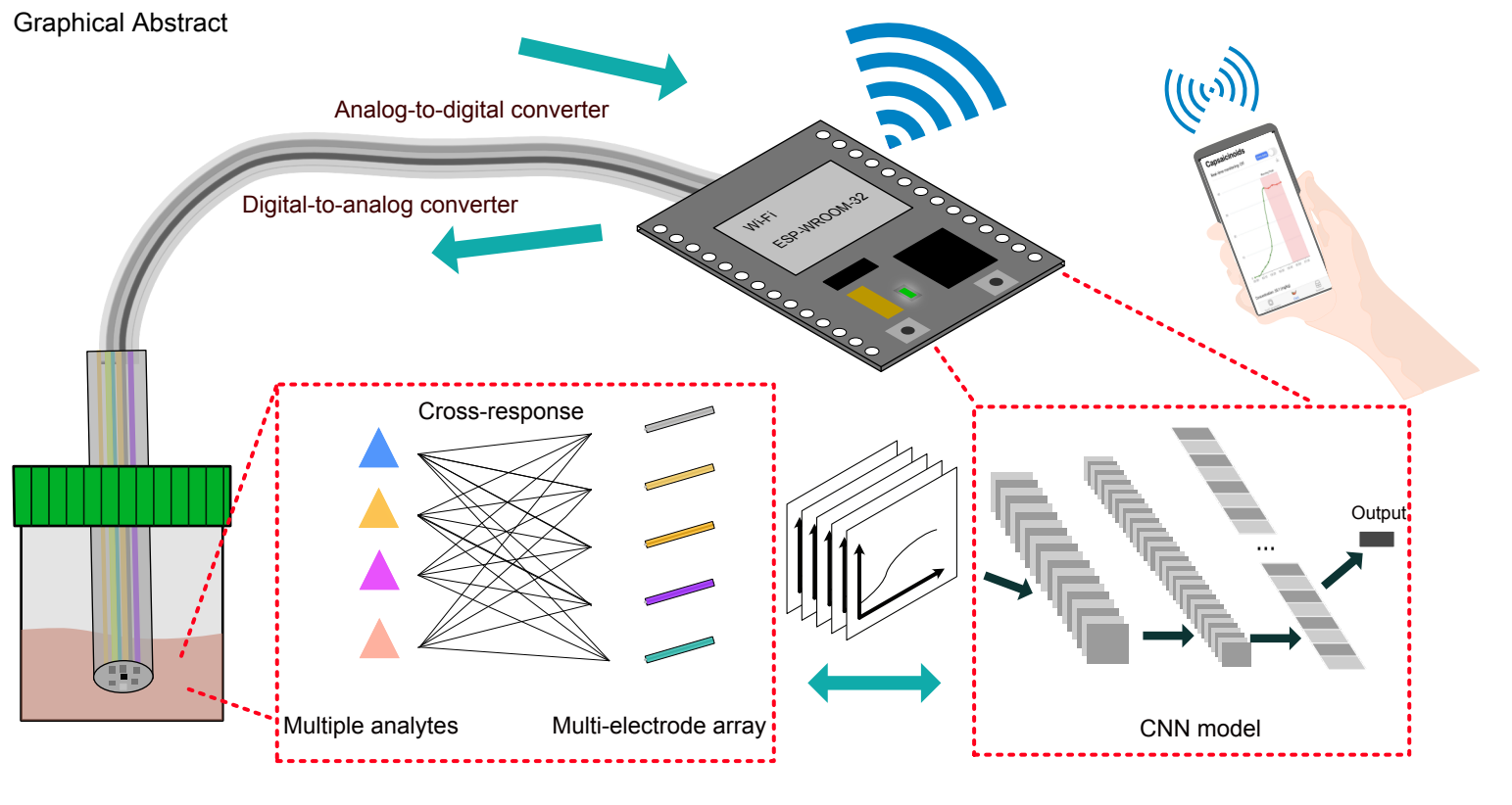
Graphical Abstract



Highlights

- Signal extraction in complex analytes relies on artificial intelligence.
- Enables quantitative prediction of target analytes by sensor arrays.
- Successful fabrication of a portable system for capsaicinoids detection in complex samples.

