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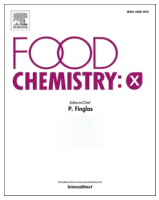

Ultrasensitive analyses of zearalenone in grain samples with a catalytic oxidation platform involving gold nanomaterials

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ABSTRACT

Zearalenone (ZEN) contamination in cereals poses a serious threat to human and animal health, yet existing rapid test methods still suffer from poor stability and low sensitivity. The studied sensor reduces inspection time while enabling applications for on-site grain inspection. Specifically, a ZEN detector that can sensitively detect ZEN content in grains was developed. Ion implantation is an effective method for modifying screen-printed electrodes (SPEs). Gold nanoparticles (AuNPs; 5–10 nm) were uniformly implanted using screen-printed electrodes as a catalytic oxidation medium to generate an electrochemical sensor. The surface structure of the modified electrode was characterized using scanning electron microscopy and X-ray photoelectron spectroscopy. The results showed that differential pulse voltammetry had good linear electrochemical response to ZEN at 10 ng/kg to 10 mg/kg, with a detection limit of 1.1 ng/kg. We used AuNP-SPE sensors to detect ZEN in grain samples such as maize and oats.

1. Introduction

Zearalenone (ZEN) is mainly found in grains that are susceptible to fungal contamination such as maize, wheat, sorghum, and rice (Zinedine, Soriano, Molto, & Manes, 2007). Exposure to ZEN or its inadvertent ingestion can seriously threaten human health by compromising the immune, nervous, and reproductive systems (Zhang, Chen, Lu, Yu, & Zhang, 2023). According to worldwide statistics from the Food and Agriculture Organization of the United Nations, up to 25% of global crops are contaminated with mycotoxins(Eskola et al., 2019), and ZEN is a common contaminant in corn, other grains, and grain-based foods (Nathanail et al., 2015; Vanheule et al., 2014). According to the European Union, the limits for ZEN in unprocessed grains and processed foods are 200 μg/kg and 75.0 μg/kg respectively to regulate mycotoxin contamination in grain (Appell, Compton, & Bosma, 2022). However, in some areas of China, the detection rate of ZEN in maize is close to half of all samples (A. Li, Hao, Guan, Wang, & An, 2022), which constitutes a serious threat to human health. Therefore, there is an urgent need for a sensitive and effective assay to detect ZEN in grain.

To date, numerous methods have been used to detect ZEN in grains. Traditional detection methods such as ultra-high performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS), mass spectrometry, or thin-layer chromatography are often used for detection, but these methods often require complex sample pretreatment and are time-consuming, and costly. Immunoassays save time, provide easy detection, and are the most commonly used rapid detection method for ZEN (Liu et al., 2021). An electrochemical (EC) sensor is a portable, easy to operate, and sensitive analysis tool (Du, Xie, & Wang, 2021). In addition, EC sensor-based detection methods have attracted attention due to their advantages such as cheap price, low detection limit, and rapidity (Yan et al., 2022). ECs sensors based on enzyme-labeled antibodies, adaptors, and molecularly imprinted polymers are common, but they are still limited by their sensitivity to external environmental influences and their high cost (Xiang et al., 2018). Recently, screen-printed electrodes have been used in sensor research to provide portable, sensitive, and low-cost analysis and detection (Paimard, Ghasali, & Baeza, 2023). Sensors based on screen-printed electrodes (SPEs) can achieve fast and simple analysis while also meeting the requirements of low-cost mass production.

Gold nanoparticles (AuNPs) have the advantages of chemical stability, good biocompatibility, superior conductivity, good catalytic performance, and large surface-to-volume ratio among numerous metal

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materials, making them the most commonly used in sensor development (Gupta & Kumar, 2023). They can achieve direct electron transfer between the analyte and electrode base, promoting electrode reactions (Caetano, Felippe, Zarbin, Bergamini, & Marcolino-Junior, 2017). Ion implantation is an excellent material surface modification technology that does not require any binders or chemicals during use. It is environmentally friendly, easy to operate, stable in batch preparation, and able to achieve industrial production rates (Gupta & Kumar, 2023; Pravesh, Dahiya, Singh, & Singh, 2023).

