

Portable electrochemical biosensing platform for rapid, on-site detection of zearalenone mycotoxin in grain corn and animal feed

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Manuscript received: 19 March 2026 | Accepted: 24 August 2026 | Published online: 28 September 2026

Abstract: Zearalenone (ZEA) is a heat-stable estrogenic mycotoxin produced by *Fusarium* species that frequently contaminates cereal grains and animal feed, posing severe threats to food safety, livestock health, and agricultural economies. While laboratory-based detection methods are highly accurate, they are often time-consuming and unsuitable for the real-time, on-site applications required by smart agricultural systems. Here, we present a portable electrochemical biosensing platform designed for rapid, decentralized ZEA quantification in raw grain corn and processed poultry feed. Screen-printed carbon electrodes (SPCEs) were functionalized with a conductive polyaniline/gold nanoparticle (PANi/AuNP) nanocomposite, providing a high-surface-area matrix for stable anti-ZEA polyclonal antibody immobilization and accelerated electron transfer. Using differential pulse voltammetry (DPV) with an optimized 1:5 matrix-to-buffer dilution ratio to minimize matrix effects, the platform achieved a linear detection range of 0–200 µg/kg (ppb) with limits of detection (LOD) of 36.88 ppb in buffer and 57.66 ppb in matrix, aligning with international regulatory thresholds. The biosensor demonstrated high analytical accuracy with spiked recoveries between 80.19% and 93.92%, while functionalized SPCE strips maintained robust storage stability at room temperature for 14 days. The sensor strips were coupled with the handheld MARDIsense reader and a smartphone application for automated data processing and real-time cloud logging. Validation across 52 real-world agricultural samples ($n=37$ raw grains; $n=15$ feed/kernel matrices) demonstrated 100% categorical agreement and strong quantitative correlation ($r=0.931$, $p<0.001$) relative to reference ELISA. Crucially, epidemiological profiling revealed low contamination in pre-harvest field grains (94.6% <50 ppb) contrasting with severe contamination in processed poultry feed (50% > 100 ppb). This highlights the platform's vital utility for prioritizing post-harvest feed mill surveillance to safeguard the food supply chain.

Keywords: electrochemical biosensor, grain corn, mycotoxin, portable detection, zearalenone

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Citation: Mohd Said, N. A., Awaludin, N., Masdek, E. M., Zulkepli, S. A., Ramli, S. N. M., Talib, M. A. A., Nor, M. E. M., Hashim, H., Karim, N. A., Ismail, M. R., & Razali, H. (2026). Portable electrochemical biosensing platform for rapid, on-site detection of zearalenone mycotoxin in grain corn and animal feed. *Journal of Smart Farming and Food Security*, 2(2), 128–148. <https://doi.org/10.51200/jsffs.v2i2.7498>

1. Introduction

Mycotoxin contamination remains a critical challenge for global grain production and food safety. Environmental conditions such as high humidity, poor aeration, and warm temperatures, a characteristic of tropical climates like Malaysia, accelerate fungal proliferation which lead to subsequent synthesis of toxic secondary metabolites (Fumagalli et al., 2021). These contaminants enter the agricultural supply chain during cultivation, harvesting, and storage, posing severe health risks to humans and animals. Long-term exposure to even low concentrations of mycotoxins is linked to chronic conditions, including hepatitis, jaundice, and various cancers. Furthermore, as animal feed serves as a primary vector for toxins to enter the human food chain, rigorous monitoring is essential to ensure compliance with regulatory frameworks, such as the Malaysian Food Act 1983, which establishes strict Maximum Residue Limits (MRLs). Globally, maize is an essential agricultural commodity with annual production exceeding 1.2 billion metric tons (FAO, 2024; USDA-FAS, 2024). In Southeast Asia, nations such as Malaysia import approximately 4 million tonnes of grain corn annually (valued at over MYR 4 billion) primarily to sustain poultry and livestock feed industries, rendering the regional economy highly vulnerable to mycotoxin-induced supply disruption (USDA-FAS, 2023).

Among the diverse array of mycotoxins, zearalenone (ZEA) stands out as a pervasive contaminant in cereal crops, primarily produced by *Fusarium* species such as *F. graminearum*, *F. culmorum*, and *F. verticillioides*. These fungi frequently infect staple grains, including corn, wheat, barley, and oats (Rai et al., 2020). A defining challenge of ZEA is its high chemical stability, which allows it to withstand varying storage conditions and high-temperature food processing, thereby posing a "permanent" threat throughout the agricultural supply chain. The global prevalence of ZEA is significant; a ten-year survey reported that 30–40% of all crops are contaminated, with maize-based products showing a heightened prevalence of approximately 44% (Gruber-Dorninger et al., 2019). Regional data further underscore the vulnerability of maize production systems. For instance, recent monitoring programs have identified ZEA in over 60% of corn samples in Asia and more than 63% in North America (McDougal, 2025). In South Africa, 62% of commercial maize samples across three provinces were found to be contaminated with ZEA and its metabolites (Nji & Mwanza, 2024). Meanwhile, the Department of Veterinary Services in Malaysia recently found that *Fusarium*-derived toxins were present in 100% of grain corn samples collected across Peninsular Malaysia (Wan Syahidah et al., 2021).

The toxicity of ZEA is primarily rooted in its structural similarity to the natural hormone 17 β -estradiol. This allows the toxin to bind to estrogen receptors, inducing hyperestrogenism and severe reproductive disorders in livestock. Swine are particularly sensitive, often exhibiting infertility, stillbirths, and endometrial hyperplasia following exposure (Zinedine et al., 2007). With the escalating pressures of climate change and environmental pollution, the risk of *Fusarium* outbreaks is expected to rise, necessitating the development of more robust and rapid detection technologies to safeguard the livestock industry and public health.

Currently, the "gold standard" for ZEA analysis relies on laboratory-based techniques such as High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Mass Spectrometry (LC-MS). While these methods offer high sensitivity and specificity, they are hindered by expensive instrumentation, the need for specialized personnel, and time-consuming sample preparation (Wang et al., 2022). Enzyme-Linked Immunosorbent Assays (ELISA) provide a higher-throughput alternative but remain laboratory-dependent and susceptible to matrix interference and light sensitivity, limiting their utility for on-site, real-time assessments (Curulli, 2021).

To overcome the limitations of conventional analytical methods, biosensor technologies have emerged as a highly effective alternative for rapid and portable mycotoxin detection. A biosensor is an analytical device that integrates a biological recognition element with a

physicochemical transducer to produce a measurable signal proportional to the concentration of a target analyte (Meliana et al., 2024). Biosensing platforms can be broadly categorized based on their transduction mechanisms into optical, piezoelectric, thermal, and electrochemical systems. Among these, electrochemical biosensors are widely being reported over optical biosensor in various application owing to their high sensitivity, rapid response times, low operational cost, and excellent potential for miniaturization into portable devices (Li et al., 2021; Rizzotto et al., 2023; Wang et al., 2022). Such features make electrochemical biosensors especially suitable in food safety analysis namely for on-site mycotoxin monitoring throughout the agricultural supply chain.

Electrochemical biosensors utilize diverse biorecognition elements as bioreceptors, including antibodies (Foubert et al., 2019; Goud et al., 2019), aptamers (Azri et al., 2021), and molecularly imprinted polymers (MIPs) (Radi et al., 2020). These bioreceptors selectively bind to their specific target analytes, converting biological recognition events into measurable, quantifiable electrical signals. By enabling rapid analysis with minimal sample preparation, these devices are uniquely suited for point-of-need and field-based applications. Furthermore, integrating portable electrochemical devices with the Internet of Things (IoT) offers distinct operational advantages, such as real-time data management and remote surveillance (Bianchi et al., 2021; Jusoh et al., 2022; Umapathi et al., 2022). Addressing the critical need for field-deployable technology, this study utilizes high-affinity polyclonal antibodies (pAbs) anchored on functionalized screen-printed carbon electrodes (SPCEs). While monoclonal antibodies and aptamers are widely researched, pAbs offer distinct diagnostic advantages in complex agricultural matrices due to their multi-epitope recognition capability, robust sensitivity, cost-effectiveness, and rapid development (Ascoli & Aggeler, 2018).