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ABSTRACT

The accelerated development of communication platforms, including the Internet of Things (IoT) and fifth-generation (5G) wireless communication networks, has enabled the construction of smart chemical sensor networks for real-time monitoring of chemical safety and personal health. However, this application scenario requires a combination of various characteristics of sensors, including portability, low cost, superior sensitivity, and high intelligence. Existing pattern recognition methods discard much of the hidden chemical fingerprint information, making the application of chemical sensors to detect samples with complex compositions poor. In this study, we constructed a signal array of five metal electrodes to obtain more differentiated responses with the detection of capsaicin in stew, combined with artificial intelligence for feature extraction of complex signal data to build a convolutional neural network model, and the results showed that satisfactory predictions (RMSE=5.407) were obtained in an independent test sample. This successfully presents a strategy as well as practice for portable sensors combined with artificial intelligence algorithms for rapid quantitative detection of complex analytes.

1. Introduction

Since early times, spicy food of agricultural products have been an indispensable part of the human diet (Sanatombi and Rajkumari, 2020). Capsaicinoids, which are responsible for the spiciness in food, can commonly enhance taste and appetite, and increase palatability and consumption. However, some people are highly sensitive to capsaicinoids. Individuals with capsaicinoids sensitivity must pay special attention to capsaicinoids concentration because it may damage their gastrointestinal organs and skin. (Magnusson and Koskinen, 1996; Chiang et al., 2021). For example, among patients with palmar hyperhidrosis (13.2%) of them may notice increased sweating when smelling or eating spicy foods in long-term follow-up record (Chang et al., 2007). Thus, food producers, consumers, and others who seek to judge the spiciness of food urgently need a suitable capsaicinoids concentration detector/tool. Capsaicinoids contain more than 20 compounds, of which the content of capsaicin and dihydrocapsaicin reaches 90% (Lyu et al., 2019). Capsaicin and dihydrocapsaicin levels are commonly measured to convert to capsaicinoids concentration. Several methods have been proposed for the qualitative and quantitative of capsaicinoids (Shafiee et al., 2022), including those based on molecules' physical properties, such as electrical conductivity (Naskar et al., 2021; Fang and Duan, 2022), visible optical absorption (González-Zamora et al., 2015), long performance liquid chromatography (HPLC)

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(Werner et al., 2021) and gas chromatography (GC) (Danis and Unal, 2021). However, as the currently accepted standard, HPLC requires sample preparation and pre-treatment steps with the addition of longer analysis times. GC involves complicated procedures, high time consumption and relatively high cost. Despite its versatility on detecting items base on chromogenic reaction, visible optical absorption lacks high separation capacity and detection level. A review by Cheng et al. (2022) presented several published studies reporting the pretreatment and analysis strategies of capsaicinoids in Food.

In recent years, electrochemistry has attracted great interest in the detection of capsaicinoids. In a study conducted by Naskar et al. (2021), a molecular imprinted poly β cyclodextrin embedded graphite electrode showed better performance in the detection of capsaicin, with a minimum detection limit of 0.007 μM, and a satisfactory result was obtained in the practical application of a green pepper extracts. In another similar study by Soleh et al. (2020),a portable electrochemical sensor for the direct detection of capsaicin in pepper samples was fabricated, which is basically a disposable electrochemical paper-based analytical device (ePAD) modified with N-doped graphene nanoplatelets. This sensor can provide linearity in the concentration range of 1-100 μM and with a detection limit of 0.37 μM. However, most conventional electrochemical detection methods are based on a linear correlation between the peak of the cyclic voltammetry (CV) curve inflection point and the capsaicinoids content. They are suitable for simple sample components, but are susceptible to interference when faced with complex sample detection, which seriously affects the detection performance. In addition, the voltammetric signal is not efficiently decomposed and transformed (converted). The traditional conventional method only extracts the vertex inflection points of the signal as eigenvalues, and directly ignores the signals other than the vertex inflection points, thus ignoring the useful detailed data contained or hidden in it. The development of artificial neural networks and artificial intelligence has brought many new perspectives (Gil et al., 2014). In addition to research in materials and device engineering to improve sensor performance, pattern recognition algorithms based on an artificial intelligence (AI) "training process" for deep learning of input values have proven to be an efficient strategy for decoding complex sensing signals according to Song et al. (2021); Li et al. (2018).

In present work, we report a strategy of multi-electrode array (MEA) detecting platform capable of selectively quantifying capsaicinoids in the presence of many analytes. Five common bare metal electrodes as working electrodes were used to scan the complex liquids from pepper food processing. A simple and effective feature matrix extraction method is proposed to eliminate the electrical signal offset caused by noise and obtain CV data with uniform dimensions. Our unique approach of cross-response signal through MEA combined with a convolutional neural network (CNN) algorithm (Guo et al., 2022) enabled microcontroller allows simple and visualization the capsaicinoids detection via smartphone without any external pre-treatment of samples.