This study optimized the ion implantation method and used AuNPs to modify the SPE to prepare a sensor for detecting zearalenone in grain. We hypothesized that the detection method might have higher sensitivity than traditional detection methods, such as UPLC-MS/MS (Arroyo-Manzanares et al., 2018), or emerging rapid detection methods such as IUCNP's ICA (Y. Chen, Shen, Wang, Zhang, & Zhu, 2023), SERS (R. Chen et al., 2021), p-dcFLISA (Zhou et al., 2022), and other electrochemical detection methods such as the aptamer sensor (Zhang et al., 2023), and the chemiluminescence aptasensor (Guan et al., 2023). Based on this comparison, it can be clearly concluded that the proposed detection method has better analytical performance than previously reported ZEN analysis methods.

The differential pulse voltammetry detection method improves the sensitivity of the sensor, and ultimately the developed sensor can be successfully applied to achieve sensitive zearalenone analysis in grain.

2. Material and methods

2.1. Chemicals and reagents

ZEN was obtained (99% purity) from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China) and SPEs from Metrohm-Dropsens (Shanghai, China). First, 0.1 M phosphate buffer saline (PBS) at different pH values was prepared by mixing 0.1 M NaH_2PO_4 and Na_2HPO_4. Possible interfering compounds, e.g., glutamic acid, cystine, tartaric acid, fructose, sucrose, and vitamin B2, were prepared at 10 g/L in ultrapure water. Glutamic acid, cystine, and vitamin B2 were then dissolved in an alkaline solution. SPEs were cleaned with ultrapure water and dried with N_2 gas before use to prevent electrode oxidation. DPV and cyclic voltammetry are commonly used to determine the electrochemical characteristics of SPEs. Comparatively, DPV is more sensitive than cyclic voltammetry, and therefore we used DPV to quantify ZEN. The potential range was 0 to 0.8 V, and the scanning speed was 100 mV S^{-1}. All experiments were conducted at room temperature.

2.2. Instruments

All electrochemical measurements were performed using a CHI660E electrochemical workstation (Chenhua Instrument Company, Shanghai, China) in a three-electrode system. The reference electrode and electric contacts were made of silver. The pH values of the buffer solutions were determined using a pH meter (Mettler Toledo Instrument Company, Shanghai, China). The acceleration voltage during Au ion implantation was 10 keV, and the injection dose was 1×10^{17} cm^{-2}. We used scanning electron microscopy (SEM; SU8020 microscope, Hitachi, Japan) to observe the surface morphology. X-ray photoelectron spectroscopy (XPS) was performed using a Thermo Escalab 250 XI (Thermo Scientific, Britain, Europe) with an Al Ka X-ray source.

2.3. Grain sample preparation

All grain samples were purchased from a local market. Sample preparation was performed as reported by (Lu et al., 2021) with modifications. First, 10 g of regular corn feed, moldy corn, and moldy oats were sampled and ground for 10 min to obtain a powdered sample. After mixing, 2 g of each sample was weighed and placed in a 50 mL centrifuge tube. Next 20 mL PBS buffer solution was then added, and the mixture was sonicated for 20 min and centrifuged for 10 min at 25,000 $\times g$. Finally, 0.22 μM microporous membrane filters were used to filter the supernatant after centrifugation to obtain the treated test solution.

3. Results and discussion

3.1. Validation of electrode modification

3.1.1. Surface characterization

AuNPs are easily synthesized, have high reaction interface activity, reduce the energy barrier of electrochemical redox, and have adequate electrochemical performance (Ju et al., 2017). AuNPs are commonly used metal modification materials. The surface morphology and microstructure of the bare and AuNP-SPE were characterized by SEM.

Bare SPE surface characteristics are shown in Fig. 1A, and AuNP-SPE surface characteristics are shown in Fig. 1B. In the SEM results for bare SPE, we observed spherical structures of varying sizes, with diameters ranging from 100 to 400 nm. Ion implantation modified the electrode surface structure, resulting in a larger and denser arrangement of carbon elements. Specifically, in the AuNP-SPE, we observed spherical structures of approximately 5 to 10 nm, which were densely and uniformly deposited on the surface. According to previous studies, the size, shape, and morphology of AuNPs affect the intrinsic performance of nanomaterials (El-Sayed et al., 2017; Wu et al., 2019a; Wu et al., 2019b).