Accordingly, the primary objective of this study was to develop, optimize, and evaluate a portable label-free electrochemical immunosensor for the rapid and on-site quantitative detection of zearalenone (ZEA) in grain corn. Specifically, this research aimed to fabricate a polyaniline/gold nanoparticle (PANi/AuNP) conductive interface on screen-printed carbon electrodes to facilitate anti-ZEA polyclonal antibody immobilization, characterize the label-free signal-off mechanism via differential pulse voltammetry across regulatory concentration thresholds (0–200 ppb) to establish detection limits in both buffer and grain corn matrices, empirically evaluate antibody specificity against co-occurring mycotoxins (Aflatoxins, Ochratoxins, and Fumonisin), and validate the analytical accuracy of the portable biosensing platform against benchmark ELISA measurements. By optimizing the biosensor interface for real-world feed samples, this platform provides a practical surveillance tool capable of mitigating the economic and health risks associated with mycotoxin contamination.

2. Materials and Methods

2.1 Chemicals and reagents

Anti-ZEA polyclonal antibodies (pAbs) were produced in-house at the Biotechnology & Nanotechnology Research Centre, MARDI with approval from the MARDI Animal Ethics Committee (Approval No. 20230622/R/MAEC00139). Phosphate-buffered saline (PBS) tablets and dimethyl sulfoxide (DMSO) were obtained from Sigma. Potassium hexacyanoferrate (III) (ferricyanide) and potassium hexacyanoferrate (II) trihydrate (ferrocyanide) were sourced from Sigma-Aldrich (USA and Japan, respectively). Polyaniline emeraldine salt (PANi) and 20 nm gold nanoparticles (AuNPs) were purchased from Aldrich (USA), while dry milk powder was obtained from Santa Cruz Biotechnology (USA). Zearalenone (ZEA) analytical standards were purchased from Trilogy® Analytical Laboratory via R-Biopharm AG (Darmstadt, Germany). The ZEA–horseradish peroxidase conjugate (ZEA–HRP) was obtained from Creative Biolabs (Shirley, NY, USA), whereas additional mycotoxin standards were sourced from Sigma-Aldrich (St. Louis, MO, USA) or Romer Labs Diagnostic GmbH (Tulln, Austria). All chemicals and

reagents used were of analytical grade or higher. Screen-printed carbon electrodes (SPCEs), integrating a 4 mm circular working electrode with counter and reference electrodes in an 8-pin contact well configuration, along with a portable electrochemical reader, were supplied by Biogenes Technologies Sdn. Bhd. (Malaysia).

2.2 Cross-Reaction Study

The specificity of the anti-zearalenone (ZEA) antibody was evaluated via competitive enzyme-linked immunosorbent assay (ELISA) against ZEA and nine co-occurring mycotoxins: Aflatoxins (AFB1, AFB2, AFG1, AFG2), Ochratoxins (OTA, OTB), and Fumonisin (FB1, FB2, FB3) at 20, 50, and 100 ppb. Microplate wells were coated with 100 μ L of 0.1 mg/mL anti-ZEA antibody overnight at 4 °C, washed three times with phosphate-buffered saline containing 0.05% Tween-20 (PBST), and blocked with 250 μ L of 0.05% non-fat dry milk for 1 h at 37°C. Following a PBST wash, 50 μ L of mycotoxin standard solution and 50 μ L of ZEA-HRP conjugate were added per well and incubated for 2 h at 37 °C. After a final washing step, 100 μ L of 3,3',5,5'-tetramethylbenzidine (TMB) substrate was added for color development in the dark at room temperature. Optical density was measured at 370 nm using a microplate reader.

2.3 Modification of SPCE and standard curve determination

The SPCEs were modified by depositing 10 μ L of a pre-optimized mixture of polyaniline emeraldine salt (PANi) and 20 nm gold nanoparticles (AuNPs) onto the carbon working electrode, followed by incubation for 30 min. Excess liquid was removed and the electrodes were dried using an air blower. Subsequently, 10 μ L of anti-ZEA antibody (0.07 mg/mL) was applied to the functionalized SPCEs and incubated for 1 h. The electrodes were then washed three times with 100 μ L PBS and rinsed with deionized water. To minimize nonspecific binding, 50 μ L of blocking buffer (0.05% dry milk in 0.01 M PBS) was applied and incubated for 30 min. After washing, 10 μ L of serially diluted mycotoxin solutions at five concentrations (0–200 ppb) were immobilized on the working electrode surface and incubated for 2 h. The SPCEs were washed again, and 100 μ L of 5 mM redox solution (ferricyanide/ferrocyanide in 0.1 M KCl) was added for electrochemical analysis. Current peaks were recorded using a portable electrochemical reader connected to the MARDIsense app. (Figure 1).



Figure 1. Portable electrochemical biosensor platform. Photograph showing the handheld MARDIsense reader fitted with a modified screen-printed carbon electrode (SPCE) (right) wirelessly paired via Bluetooth to the custom MARDIsense mobile application on a smartphone (left).

2.4 Sample preparation for matrix effect study

Control grain corn samples were first sterilized using 30% (v/v) sodium hypochlorite solution to eliminate potential mycotoxin contamination. For extraction, 1 g of ground corn sample was mixed with 5 mL of 80% (v/v) aqueous acetonitrile and shaken for 3 minutes to ensure efficient mycotoxin extraction. The mixture was filtered using filter paper, and the filtrate was prepared in a series of dilutions (undiluted, 1:1, 1:5, 1:10, 1:20, and 1:100) for matrix background analysis using the electrochemical biosensor system.

2.5 Method validation and recovery study

The accuracy and precision of the proposed analytical method were assessed through a recovery study conducted on blank grain corn samples. ZEA working standards were spiked into the matrix at three distinct concentrations: 20, 60, and 100 ppb. These specific levels were selected to reflect the low-to-moderate contamination range typically encountered in natural occurrences and to align with the maximum limits (MLs) established by international regulatory frameworks, such as the European Commission (Regulation EC No 1881/2006), which sets a limit of 100 ppb for ZEA in maize intended for direct human consumption.

The extraction was performed using a 1:5 (w/v) sample-to-solvent ratio. Each concentration level was analyzed in triplicate to ensure reproducibility. The percentage recovery ($R\%$) was calculated using the following equation:

$$R\% = \left(\frac{C_{\text{recovered}}}{C_{\text{spiked}}} \right) \times 100$$

where $R\%$ is the percentage recovery of the analyte in the spiked sample, $C_{\text{recovered}}$ is the measured concentration of the analyte determined experimentally in the spiked sample, and C_{spiked} is the known concentration of the analyte deliberately added to the sample.

2.6 Application to real samples

Grain corn samples were collected from experimental plots at the Industrial Crop Research Centre across six locations in Malaysia: Seberang Perai, Bachok, Serdang, Genting Sepang, Pontian, and Malacca. Grain corn samples were collected from experimental plots at the Industrial Crop Research Centre across six locations in Malaysia: Seberang Perai, Bachok, Serdang, Genting Sepang, Pontian, and Malacca. To evaluate the versatility and analytical performance of the biosensor platform across diverse sample matrices, commercial corn kernels and commercial animal feed products were additionally procured from local retail markets and an online e-commerce platform (Shopee, Malaysia).