2. Materials and methods

2.1. Materials and chemicals

Duck wing, capsicum frutescens (chili type) and seasoning packets (include star anise 20.0 g, cloves 4.5 g, cinnamon 15.0 g, orange peel 4.0 g, sugar 25.0 g) were purchased from the local wholesale market (Wuhan, China). All reagents used were of analytical grade and purchased from Sigma Aldrich (Poole, UK) unless specified otherwise.

2.2. Cooking procedure

In a pot containing 5.0 L of pure water, we added 75.0 g of salt, 50.0 g of soybean oil, 1250.0 g duck wing, 100.0 g of capsicum frutescens and a seasoning packet (Becker et al., 2016). The cooking temperature was set at 60 °C, which took approximately 2 h to the samples to reach the core temperature of 60 °C. Upon boiling of the soup, the brine were collected at intervals of about 3 min within 2 hours. This process was repeated 5 times and a total of 150 samples were collected.

2.3. Capsaicinoids analysis

The ultra performance liquid chromatography (UPLC) analyses were performed with an ACQUITY UPLC H-Class(Waters, Milford) with autosampler interfaced to a PC. The UPLC separations were obtained using an Acquity UPLC HSS T3 reverse column (2.1mmx100mm, 1.8μm) operating at 25 °C in gradient mode. The detection wavelength was 280 nm. The mobile phase was A (acetonitrile), B (H_2O+0.1% formic acid). The gradient programming was: t = 0 min, A: 30%∼60%, t = 10.0 min, A: 60%∼62%, t = 12.0 min, A: 62%∼70%, t = 14.0 min, A: 70%∼30%, t = 15.0 min, A: 30% (hold 1 min). The injection volume was 2 μL and the flow rate was set at 0.2 mL/min.

The prepared brine was poured into a separatory funnel to obtain the upper oil phase. After cooling at room temperature, 2.0 g of the upper oil sample were collected in a 50 mL centrifuge tube, 10 mL of acetonitrile-saturated n-hexane solution was added and vortexed for 1 min. Then 10 mL of n-hexane-saturated acetonitrile solution was added into the centrifuge tube and sonicated for 2 min for the extraction. The mixture was allowed to stand for 10 min before centrifuging at 8,000 r/min for 5 min. The acetonitrile layer was removed and 4 mL of n-hexane-saturated acetonitrile solution was added for extraction twice. The extracts of the acetonitrile layers were combined, dried in a water bath at 45°C and dried with nitrogen. The volume of acetonitrile was adjusted to 1 mL, and the solution was passed through a 0.22 μm organic filter membrane, 2 μL of which was injected into the UPLC system for data analysis.

Standards of capsaicin and dihydrocapsaicin were prepared at a concentration of 100 mmol/L with DMSO. A mixed gradient dilution solution of capsaicin and dihydrocapsaicin was configured with methanol solvent. For the standard curve, the standard concentrations were plotted as horizontal coordinate and the peak area of the UPLC analysis as vertical coordinate.

2.4. Assembly of portable detecting platform and electrochemical detection

The portable device consisted of a microcontroller unit (MCU; ESP32), a Wi-Fi module, multi-electrode array, many general-purpose input/output (GPIO) pins: analog-to-digital converter (ADC) pins, digital-to-analog converter (DAC) pins, GND pins. Formation of one circuit loops: MEA-DAC, pin-GND, glassy carbon electrode (GCE). As shown in Figure 1(A), five bare metal electrodes consisting of MEA with each sensing electrodes (Ti, Cu, Ge, Wu and Ag) were integrated into a modified centrifugal tube. To collect the sensing responses of the capsaicinoids sensors in real time, a module capable of simultaneously driving the MEA is required. The ESP enables the DAC module to provide periodic voltage changes to the MEA. Each working electrode, auxiliary electrode GC, and electrolyte (complex analytes) in the MEA form a circuit, and the current detection sensor captures real-time current change values and returns them to the ESP32 via the ADC module. Figure 1(B) shows a simplified overview of the operation of the MEA-based portable capsaicin detecting platform. This system allows simultaneous CV curve scanning of the MEA as well as data feedback.