3.1.2. XPS

We used XPS to evaluate the chemical structural changes in SPEs before and after modification by gold nanoparticles, specifically surface elemental composition and chemical states (Keerthi et al., 2022). Fig. 1C shows that, within the same energy range, there was no Au peak in bare SPEs. After ion implantation, there was a clear Au peak in the AuNP-SPE, and Au 4f could be clearly observed. Fig. 1D shows that Au ($4f_{7/2}$) at 84.70 eV and Au ($4f_{5/2}$) at 88.30 eV were visible and that AuNPs were successfully implanted on the SPE surface (Devi et al., 2019; Kong et al., 2019; Luo et al., 2016).

3.2. Optimization of experimental conditions

To achieve more sensitive detection results, we optimized the buffer pH for electrochemical detection. The effect of pH on the electrochemical behavior of the AuNP-SPE was studied by cyclic voltammetry at pH 4–8. The peak current increased with increasing pH value. At pH 7.4, cyclic voltammetry detected the highest peak current. At pH > 7.4, the peak current decreased (Fig. 2). Therefore, pH 7.4 provided the best electrochemical detection condition required by the electrode, which is consistent with past studies (Cardoso, Moreira, Fernandes, & Sales, 2016). Therefore, subsequent experiments were conducted with PBS at pH 7.4.

3.3. Electrochemical detection of sensors

The sensor manufacturing process was monitored using CV and DPV. As shown in Fig. 3A, the position of the current peak can scarcely be observed for bare SPEs, whereas a pair of obvious current peaks can be observed after modification with gold nanoparticles. Consistently with the CV results, the DPV results showed a significant oxidation current peak on the screen-printed electrode modified with gold nanoparticles. Use of the modified sensor to detect ZEN will significantly increase the peak current.

The smaller size of the gold nanoparticles leads to an increase in the specific surface area, which in turn leads to dynamic single-atom generation and exposure of more surface groups, enabling them to have enzyme-like catalytic oxidation properties.

For example, Wu et al. successfully prepared AuNPs with small particle size and found that they exhibited excellent catalytic activity in CO oxidation (Wu et al., 2019a; Wu et al., 2019b). The XPS results, along