For sample extraction, 5 g of finely ground sample was mixed with 250 mL of an 80% (v/v) aqueous acetonitrile solution. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was diluted 1:5 (v/v) with PBS to minimize matrix effects. A 10 μL aliquot of the diluted extract was deposited onto the working surface of the SPCE surface and incubated for 2 hours at room temperature. Following a washing step to remove unbound substances, 100 μL of 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox probe solution was applied for electrochemical quantification via differential pulse voltammetry (DPV).

2.7 Validation study

The RIDASCREEN® FAST Zearalenone ELISA test kit (R-Biopharm, Germany) was used to validate the analytical results. The assay was performed according to the manufacturer's instructions.

2.8 Storage stability evaluation of functionalized SPCEs

The storage stability of the fully functionalized SPCEs was evaluated over a 14-day testing period. Screen-printed carbon electrodes were prepared up to the blocking step following the aforementioned protocol (PANi/AuNP deposition, anti-ZEA antibody immobilization, and blocking with 0.05% dry milk in 0.01 M PBS). Following the post-blocking washing step with 100 μ L PBS and deionized water, the modified electrodes were gently air-dried and stored in sealed, airtight containers containing silica gel desiccant to prevent ambient moisture degradation. To determine optimal storage conditions, the functionalized SPCE strips were divided into two cohorts and stored at two distinct temperatures: room temperature (25°C) and refrigerated storage (4°C).

At designated temporal intervals (Days 0, 1, 3, 7, 10, and 14), triplicate SPCE strips from each storage temperature were retrieved to assess electrochemical responsiveness. Each stored electrode was incubated with 10 μ L of a fixed ZEA control standard (100 ppb) for 2 h at room temperature. After washing away unbound toxin, 100 μ L of the 5 mM ferricyanide/ferrocyanide redox probe in 0.1 M KCl was applied, and peak current responses (I_t) were recorded using the portable reader. Performance retention was calculated by comparing the peak current output on day t against the baseline current recorded on Day 0 (I_0) using the following equation:

$$\text{Signal Retention (\%)} = \frac{I_t}{I_0} \times 100$$

where I_0 represents the initial baseline peak current measured on Day 0, and I_t denotes the peak current measured after t days of storage.

2.9 Statistical Analysis

All electrochemical measurements, calibration determinations, storage stability evaluations, and spiked recovery assays were performed in triplicate ($n=3$). Experimental results are expressed as the $\pm$ standard deviation (SD). Assay precision and repeatability were evaluated using the Relative Standard Deviation (RSD, %):

$$\text{RSD (\%)} = \frac{(\text{SD})}{(\text{mean})} \times 100$$

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the standard equations:

$$\text{LOD} = \frac{3.3 \sigma}{S}$$
$$\text{LOQ} = \frac{10\sigma}{S}$$

where σ represents the standard deviation of the blank signal response and S denotes the slope of the linear calibration plot.

Data processing, linear regression modelling, and statistical testing were performed using Microsoft Excel and Python (SciPy/Statsmodels modules). For real-world method validation against the reference microplate ELISA kit across agricultural matrices ($N=52$), categorical diagnostic agreement was evaluated. For the strictly quantifiable positive sample cohort ($n=11$), quantitative comparability was determined using Ordinary Least Squares (OLS) simple linear regression ($y = mx + c$) and Pearson product-moment correlation analysis (r and R^2) to assess proportional and constant bias. Two-tailed paired t -tests were conducted to evaluate

numerical differences between biosensor-derived and ELISA-derived ZEA concentrations. Statistical significance was defined as $p < 0.05$.

3. Results and Discussion

3.1 Specificity and cross-reactivity evaluation of anti-ZEA polyclonal antibodies

The production and characterization of the anti-ZEA polyclonal antibodies (pAbs) were previously described by Said et al. (2025). In order to validate the selectivity of the in-house anti-ZEA pAb, a cross-reactivity study was conducted employing 0.1 mg/mL of purified antibody (third bleed batch, Rabbit 2), against nine common co-occurring mycotoxins namely Aflatoxins (AFB1, AFB2, AFG1, AFG2), Ochratoxins (OTA, OTB), and Fumonisin (FB1, FB2, FB3) at concentrations of 20, 50, and 100 ppb. Cross-reactivity percentages were derived from relative absorbance values, assigning 100% reactivity to the target analyte, zearalenone.

As illustrated in Figure 2, the anti-ZEA pAb demonstrated 100% reactivity toward zearalenone across all tested concentration levels. In contrast, non-target mycotoxins displayed minimal cross-reactivity, remaining between 12.76% and 18.97%. At 20 ppb, the maximum cross-reactivity observed across all non-target species was 18.97% for AFB2 and 18.93% for FB3. As the concentration increased to 100 ppb, interference values further decreased to between 12.90% and 15.68%.



Figure 2. Cross-reactivity analysis of the anti-ZEA polyclonal antibody (0.1 mg/mL, third bleed, Rabbit 2) against target and non-target mycotoxins at 20, 50, and 100 ppb. The antibody exhibits 100% reactivity toward zearalenone with minimal cross-reactivity (<19%) to non-target compounds.

These findings confirm that the antibody used in this study has strong binding affinity for ZEA with negligible background interference from major structurally distinct mycotoxins. Although direct cross-reactivity against zearalenone metabolites and structural analogs (e.g. α -zearalenol, β -zearalenol, α -zearalanol, and β -zearalanol) was not empirically quantified in this study, polyclonal antibodies frequently display partial cross-recognition toward closely related analogs (Thongrussamee et al., 2008; Wang et al., 2021). In biosensing, group-selective recognition of ZEA along with its primary metabolites is beneficial for assessing total zearalenone burden. Concurrently, the low response (<19%) toward distinct toxin classes

ensures that the immunosensor prevents false-positive signals in complex real-world agricultural matrices where Aflatoxins, Ochratoxins, or Fumonisin co-exist.

3.2 Electrochemical characterization of the antibody-modified SPCE

The key to achieving high sensitivity lies in the effective immobilization of anti-ZEA pAbs onto the screen-printed carbon electrode (SPCE) surface. We achieved this using a high-performance conductive nanocomposite composed of polyaniline (PANI) polymer and gold nanoparticles (AuNPs). This specific PANi/AuNP network was selected because it significantly enhances electrode conductivity while providing a vastly increased effective surface area for biomolecule attachment. Additionally, the excellent biocompatibility and high surface-to-volume ratio of AuNPs facilitate stable, high-density antibody loading (Yagati et al., 2018). The formation of this robust conductive network ensures stable immobilization throughout the sensing process.

To confirm successful surface functionalization, we evaluated the electrochemical characteristics of the SPCE before and after modification using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). While CV provides a comprehensive profile of the electrode's overall redox behavior, DPV offers the superior sensitivity required for detection, utilizing small potential pulses to identify subtle fluctuations in current. The voltammetric measurements revealed distinct current increments and potential shifts (Figure 3), directly confirming the sequential layering process. Furthermore, the inclusion of the PANi network markedly improved surface conductivity, as evidenced by significantly elevated peak currents observed in both voltammetric techniques.