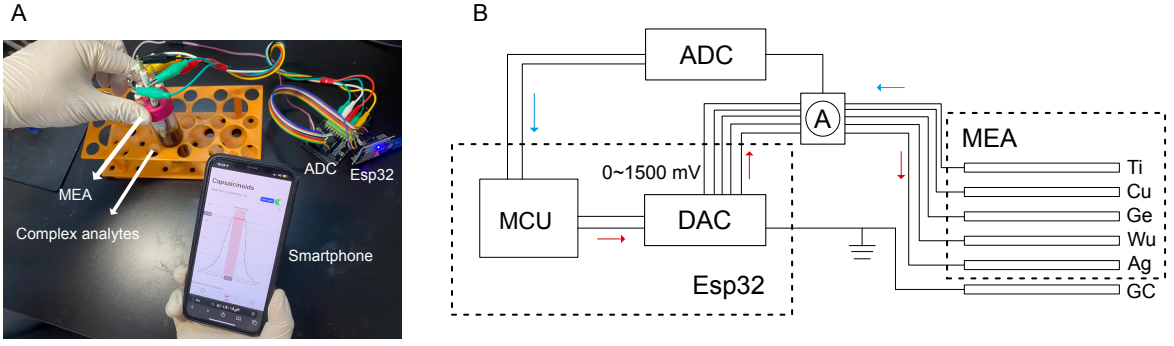

Figure 1: (A) Image of a smartphone-enabled capsaicin detecting platform. (B) Simplified overview of the sensor control for the gas test. MEA is excited by a DC voltage (0~1500 mV) from the DAC module, and the resistance data is converted to the current. ADC module is used to return a sensor response data to MCU.

All electrochemical measurements were carried out using a portable multi-electrode array device. Multi-electrode array was used as a working measurement and to send and receive electrical signals via the GPIO pin connected to the smart chip Esp32. Electrochemical measurements were performed using a 20 mL soup (as electrolyte solution) containing various concentrations of capsaicin covering all the electrodes of the multi-electrode array. The Esp32 chip could control and read the potential signal of the multi-electrode array by converting the digital signal and analog signal through 2 modes: digital-to-analog conversion and analog-to-digital conversion. CV was carried out by scanning between 0 mV and +1500 mV at a scan rate of +3 mV/ms.

2.5. Construction of multidimensional eigenvalue matrix.

The statistical analysis of the electrochemical data was performed using the python3.9 libraries such as NumPy, Pandas, Scikit-learn, Matplotlib etc. The response signal of the MEA is the original voltammetric signal, and the conventional eigenvalue extraction method is to directly select the inflection point or extreme point of the signal (Lu et al., 2021). However, the conventional method is not suitable for the multidimensional eigenvalue extraction, because the inflection point and extreme point of the signal are not always the same. CV was carried out by scanning between 0 mV and +1500 mV at a scan rate of +3 mV/ms, the data points of the CV curve are theoretically 500-dimensional vector according to the design in the experiment. However, due to the interference of different electrodes and environmental factors, the data change steps from 0 to 1500 mV operating potential data are inconsistent, resulting in the completion of the scanning CV curve earlier or later, even the same electrode exists between different samples. In artificial intelligence, a feature vector is an n-dimensional vector that describes the computational characteristics of some entities, and the vector dimensions are usually required to be consistent to form an eigenmatrix during model training (Singh and Singh, 2021). The extraction of feature variables in this experiment was performed following Xing et al. (2020) method with some modifications. In mathematics (Figure 2), the columns of the matrix X represent different sensors in the MEA, each row represents the sampling point with increasing potential (from 0 to 1500 mV), and each data point corresponds to the potential and current value of the point. The extraction of the feature matrix requires that a matrix dimension n be given, and n should be less than the smallest row in the matrix X. Shown here is a representative example of n = 450. The eigenvalue matrix is a 450-by-5 matrix. The eigenvalue matrix is calculated by the following steps: (1) The interval 0∼1500 is evenly divided into 450 parts, which means that the expected matching potential values are $\left[3.33, 6.67, ..., 1500\right]$(computed by $\frac{1500}{450}\left[1, 2, ..., 450\right]$). (2) The match current values in each column in matrix X at the closest approximation in potential values by the matching potential values from step 1. (3) The matching current value obtained in step 2 constitutes a 450-by-5 eigenmatrix.

The method we propose to extract the eigenvalue matrix is a simple algorithm is a simple algorithm to convert multiple dimension variables into a unified dimension. It is worth noting that this method is only used to reduce dimension to a unified level but cannot be used for the extension. In addition, due to differences in equipment, samples, and environments, the optimal matrix dimension n is usually uncertain. Given different matrix dimensions n, eigenmatrix for various situations can be obtained.