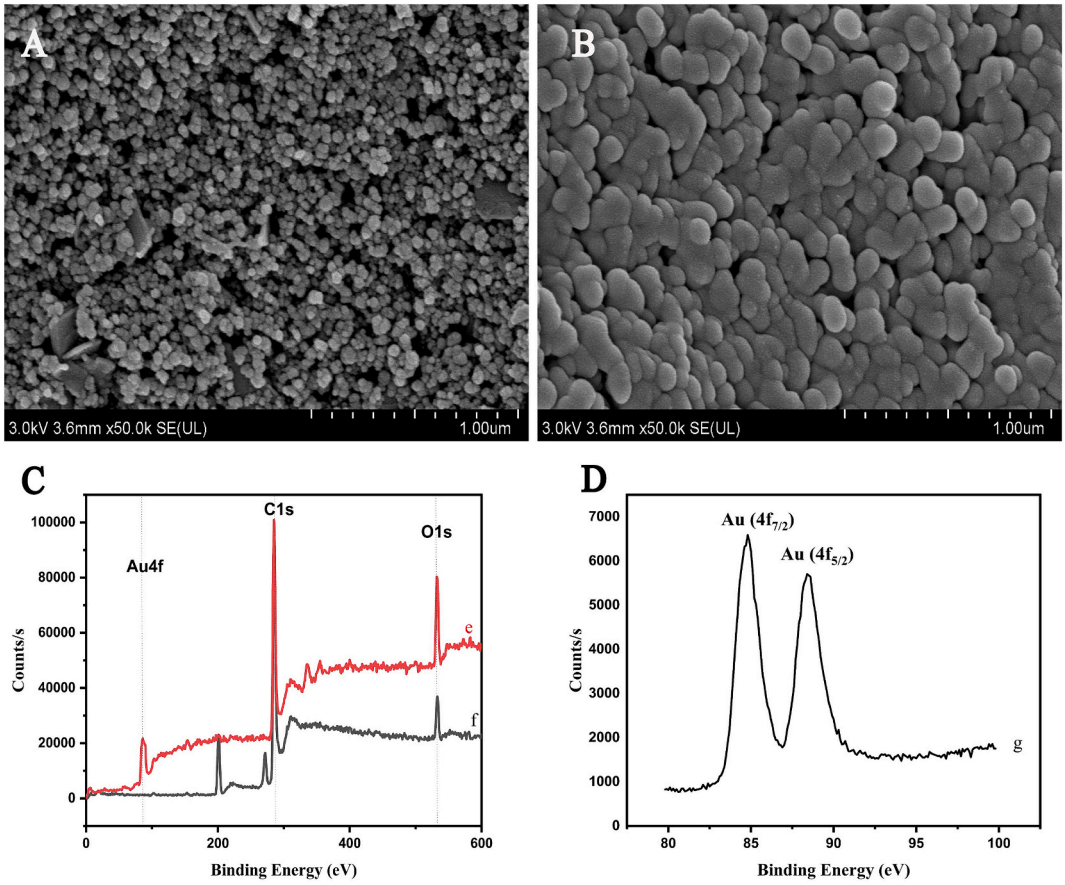

Fig. 1. SEM images of bare SPE (×50 k)(A) and of AuNP-SPE (× 50 k)(B). XPS images of the AuNP-SPE sensor (C), Au 4f (D); AuNP-SPE (e), bare SPE (f), AuNP-SPE (g).

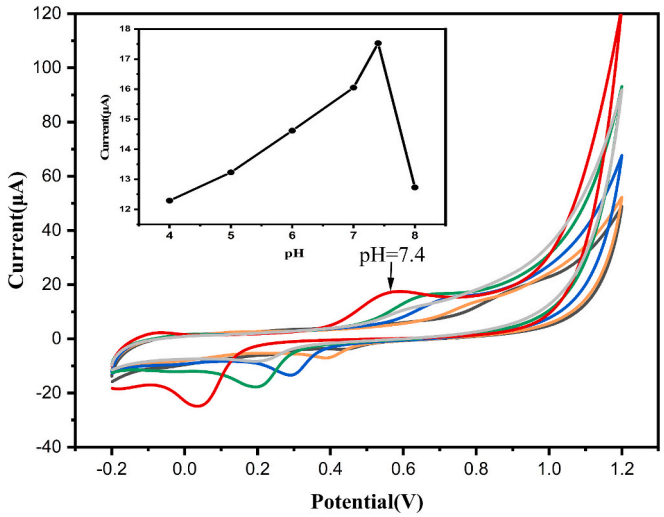

Fig. 2. Cyclic voltammograms (CVs) of 0.1 M PBS of different pH values. Inset: the influence of pH on the peak current.

with the CV and DPV electrochemical assays discussed earlier, revealed a notable rise in the oxidation peak current of AuNP-SPE/ZEN. This suggests the active involvement of tiny AuNPs in the oxidation catalytic process of zearalenone. In the DPV assay, the zearalenone double bond was oxidized when the applied potential was 0.5 V. Based on previous studies (Wang et al., 2023; Yu, Pang, Yang, Liao, & Shen, 2023), the location of oxidation was determined as shown in Fig. 3C.

The CV and XPS results show that SPE modified with gold nanoparticles was involved in the electrocatalytic oxidation of zearalenone (Fig. S1). At approximately 0.6 V, the increase in oxidation current was attributed to the reaction between Au^0 and zearalenone. The kinetic parameters of the AuNP-SPE in ZEN standard solution were obtained by cyclic voltammetry at different scanning speeds (Fig. 3B). The oxidation peak potential moved positively with increasing scanning rate, whereas the reduction peak potential moved negatively. Therefore, the peak current values of oxidation and reduction were positively correlated with scanning rate. The obtained equations and correlation coefficient were $I_{pa} = 0.1892\ v + 8.6921$ and $R^2 = 0.9930$, respectively, indicating that the reaction between the electrode and ZEN is a typical adsorption-control process (K. Li et al., 2023).