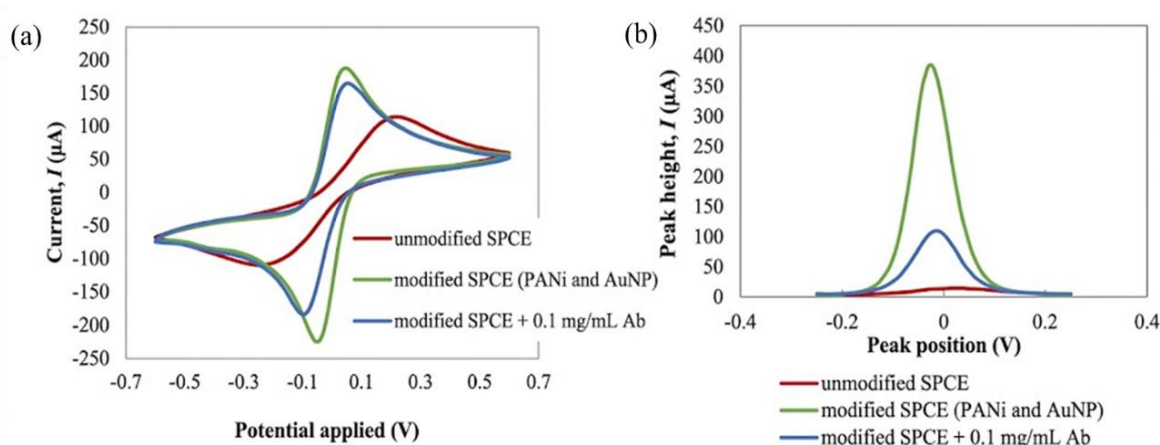


Figure 3. (a) Cyclic voltammograms (CV) and (b) differential pulse voltammograms (DPV) recorded for the SPCE before and after modification in a 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox solution (containing 0.1 M KCl). Scan rates: 50 mV/s (CV) and 2 mV/s (DPV).

Quantitative assessments of the anodic peak current (I_p) peak-to-peak separation (ΔE_p), and peak current readings derived from CV and DPV are summarized in Table 1. The anodic peak current increased from 96.67 μA to 196.32 μA following SPCE modification, indicating a substantial enhancement in surface conductivity. The incorporation of AuNPs contributed to this current elevation through their inherent high conductivity and by providing additional anchoring sites for antibody immobilization. A concurrent decrease in ΔE_p suggests more efficient electron transfer kinetics at the modified electrode interface. However, upon the addition of the anti-ZEA antibodies, a marginal attenuation in peak current and a slight shift in ΔE_p were observed. This decrease is attributed to the formation of an insulating biomolecular layer (antibodies) that partially obstructs the conductive sites; nevertheless, the overall conductivity of the SPCE remained robust and was not significantly compromised.

Table 1. Electrochemical characterization of the SPCE before and after modification with PANi and AuNPs.

SPCE	Cyclic voltammetry (CV)		Differential pulse voltammetry (DPV)
	Peak current, I_p (μA)	Peak separation, ΔE_p (V)	Peak current, I_p (μA)
Unmodified (red line)	96.67	0.40	14
Modified with PANi and AuNP (green line)	196.32	0.09	370
Modified with PANi + AuNP + anti-ZEA pAb (blue line)	164.0	0.14	103

The DPV analysis yielded patterns consistent with the CV data. The DPV peak current signal increased significantly following PANi/AuNP modification, reinforcing the enhanced sensitivity and electron transfer capabilities of the modified surface. The high sensitivity of the DPV technique also allowed for the detection of minor current variations resulting from antibody immobilization, where a slight decrease in current was recorded, corresponding to the formation of the biolayer on the SPCE. However, the resulting signals remained stable and well-defined, validating that the modified electrode maintains optimal electrochemical performance even after the bioconjugation process.

3.2.1 Sensing mechanism of the ZEA immunosensor

The sensing mechanism of the electrochemical immunosensor is based on the label-free detection and specific interaction between zearalenone (ZEA) toxin and immobilized anti-ZEA polyclonal antibodies (pAbs) measured by electrochemical DPV technique. DPV has been widely used in electrochemical biosensor for label-free immunosensor as it is able to detect Faradaic changes on electrode surface when antibody-antigen binding takes place (Aymard et al., 2022; Zambry et al., 2025). The detection mechanism can be described as an indirect, signal-off immunosensing process in which the DPV peak current decreases with increasing ZEA concentration.

The SPCE modification via PANi/AuNP composite provides an electrochemically active and conductive interface with an increased effective surface area for antibody immobilization. The AuNPs also provide favourable sites for the immobilization of anti-ZEA pAbs through non-covalent interactions between the antibody molecules and the gold surface. Following antibody immobilization, the electrode surface contains recognition sites that can selectively interact with ZEA molecules present in the sample.

In the absence of ZEA, the ferri/ferrocyanide redox probe can readily approach the modified electrode surface and undergo electron-transfer reactions. Consequently, a relatively high DPV peak current is obtained. When a sample containing ZEA is introduced, ZEA molecules specifically bind to the immobilized anti-ZEA pAbs, forming antibody-ZEA complexes at the electrode interface. This binding event increases the steric hindrance at the electrode surface and creates an additional interfacial layer that partially restricts the access of the charged ferri/ferrocyanide species to the electroactive surface. The antibody-ZEA complex therefore acts as a diffusion and electron-transfer barrier, resulting in a reduction in the faradaic current generated by the redox probe. Thus, the magnitude of the DPV response exhibits an inverse relationship with ZEA concentration (Figure 4).

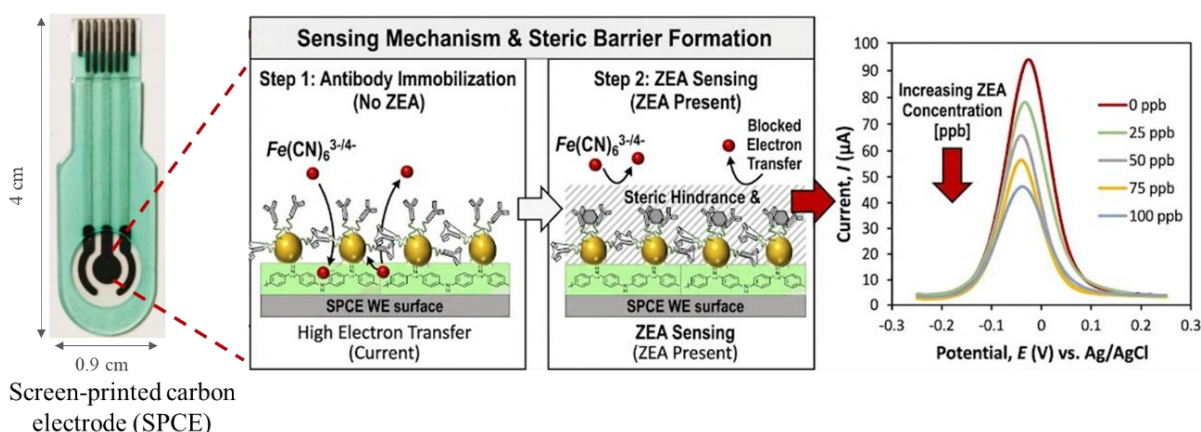


Figure 4. Schematic representation of the anti-ZEA/AuNP/PANi/SPCE immunosensor fabrication and its signal-off sensing mechanism, displaying a progressive decrease in DPV peak current as ZEA concentration increases.

The improved electrochemical response following PANi/AuNP modification and the concentration-dependent decrease in DPV peak current following ZEA exposure, provide evidence supporting the proposed signal-off immunosensing mechanism. The mechanism is particularly suitable for a portable platform because the recognition event is converted directly into a measurable current response without requiring a secondary enzymatic or redox-labelled reporter.