2.6. Capsaicinoids quantification with CNN and deep neural network

Our proposed capsaicin regression quantification system is completely based on the CNN method. The CNN model was trained on the data of the experiment, and the trained model was used to predict the concentration of capsaicinoids in the samples. We use the PyTorch framework to construct the CNN model because it can be easily implemented

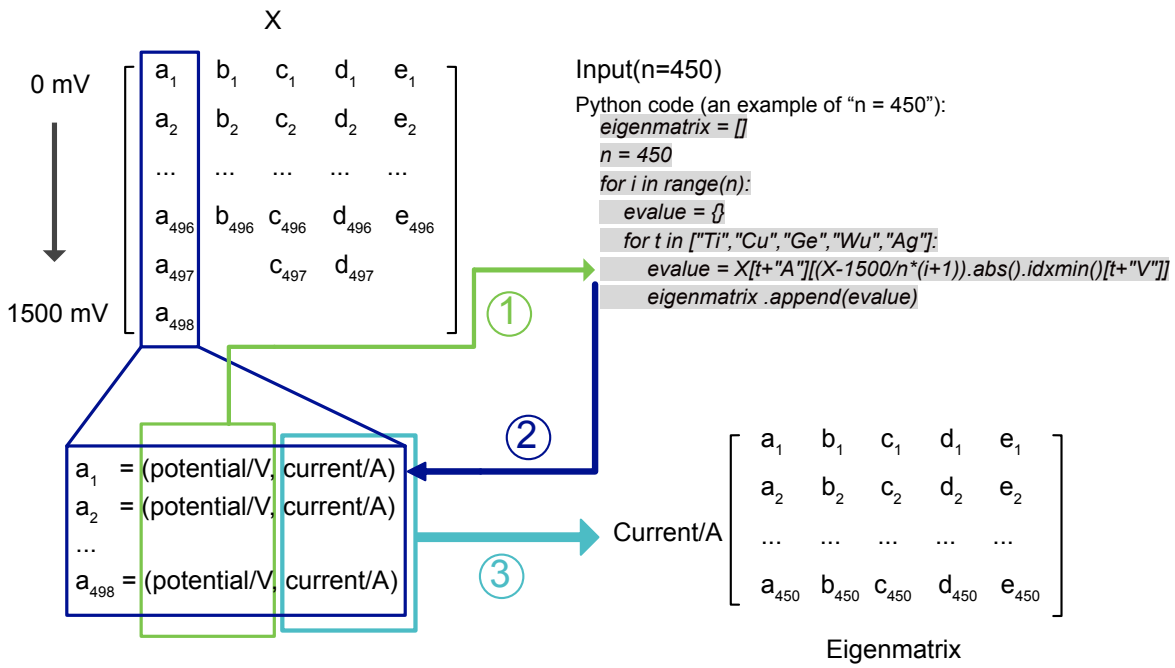

Figure 2: Schematic process of the calculation for the multidimensional eigenvalue matrix.

on the python platform and can dynamically adjust the model without building from scratch. Thus, people with basic knowledge of python can utilize it to build their own deep learning models (Sen and Sawant, 2021). The following flowchart (Figure 3) illustrates the overall flow of the approach. Deep Neural Network consists of three types of neuron layers: convolutional layer, pooling layer and fully connection (FC) layer (LeCun et al., 2015). Convolutional layers are used to extract features from the input data, and pooling layers are used to reduce the dimensionality of features. The concentration of capsaicinoids in the samples was determined using the FC layer.

In this network, the eigenmatrix (450,5) is directly used as the input layer after normalization. Apply the 1D convolution over an input signal composed of 5 input channels. The first convolution layer (1DConv Layer 1) is 446 with the 20 feature maps, followed by activation function named ReLU (Rectified Linear Units) for the faster computation. Then, the inputs are transferred into the second convolutional layer (1DConv Layer 2) and ReLU, followed by Max Pooling at 2 with the stride at 2. Thereafter, they will pass the Fully connection layer and output Layer, which is determined as Softmax to capsaicinoids quantification as presented in Figure 3.

To train the CNN model, we used the Adam optimizer (Kingma et al., 2015) converging at a faster speed to improve the efficiency of the training process with an initial learning rate of 0.01 and a mini-batch size of 7. The parameters of CNN model are updated efficiently with the backpropagation and stochastic gradient descent method (Amari, 1993). In total, 600 training epochs were run. The epoch refers to the iteration over the entire training set. This study used a