3.4. Application of AuNP-SPE

Analytical ZEN detection performance was investigated under specific conditions of PBS concentration (0.1 M), pH (7.4), and scanning speed (100 mV S^{-1}) using the characteristics of sensor current variation with ZEN concentration. As shown in Fig. 4, with increasing ZEN concentration, the DPV signal increased. The calibration curve of the logarithm of ZEN concentration is shown in Fig. 4. The corresponding linear regression equation was $I_p = 0.4257\ lg\ C + 11.566$ ($R^2 = 0.9907$), and the ZEN detection limit was 0.11 ng L^{-1} based on the equation LOD = **3**Sd/b, where Sd is the standard deviation of the electrochemical response of the blank solution for 30 cycles and b is the slope of the calibration curve. Consequently, the AuNP-SPE was shown to have sensitive ZEN detection ability and can be used as an effective tool for detecting ZEN in grain.

In recent years, research on mycotoxin identification in grain

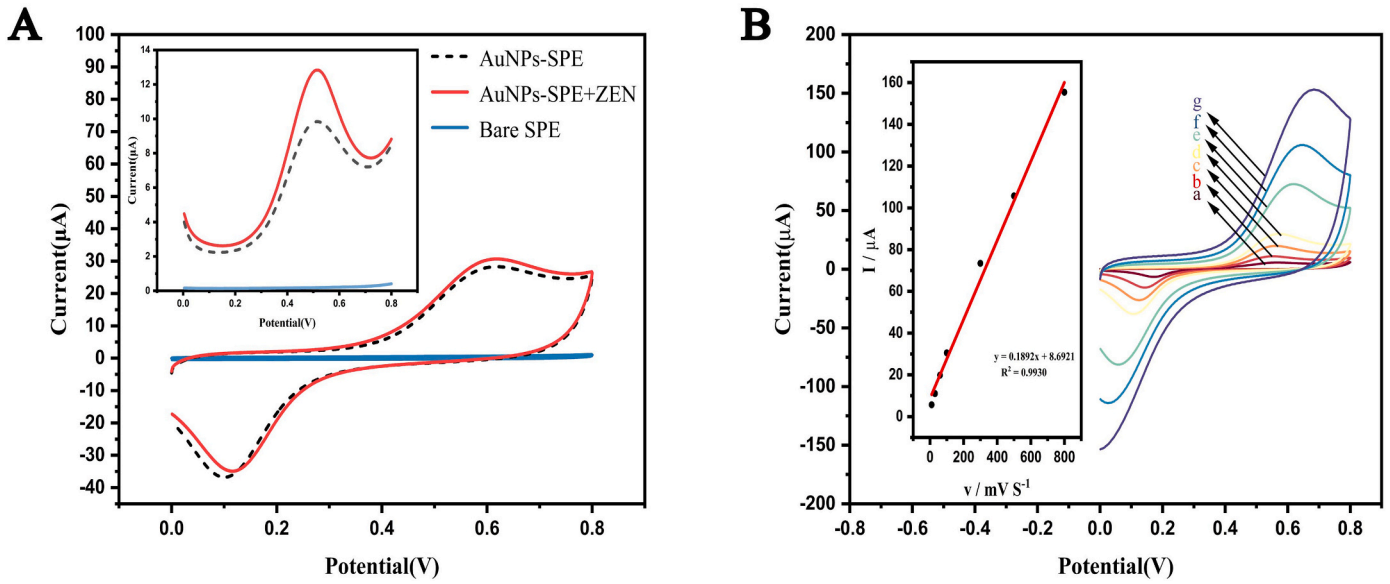

Fig. 3. (A) CVs and DPVs of ZEN (0.1 mg L^{-1}) in 0.1 M PBS (pH 7.4) of the bare SPE and AuNP-SPE. (B) The CV of 10 mL PBS in 0.1 M ZEN on the AuNP-SPE at different scan rates (a to h: 10, 30, 60, 100, 300, 500, and 800 mV S^{-1}).