3.3 Evaluation of matrix effects

The matrix effect was evaluated to assess the performance of the electrochemical biosensor in grain corn sample extracts across various dilution ratios (undiluted, 1:1, 1:5, 1:10, 1:20, and 1:100). The electrochemical current generated from each diluted sample was compared directly with that of a standard buffer solution (0.01 M PBS, $I = 328.47 \mu\text{A}$). As shown in Figure 5, both the undiluted matrix (381.28 μA) and the 1:1 dilution (372.44 μA) produced noticeably higher current responses than the buffer baseline, indicating significant positive matrix interference. This elevated background current is primarily attributed to the presence of endogenous electroactive constituents naturally occurring in raw corn extracts (e.g., phenolic acids, organic acids, flavonoids, and ascorbic acid) that undergo non-specific redox reactions at the electrode surface (Forzato et al., 2020; Ziyatdinova et al., 2021), as well as high concentrations of macro-biomolecules (such as proteins and lipids) that alter double-layer capacitance and background charging current (Robin & Gerber-Lemaire, 2023; Seaton et al., 2020).

The dilution ratio producing a current response closest to the reference buffer system (indicated by the red dashed line in Figure 5) was considered to exhibit minimal matrix interference. Consequently, a 1:5 dilution ratio (313.81 μA) was selected as optimal, exhibiting an effective balance between mitigating matrix-induced current enhancements and maintaining sufficient target analyte concentration for sensitive detection. From a practical standpoint, the 1:5 dilution also reduces the residual proportion of organic extraction solvents, such as acetonitrile. In electrochemical sensing applications, maintaining organic solvent content <40% is recommended to avoid altering electrolyte conductivity, destabilizing the recognition layer, or interfering with signal transduction (Zhang et al., 2000).

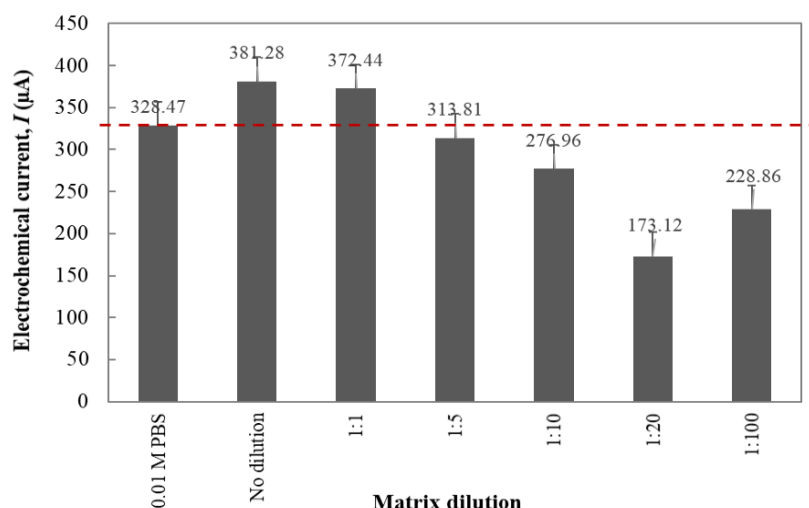


Figure 5. Electrochemical current response of grain corn extracts at different dilution ratios (antibody concentration: 0.07 mg/mL) measured using the portable device. The red dashed line represents the reference electrochemical current obtained using 0.01 M PBS in the absence of grain corn matrix and serves as the buffer reference for evaluating the matrix effect at different dilution ratios.

3.4 Development of standard curves

Standard calibration curves for ZEA were established in two analytical systems: a buffer solution and a grain corn matrix. For the grain corn matrix, a dilution ratio of 1:5 was applied based on optimized conditions determined in the matrix effect study. Using a modified SPCE (0.07 mg/mL antibody concentration), a linear standard curve for ZEA concentrations ranging from 0 to 200 ppb was established, yielding a limit of detection (LOD) of 36.88 ppb, a limit of quantification (LOQ) of 111.74 ppb, and a coefficient of determination (R^2) of 0.9611 (Figure 6a). Similarly, linear regression analysis of the results from the 1:5 diluted grain corn matrix produced a high R^2 value of 0.962 (Figure 6b), demonstrating strong linearity between the electrochemical current response and ZEA concentration. This calibration curve yielded an LOD of 57.66 ppb and an LOQ of 166.23 ppb. While the detection limit in the grain corn matrix is slightly higher than in the buffer system, as expected due to matrix components that partially affect the electrochemical signal, the strong linearity indicates that the 1:5 dilution effectively reduces matrix interference while maintaining sufficient analyte concentration for reliable detection.

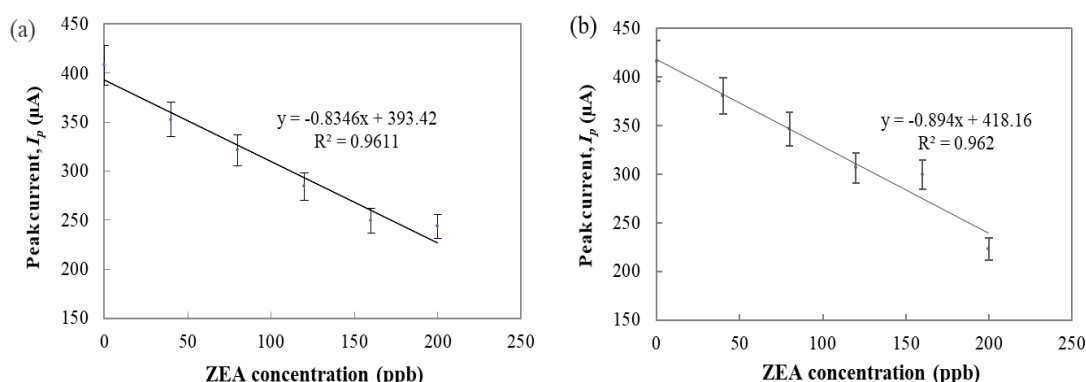


Figure 6. Calibration curves for ZEA obtained using the portable electrochemical device (0.07 mg/mL antibody) in: (a) buffer solution (LOD=36.88 ppb, LOQ=111.74 ppb); and (b) grain corn matrix at a 1:5 dilution (LOD=57.66 ppb, LOQ=166.23 ppb).

The selected calibration range of 0–200 ppb reflects realistic contamination levels of ZEA commonly reported in cereals and animal feed. International regulatory guidelines establish maximum permissible levels of approximately 50–100 µg/kg (ppb) for ZEA in cereal-based food and feed products, making this range particularly relevant for practical monitoring (Jiang et al., 2024). Consequently, the developed biosensor is capable of quantifying ZEA concentrations near and above regulatory thresholds, enabling direct application in food safety screening and agricultural quality control.

To contextualize the analytical performance of the developed biosensor, its operational metrics were compared against recently reported electrochemical ZEA sensors in literature (Table 2).

Table 2. Comparison of electrochemical sensing strategies for ZEA detection.

Biosensing Platform	Recognition Element	Linear Range	Detection Limit (LOD)	Fabrication Complexity & Portability	Matrix Application	Ref.
Graphene/MOF Nanocomposite	Sandwich Antibody / Peptide	10 fg/mL – 100 ng/mL	0.57 fg/mL	High (Multi-step synthesis, benchtop equipment)	Corn, Wheat	Jiang et al. (2024)
Cu-MOF / Fe ₃ O ₄ -GO	Direct Oxidation	159 – 2865 ng/mL	23.14 ng/mL	Moderate (Porous nanostructure modification)	Corn sample	Zeng et al. (2022)
Molecularly Imprinted Polymer (MIP)	Synthetic Cavities	1.0 × 10 ⁻⁴ – 10 ng/mL	3.4 × 10 ⁻⁵ ng/mL	High (Polymerization process, laboratory-bound)	Corn, Milk	Guo (2024)
PANi/AuN P/SPCE (This Work)	Polyclonal Antibody (pAb)	0 – 200 ppb	36.88 ppb (Buffer) 57.66 ppb (Matrix)	Low (Single-step modification, portable handheld reader)	Grain Corn (1:5 dilution)	Present Study

As summarized in Table 2, various advanced sensing strategies have achieved ultralow detection limits. For instance, sandwich-type nanocomposites and molecularly imprinted polymers reach femtogram-to-picogram detection thresholds through multi-stage signal amplification and engineered high-surface-area nanostructures (Jiang et al., 2024; Guo, 2024). However, these platforms have clear analytical trade-offs. Achieving sub-picogram sensitivity typically requires tedious multi-step chemical syntheses, expensive nanomaterial labels, long incubation protocols, and reliance on benchtop electrochemical workstations. Furthermore, such extreme sensitivity often exceeds practical requirements for agricultural monitoring, where regulatory action levels fall within the low parts-per-billion range (50–100 ppb).