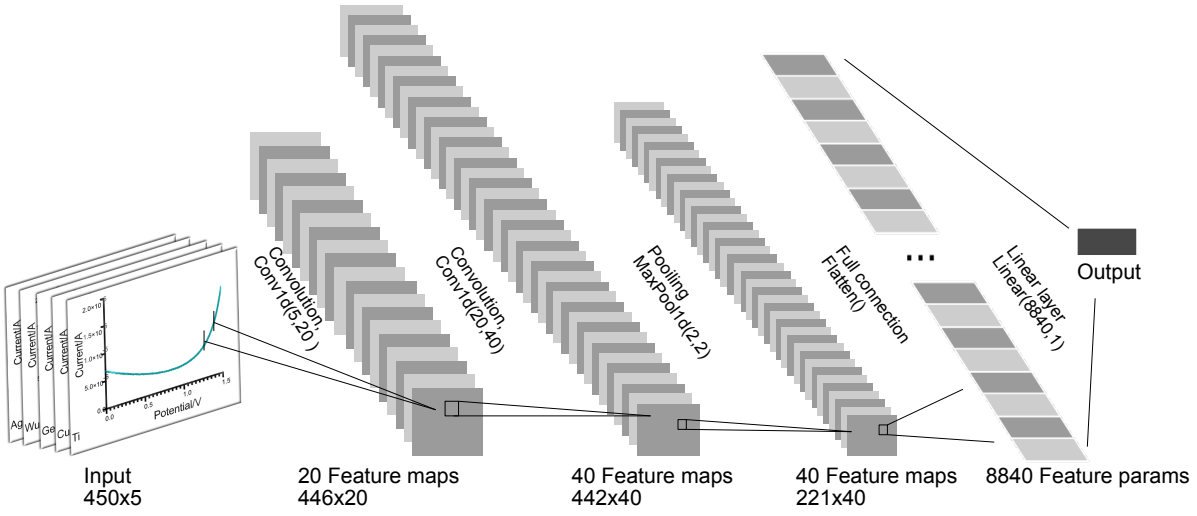

Figure 3: Convolutional neural network architecture for capsaicinoids quantification.

random shuffle method. First, the samples were randomly divided into 5 equal sets. Four groups were used as training models, and one group is used to test the CNN model performance.

3. Results and discussion

3.1. Analysis of capsaicinoids in the brine stewing process

It was reported that capsaicin and dihydrocapsaicin account for about 90% of the capsaicinoids content in peppers (Lyu et al., 2019), therefore, capsaicinoids content is often calculated from the content of capsaicin and dihydrocapsaicin. Two standard curves (Figure 4(A,B)) were created by using Graphpad Prism 8 (GraphPad, prism software), the line equation for the capsaicin standard curve was $y = 5978x - 4278(R^2 > 0.99)$ and the line equation for the dihydrocapsaicin standard curve was $y = 4967x - 8260(R^2 > 0.99)$. During the stewing process, spices were added and allow to boil. The brine samples were taken at intervals of about 6 min as the detection object, UPLC was used to analyze the content of capsaicinoids in the brine samples with different stewing times. As shown in the Figure 4(C), the change of total capsaicinoids during the stewing process generally indicated a fluctuating increase, and it was slowly released into the stewed soup in the first 36 min. The total capsaicin content in the stewed soup significantly increased from 36 to 78 min, reaching a maximum peak. After 78 min, it gradually tends to fluctuate and balance with the prolongation of stewing time. In the early stage of braised cooking, most of the capsaicinoids dissolved in the braised soup may be adsorbed into the meat. A very small part of it was decomposed by heat, because capsaicin had good stability at a high temperature of 100°C (Hachiya et al., 2007). In the later stage of braised cooking, on the one hand, the content of capsaicinoids in meat and braised soup reached a dynamic adsorption equilibrium. On the other hand, total capsaicinoids was dissolved and almost completely released from peppers, so its content in braised soup