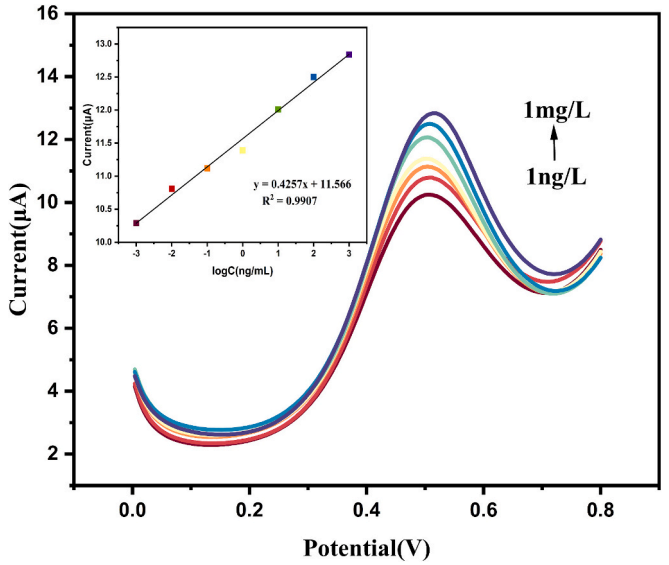

Fig. 4. DPVs of ZEN with different concentrations of the AuNP−SPE. Inset: the influence of pH on the peak current. ZEN concentrations ranged from 1 ng L^{-1} to 1 mg L^{-1}. The concentration of the next one is 10 times that of the previous one.

matrices has been increasing, and various sensors have been used to detect ZEN. Table 1 lists the electrochemical sensors developed to detect zearalenone in grains or nuts. Compared to these previous sensors, the sensor developed in the current study was revealed to have higher sensitivity and a wider detection range.

3.5. Selectivity, reproducibility and stability

To evaluate the selectivity of the developed sensor, we used AuNP-SPEs to detect various foreign species. The results indicates that the concentrations of 50 times of fructose, sucrose, glutamic acid, cystine, tartaric acid, and vitamin B2, and the concentrations of 100 times anhydrous ethanol can not affect the determination of ZEN (Fig. 5A). Only ZEN resulted in a significant electrochemical response, indicating that our developed sensor has high anti-interference ability.

Table 1
A comparison of the analytical methods for the detection of zearalenone.

Method	Samples	Linear Range	LOD	Ref
Aptasensor	maize oat	0.01–100 ng/mL	0.12 pg/mL	(Zhu et al., 2023)
Immunosensor	maize	10–1000 μg/kg	3.6 μg/kg	(Yin et al., 2022)
SERS aptasensor	maize	3–200 ng/mL	6.4 ng L^{-1}	(Guo et al., 2023)
QB-based mICA	maize	–	10 ng/mL	(Duan, Li, Shao, Huang, & Xiong, 2019)
Indirect ELISA	maize	–	0.22 μg/L	(Ma et al., 2021)
This work	maize oat	10 ng/kg - 10 mg/kg	1.1 ng/kg	

The results of 30 CVs of the same AuNP-SPE on the first day and after 90 days as well as the peak currents of four different AuNP-SPEs at the same time were investigated (Fig. 5B). After 30 consecutive scans of the first day and after 90 days, the RSD was 0.25% and 0.33%, indicating that the electrode has good anti-fouling ability. The histogram shows that the AuNP-SPEs had high stability and that the peak current of the different electrodes undergoing four DPVs remained almost unchanged (RSD < 1%).

3.6. ZEN analysis in real sample

Maize and oats have a high zearalenone detection rate, and therefore we selected these two grains as representative of actual samples and determined their zearalenone content using the electrochemical sensor that we developed. To further study the feasibility of the proposed sensor, the standard addition method was used to determine ZEN in normal maize sample and oats sample (Table S1). The content of ZEN in normal sample and oats sample was determined by LC-MS, and the results showed that ZEN was not detected. Visible moldy corn and oats and edible buckwheat, rice, millet, and sorghum were individually assayed for ZEN using AuNP-SPE. The results showed that the concentration of ZEN in the actual samples determined by the proposed sensor was essentially the same as that of the conventional LC-MS method