In contrast, the biosensor developed in this work prioritizes field-deployable simplicity, cost-effectiveness, and rapid operational workflows. By utilizing an SPCE modified with a conductive PANi/AuNP composite, the platform achieves simple single-step antibody immobilization without requiring secondary labels or enzymatic reactions. When paired with a portable potentiostat, the system provides rapid on-site quantitative screening directly at

regulatory cut-offs. The matrix validation in a 1:5 grain corn extract confirms that the sensor effectively mitigates matrix suppression without requiring extensive sample clean-up columns. Therefore, while laboratory-based nanostructured sensors excel in trace-level sensitivity, the present sensor provides a balanced solution optimized for practical, decentralized agricultural testing.

3.5 Assessment of biosensor accuracy

The analytical accuracy and precision of the portable electrochemical biosensor were evaluated through a spiked recovery study within a grain corn matrix. Standard ZEA solutions were introduced at concentrations of 20, 60, and 100 $\mu\text{g/kg}$ (ppb). This specific range was strategically selected to encompass the critical regulatory "decision zone" for grain commodities; specifically, the 100 ppb level aligns with the Maximum Limit (ML) established for ZEA in maize intended for direct human consumption. By focusing on these lower thresholds rather than higher, more easily detectable concentrations, the study rigorously validates the biosensor's sensitivity and its practical utility for early-stage screening where matrix-induced bias is most challenging.

As illustrated in Figure 7, the mean recovery values ($R\%$) across the tested range varied between 80.19% and 93.92% ($93.92\% \pm 6.11\%$ at 20 ppb, $91.45\% \pm 7.76\%$ at 60 ppb, and $80.19\% \pm 4.52\%$ at 100 ppb; $n=3$). The relative standard deviations (RSD) ranged from 5.64% to 8.48%, demonstrating high reproducibility and method precision across all evaluated levels. These results demonstrate high analytical accuracy and precision, fully complying with the performance criteria established by European Commission Regulation (EC) No 401/2006 for mycotoxin analysis, which mandates acceptable recoveries of 60%–120% ($\text{RSD}_r \leq 40\%$) for levels $\leq 50 \mu\text{g/kg}$ and 70%–120% ($\text{RSD}_r \leq 25\%$) for levels $>50 \mu\text{g/kg}$. Notably, the highest recovery (93.92%) was achieved at the lowest spiking level (20 ppb), confirming the platform's efficiency at trace levels despite the complex grain matrix.

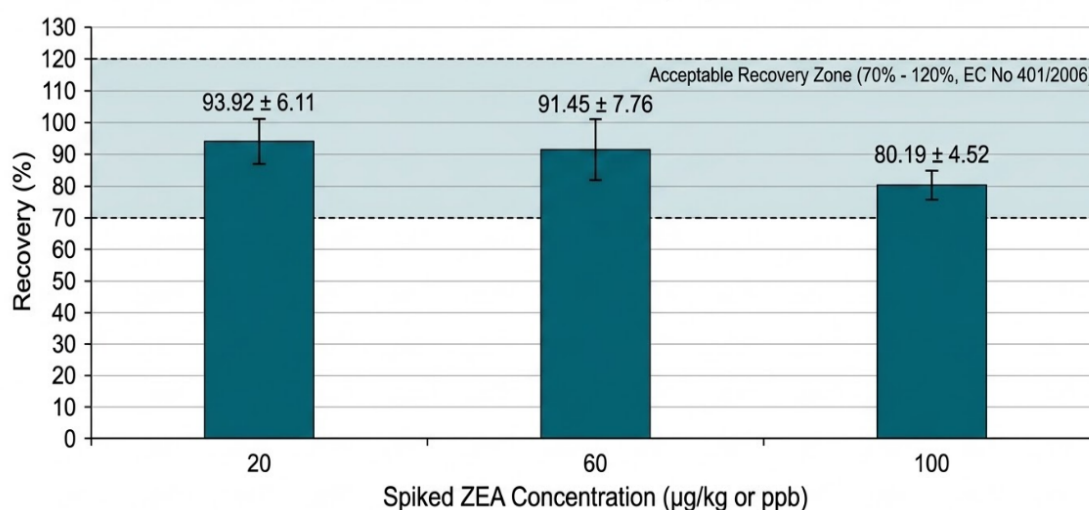


Figure 7. Percentage recovery of spiked zearalenone (ZEA) concentrations (20–100 ppb) in grain corn matrix (1:5) measured using the portable electrochemical biosensor. Error bars represent standard deviation ($\pm\text{SD}$, $n=3$). The horizontal zone indicates acceptable recovery ranges according to Commission Regulation (EC) No. 401/2006.

A slight inverse relationship was observed between the spiked concentration and recovery percentage, with the lowest recovery ($80.19\% \pm 4.52\%$, $\text{RSD } 5.64\%$) occurring at 100 ppb. This phenomenon may be indicative of interwoven electrochemical effects. First, the intricate composition of grain corn which is rich in proteins, lipids, and carbohydrates, may exert more

significant matrix interference as the analyte load increases (Jiang et al., 2024). Alternatively, the decline could signal the onset of sensor saturation; as analyte occupancy rises, increased steric hindrance or a reduction in available antibody binding sites at the electrode interface can impede binding kinetics (Urraca et al., 2005). Similar concentration-dependent variations in complex food matrices have been documented in recent electrochemical biosensing literature (Goud et al., 2019; Siva Sangu et al., 2022). Nevertheless, the robust performance across this critical regulatory range validates the biosensor as a reliable tool for rapid ZEA quantification in decentralized field settings

3.6 Validation with naturally contaminated grain corn samples

The practical utility of the integrated biosensor and portable reader platform was verified through the analysis of 52 real-world agricultural samples ($N=52$). As summarized in Table 3, the sample cohort comprised two primary matrix categories: raw grain corn ($n=37$) and processed/feed matrices ($n=15$). The raw grain samples were collected across six distinct regional production sites in Malaysia, namely MARDI Seberang Perai ($n=20$), MARDI Bachok ($n=10$), MARDI Serdang ($n=2$), Genting Sepang Plantation ($n=2$), MARDI Pontian ($n=2$), and Malacca ($n=1$). The processed/feed category consisted of commercial retail corn kernels ($n=3$) and commercial poultry feed ($n=12$), the latter incorporating crushed grain corn as a primary constituent for livestock consumption. Including crushed corn matrices alongside whole grains allowed for a thorough evaluation of the biosensor's analytical performance across varying particle sizes and complex feed formulations.

Table 3. Breakdown of agricultural grain corn and processed poultry feed samples by regional sampling location and matrix category ($N=52$).