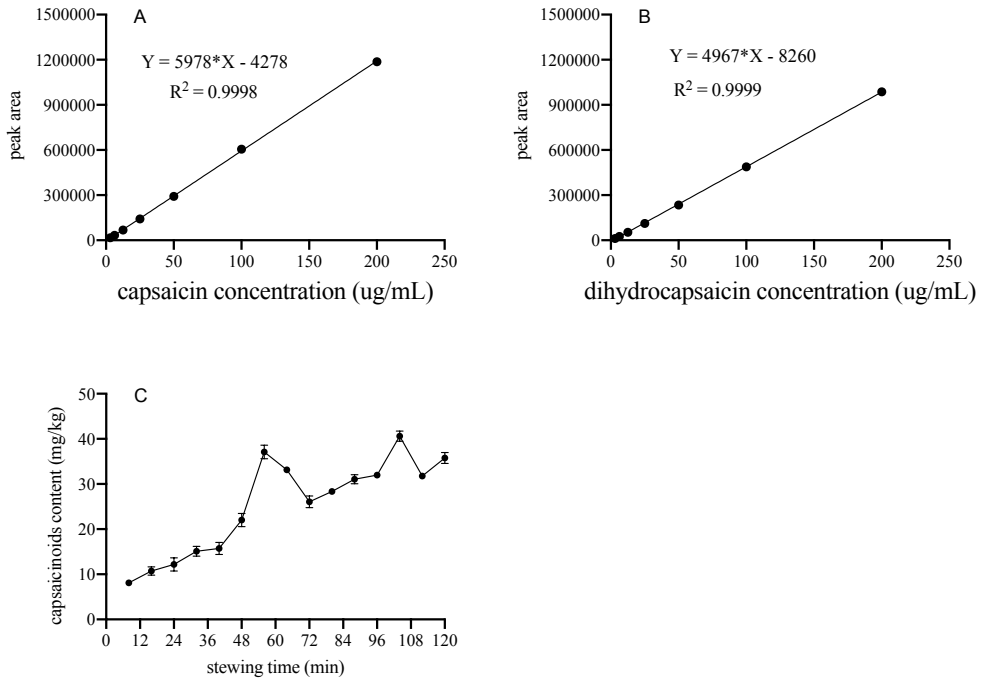

Figure 4: Standard curves of (A) capsaicin and (B) dihydrocapsaicin, (C) variation of capsaicinoids content in a low temperature cooking cycle.

tended to be in dynamic equilibrium.

3.2. Electrochemical behavior of capsaicinoids on the MEA

Voltammetry is employed to characterize the electrochemical performances of bare array electrodes in the soup for the fabrication of capsaicinoids biosensors. Figure 5(A-E) shows the CV obtained of bare array electrodes in the different stewing stages: initial, intermediate and final. Different working electrodes (or different analyte and working electrode pairs) and different stewing stages will result in different electron transfer rates, yielding differential response data. Ions in an electrolyte solution move under the action of an electric field, forming an electric current, as shown in Figure 5(F). The electrochemical redox mechanism of capsaicinoids was first proposed by Kawada et al. (1985), and later, after continuous research and improvement, Kachoosangi et al. (2008) found that the oxidation of hydroxyl groups on capsaicinoid molecules can occur through different pathways by using solutions with different pH. da Silva Antonio et al. (2019) concluded that capsaicin may undergo electrochemical redox reactions through two different ranges of pH pathways, as shown in the Figure 5(G). The first redox reaction occurs under alkaline or neutral conditions at pH 6-10, where the phenolic hydroxyl group is dehydrogenated and converted to phenoxy radicals, which are stabilized by resonance, followed by further oxidation of the conjugated radicals to phenoxy cations, a pathway involving two single electrons. The second pathway occurs in an acidic environment at pH $<$ 6, where the phenolic

hydroxyl group undergoes irreversible oxidation in a single two-electron, forming phenoxy cations. After these two pathways, the phenoxy cation is hydrolyzed to methanol and o-benzoquinone, and a reversible redox reaction between o-benzoquinone and catechol also occurs.