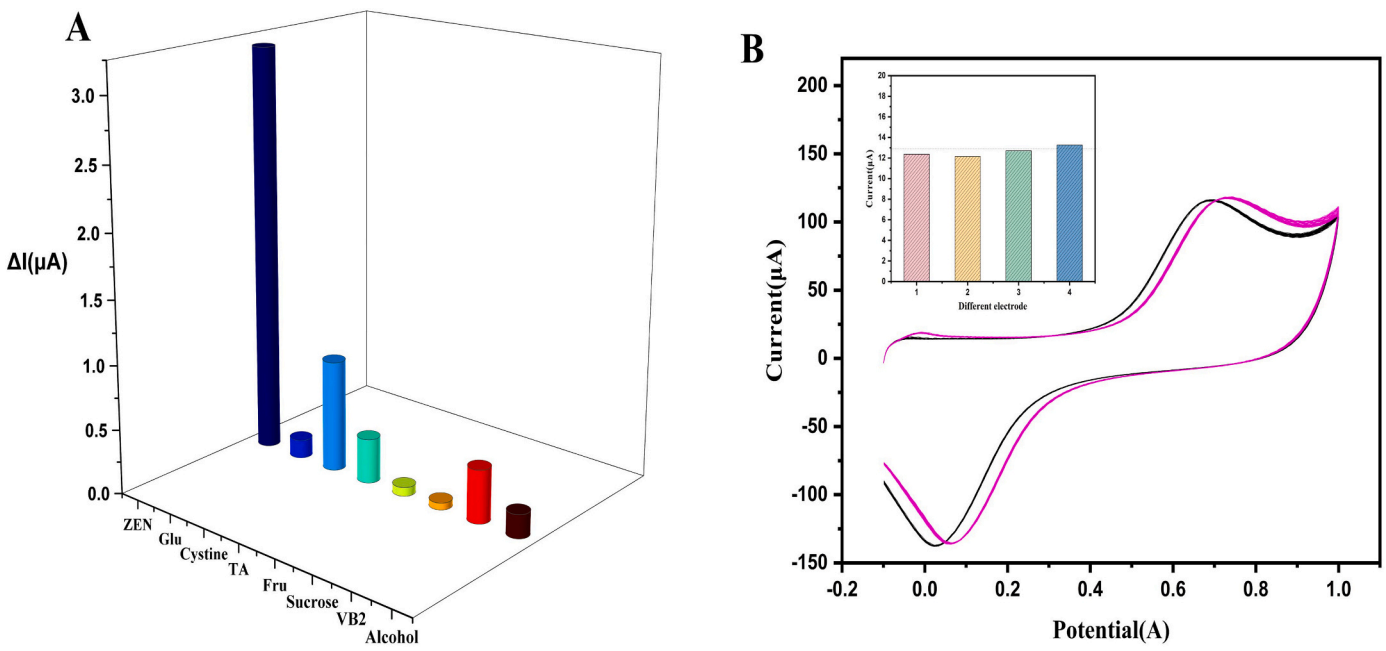

Fig. 5. (A) Detection of multiple compounds by the AuNP-SPE. The concentration of ZEN was 1 μg mL^{-1}. (B) Thirty CV results of the AuNP-SPE (Black plots: first day; Pink plots: 30 days later). Inset: Bar plots of the maximum peak current of four different AuNP-SPEs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Table S2). This indicates that the sensor developed in this study can effectively quantify the ZEN concentration in actual sample analysis with high reliability and accuracy.

4. Conclusions

The current study used electrochemical methods to detect the ZEN content in grain. Use of the ion implantation method to modify screen-printed electrodes with gold nanoparticles has improved electrode detection sensitivity. The proposed DPV detection method simplifies the complex and cumbersome sample pretreatment process and can easily, quickly, and sensitively detect ZEN content in grain, providing research ideas for onsite ZEN detection.

CRediT authorship contribution statement

Liyuan Zhao: Writing – original draft, Validation, Software, Methodology, Formal analysis. **Longzhu Zhou:** Validation, Investigation. **Dieudonné M. Dansou:** Writing – review & editing, Validation. **Chaohua Tang:** Writing – review & editing, Resources. **Junmin Zhang:** Writing – review & editing, Supervision, Funding acquisition. **Yuchang Qin:** Supervision, Funding acquisition. **Yanan Yu:** Supervision, Methodology, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101666.

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