Sample Category	Sample Origin / Sub-Type	Sample Size (n)
Raw Grain Corn	MARDI Seberang Perai	20
	MARDI Bachok	10
	MARDI Serdang	2
	Genting Sepang Plantation	2
	MARDI Pontian	2
	Malacca	1
Processed / Feed	Commercial Poultry Feed	12
	Retail Corn Kernels	3
Total	All Matrices Combined	52

Analytical results obtained from the PANi/AuNP/anti-ZEA biosensor platform were benchmarked against a commercial enzyme-linked immunosorbent assay (ELISA). ELISA was selected as the reference standard as both systems share a common immuno-analytical foundation based on specific antibody–antigen recognition for zearalenone (ZEA) sequestration. Their shared reliance on immunochemical selectivity allows for a direct evaluation of biosensor accuracy against an established laboratory standard.

3.6.1 Analytical framework and handling of non-quantifiable samples

To provide a rigorous diagnostic validation, we established clear analytical criteria for distinguishing between "Detected" (quantifiable) and "Not Detected" samples across both platforms. According to the official RIDASCREEN FAST manufacturer instructions, the Limit

of Quantification (LOQ) is established at 50 µg/kg (ppb), defining the lower operational limit of the quantifiable standard curve. The manual states that numerical estimates generated below this validated 50 ppb threshold are deemed "out of measuring range" and the results "may not be significant" for standard quantitative comparison (R-Biopharm, 2023). This focus on a robust quantifiable threshold is a characteristic of studies employing "FAST" microplate assays, prioritizing speed and regulatory concordance over extreme low-limit precision.

Our comparative analysis handled samples according to these validated diagnostic thresholds. Samples yielding ELISA results significantly below the 50 ppb LOQ were categorized collectively within the "Negative/Low ZEA" cohort. This cohort comprised strict true negatives (current out of range, ZEA Not Detected on the biosensor current output, $n=11$) and samples returning ELISA estimates between 0.3 and 45.89 ppb ($n=31$). For diagnostic accuracy calculation, all 42 samples exhibiting ZEA concentrations effectively below the operational quantifiable threshold of 50 ppb were deemed diagnostically concordant for "Negative/Low ZEA" across both platforms.

The remaining 10 samples yielded validated quantifiable numerical data significantly above the 50 ppb ELISA LOQ and within the established linear range of the biosensor, forming the Quantifiable True Positive cohort. We specifically justify this comparative statistical focus on quantifiable samples (>50 ppb) from both analytical and regulatory perspective. Analytically, a true method correlation (R^2 analysis) must operate on robust numerical data rather than extrapolated, low-confidence estimates below the standard curve's precision to avoid statistical skewing. Furthermore, maximum limits (MLs) set by regulating bodies, such as EU Commission Regulation No 1881/2006, for raw maize are significantly higher than 50 ppb. Establishing a screen-active threshold focused on samples approaching or exceeding the recognized standard LOQ (50 ppb) hence ensures the developed sensor's relevance for rapid food safety screening and effective regional surveillance.

3.6.2 Diagnostic performance and quantitative precision

Linear regression analysis was applied as the primary statistical method to validate the method correlation for the 11 quantifiable true positive samples ($n=10$). The scatter plot in Figure 8 illustrates this relationship, with a best-fit crimson regression trend line. The linear regression equation is determined as $y = 0.93x + 22.57$, with a coefficient of determination of $R^2 = 0.867$.

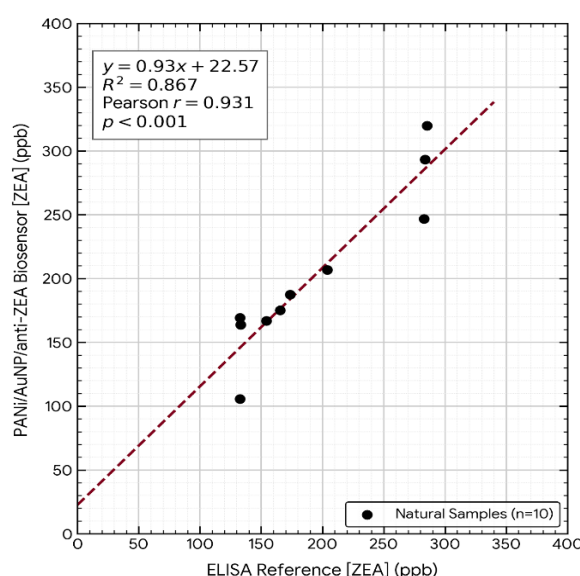


Figure 8. Linear regression analysis correlating quantifiable Zearalenone (ZEA) concentrations measured by the developed PANi/AuNP/anti-ZEA electrochemical biosensor versus standard ELISA ($n=10$).

The strong positive linear association between the two analytical methods was confirmed by calculating the Pearson Product-Moment Correlation coefficient ($r=0.931$). This robust, statistically significant agreement ($p<0.001$) on identical quantitative numerical data directly demonstrates the high accuracy of the modified SPCE surface and the specificity of the anti-ZEA pAbs, ensuring true quantitative precision rather than simple classification capability. Although the established linear dynamic range of the calibration curve is in the range of 0-200 ppb, concentrations exceeding 200 ppb were mathematically extrapolated using the linear calibration equation ($y=-0.894x+418.16$, $R^2=0.962$) (Figure 6b) and subsequently cross-validated against standard ELISA measurements.

When combining the high qualitative concordance of the 'Not Detected' cohort (42 samples) with the precise numerical agreement of the Quantifiable cohort (10 samples), the biosensor achieved 100% categorical agreement relative to ELISA. The strong quantitative correlation results demonstrated in the quantifiable cohort provide the necessary robust statistical evidence to validate the platform's quantitative accuracy for high-impact analytical applications. This demonstrates the capacity of the developed PANi/AuNP/anti-ZEA sensor as a reliable, interchangeable alternative to standard microplate ELISA for rapid, quantitative on-field monitoring of Zearalenone.

3.6.3 Divergent ZEA contamination profiles in raw grains and processed poultry feed

The diverse source breakdown of the revised $n=52$ samples offers valuable agricultural epidemiology insights into ZEA contamination trends in Malaysia, highlighting a critical disparity between field conditions and processed commodities. From the quantifiable $n=10$ positive samples (Section 3.6.2), only two were derived from raw grain corn plantations (20%). These samples were localized to MARDI Seberang Perai, with samples in the range of 130-175 ppb.

An interesting epidemiological pattern emerged from the multi-site raw grain surveillance. Out of the $n=37$ raw grain samples collected directly from six distinct production sites (MARDI plots in five regions, Genting Sepang, and Malacca), $n=35$ samples (94.6%) either returned "Not Detected" or fell effectively below the lowest significant quantifiable threshold (50 ppb). This overwhelmingly high incidence of negligible ZEA contamination across raw corn plantation plots indicates the effectiveness of existing pre-harvest agricultural practices or potentially favorable climatic conditions during the growing season (Yazid et al., 2021), which likely mitigate initial *Fusarium* ear rot infection and early toxin synthesis.

Conversely, the processed animal feed sector presented a highly concerning contamination profile. Of the commercial poultry feed samples tested ($n=12$), six (50%) were validated as highly quantifiable positives. Crucially, a significant majority of these feed samples exhibited contamination levels sharply exceeding 150 ppb, sharply contrasting with the low burden observed in the field. An additional two samples exceeding 100 ppb (105.66-166.93 ppb) were identified among the retail corn kernels. This epidemiological shift demonstrates that the primary risk pathway for ZEA entry into the Malaysian food chain is localized to post-harvest issues. Factors specific to tropical climates, including prolonged storage, inadequate drying preceding formulation, high-moisture transport environments, and high humidity during storage at feed mills, are renowned catalysts for continued *Fusarium* growth and accelerated mycotoxin synthesis within finished feed matrices.