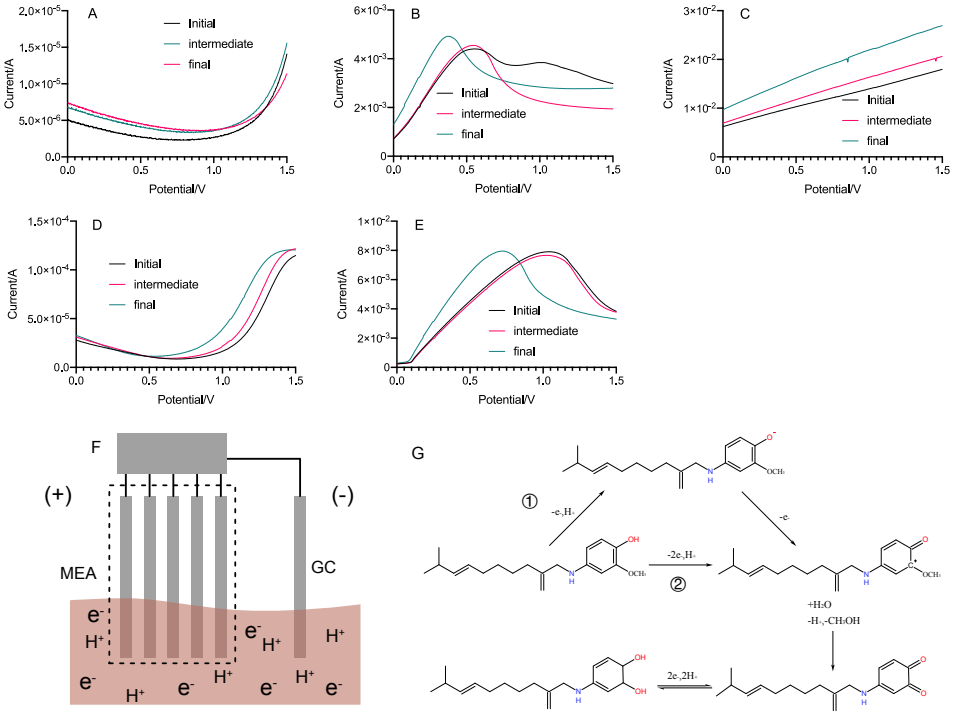

Figure 5: Electrochemical characterization measurements performed in the different stewing stages: initial, intermediate and final. Subplots (A), (B), (C), (D) and (E) represent the electrochemical characteristic curves of different metal electrodes (Ti, Cu, Ge, Wu and Ag) in the MEA, respectively. (F) Schematics illustrating the redox reaction of MEA and analyzes (G) Mechanism of the electrochemical oxidation and reduction of capsaicinoids.

3.3. Modeling and quantitative prediction.

A training loss of model is computed between the actual and the predicted values. In this CNN model, the mean squared error (MSE) loss function is used for training because it produces a trained network that approximately maximizes the peak signal-to-noise ratio (PSNR) according to Nagare et al. (2021). Figure 6 shows the loss curve and predicted curve of the best architecture (in CNN model). From the loss curve in Figure 6(A), we can observe that as the number of training epochs increases, the training and testing loss show an overall downtrend. In training loss curve, when the number of epochs reached 300, the algorithm quickly converged to an ideal state, and the training loss reached a stationary state of approximately 0.015. In contrast to the training loss curve, the test loss gradually decreased, and when the number of training epochs reached 163, the testing loss reached a stationary state of approximately 0.028. Figure 6(B) shows the test set predicted curve of the best architecture in initializing epoch to 163. The

performance of the best trained model is significant for the test set. The steady state can be evaluated from both the actual and predicted values simultaneously, therefore, the CNN model trained in this experiment shows a satisfactory fit effect (RMSE=5.407).

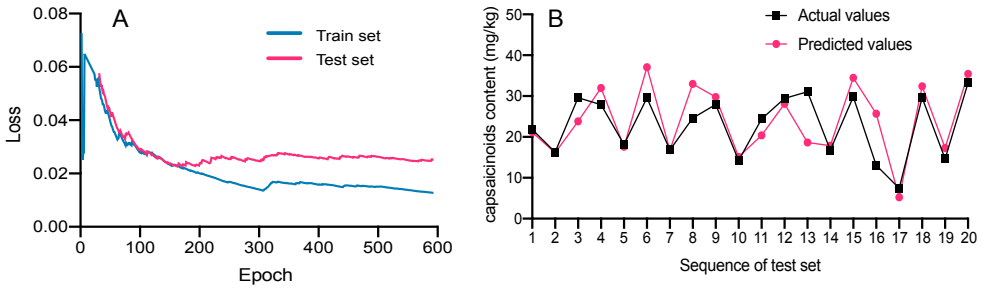

Figure 6: Loss curve and predicted curve of the best architecture. (A) Shows the loss curve of the best architecture. The blue curve represents the training loss and the pink curve represents the testing loss. (B) Represents the predicted curve of the best architecture in test set. The black points represent the actual values and the red points represent the predicted values of the capsaicinoids content.

We further studied the performance of the CNN best architecture, and found that the testing loss is different when the convolutional layers are different. Too many layers can lead to an overly complex CNN model, which is subject to overfitting (Servadio and Convertino, 2018). The prediction performance on the test set does not show higher accuracy due to the more convolutional layers included. This notion is consistent with the results of recent modeling work by Sun et al. (2021).

4. Conclusions

In summary, we have successfully developed a high performance capsaicin detection system by integrating an array of electrodes, MCU for data processing, Wi-Fi unit for wireless data transmission and smartphones for visualizationand. Notably, we used easily accessible metal electrodes as array materials, applied artificial intelligence (AI) algorithms to decode complex sensing signals, and convolutional neural network algorithms effectively extracted feature data, identified patterns of input values according to a "training process" and obtained satisfactory results. The system was able to detect capsaicinoids content in the stewed soup with a high accuracy, which is an effective practice for quantitative regression detection of target analytes in complex samples by relying on AI recognition.

In the future, big data, Internet of Things (IoT), 5G communication, and AI technologies will develop considerably. The miniaturization of sensors and real-time monitoring of intelligent data will be the key direction for the development of gas sensors. This system design could be applied also for detection of other fields, such as food safety, gas pollution, human health, etc.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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