These findings highlight that successful ZEA regional surveillance cannot solely focus on raw grain corn; rather, the critical risk hot spots existing within the finished animal feed supply chain must be prioritized. ZEA is a highly robust estrogenic mycotoxin that remains toxic through standard feed processing due to its thermal stability. In poultry, particularly chickens, dietary ZEA exposure, even at levels considered moderate for mammals, leads to severe hyperestrogenism, including pathological enlargement of the oviduct, vent swelling, cloacal

prolapse, and severe reproductive disorders in laying hens that dramatically reduce egg production and shell quality. Epidemiological data indicates that continuous low-dose exposure can lead to reduced weight gain and immunosuppression, increasing flock vulnerability to opportunistic infections and substantial economic losses in both broiler and layer operations.

Consequently, our findings demonstrate that mandatory, decentralized ZEA screening utilizing the developed portable biosensor must be prioritized at feed manufacturing and storage facilities over field plot surveillance in this region. Traditional reference methods such as HPLC and ELISA, while robust, are inherently laboratory-bound, requiring specialized instrumentation, extensive sample cleanup, and lengthy processing times that often delay results by days or even weeks. In contrast, the developed electrochemical biosensing platform achieves complete sample-to-answer quantitative detection in under 2 hours directly on-site. This rapid turnaround capability empowers feed mill operators and regulatory bodies to make immediate, real-time decisions to intercept contaminated feed stocks prior to formulation and storage, thereby establishing a viable defense against ZEA entering the animal feed supply chain.

3.7 Storage study of the modified electrode

To evaluate the shelf-life and practicality of the PANi/AuNP/anti-ZEA biosensor for decentralized field testing, storage stability was investigated under ambient room temperature (25°C) and refrigerated conditions (4°C) over extended storage periods ($n=3$). As illustrated in Figure 9, the biosensor exhibited remarkable stability when stored dry at room temperature, retaining 100.0% of its initial current response for up to 14 days.

Interestingly, storage at 4°C resulted in a more immediate decline in current response, retaining only ~80% response within the first week. This counterintuitive trend can be attributed to the physical-chemical behavior of the milk protein blocking layer and the underlying conducting polymer matrix. At lower temperatures (4°C), milk proteins (predominantly casein) are prone to hydrophobic dissociation and cold-induced structural aggregation (Fox & Brodkorb, 2008), which can sterically hinder antibody paratopes and increase charge-transfer resistance across the bio-interface. Furthermore, bringing refrigerated electrodes to ambient conditions for electrochemical testing induces micro-condensation on the electrode surface, altering the local protonation state and swelling behavior of the polyaniline (PANi) film (Stejskal et al., 2003).

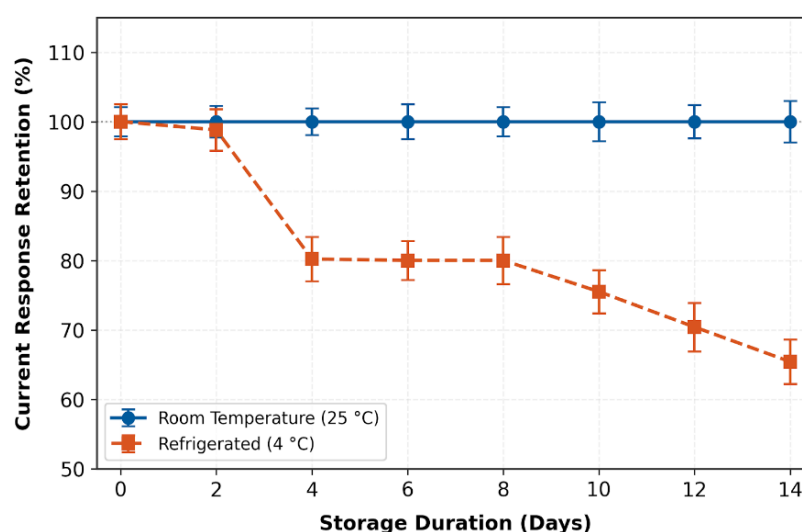


Figure 9. Storage stability of the modified PANi/AuNP/anti-ZEA biosensor stored dry under room temperature (25°C) and refrigerated (4°C) conditions over a 14-day evaluation period. Data points represent the mean current response retention (%), with error bars denoting standard deviation ($\pm$ SD, $n=3$). The dotted line indicates baseline initial response (100%).

The ability of the PANi/AuNP/anti-ZEA platform to maintain 100% analytical performance for 14 days at room temperature without requiring cold-chain logistics (4°C refrigeration) highlights its high stability and commercial suitability for on-site mycotoxin monitoring in resource-limited agricultural settings. Furthermore, as the biosensor relies on high-affinity antibody–antigen immunobinding on SPCEs tailored for rapid field screening, it is designed as a single-use disposable test strip. This eliminates the risk of cross-contamination and matrix carryover between agricultural samples, ensuring high measurement reliability during field operations.

4. Conclusion

This study successfully developed a portable electrochemical biosensor platform for the rapid, decentralized detection of zearalenone (ZEA) in grain corn and animal feed matrices. Successful surface modification of screen-printed carbon electrodes (SPCE) using a polyaniline/gold nanoparticle (PANi/AuNP) nanocomposite provided a highly conductive matrix for stable anti-ZEA antibody immobilization and accelerated electron transfer kinetics. Matrix effect evaluations established an optimal 1:5 dilution ratio, effectively mitigating background interference from complex feed components. Under optimized conditions, the biosensor achieved a matrix-specific limit of detection (LOD) of 57.66 ppb and a robust linear response up to 200 ppb aligning with international regulatory thresholds. Analytical validation demonstrated high recovery rates (80.19%–93.92%) in spiked samples, while shelf-life evaluations confirmed robust storage stability at room temperature for up to 14 days.

The practical utility of the platform was established through the analysis of 52 real-world agricultural samples encompassing raw grain corn and commercial poultry feed. Crucially, analytical profiling revealed a striking disparity in ZEA prevalence: pre-harvest field corn exhibited overwhelmingly low contamination (80.8% below the 50 ppb LOQ), whereas commercial poultry feed demonstrated high contamination rates, with 50% of tested feed samples sharply exceeding 150 ppb. Across both complex matrices, the biosensor achieved 100% categorical concordance and strong quantitative correlation relative to reference ELISA assays, confirming laboratory-grade accuracy in a field-ready format. Pairing the functionalized SPCE strip with the handheld MARDIsense portable reader and a custom mobile application enables real-time cloud logging and geospatial tracking, allowing non-specialized personnel to conduct rapid on-site testing. Ultimately, these findings demonstrate that regional surveillance must reorient from pre-harvest field plot monitoring toward high-risk post-harvest feed processing facilities, where this decentralized technology provides a scalable solution to safeguard the agricultural supply chain.

Data Availability

The data supporting the findings of this study are not publicly available due to intellectual property rights and related restrictions. The data may be made available upon reasonable request, subject to approval by the corresponding author.

Author Contributions

Nur Azura Mohd Said: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Erna Mutiara Masdek:** Methodology, Validation, Formal analysis, Investigation. **Norhafniza Awaludin:** Methodology, Project administration, Investigation. **Mohammad Rejab Ismail, Hazana Razali, Sahira Akmar Zulkepli, Syah Noor Muhammad Ramli:** Methodology, Resources, Investigation. **Mohd Afendy Abdul Talib, Mohd Effendi Mohamed Nor, Halimah Hashim, Nurul Afza Karim:** Resources.

Acknowledgement

The authors would like to acknowledge the Ministry of Agriculture and Food Security (KPKM) for the financial support provided under the 12th Malaysia Development Plan Fund (Grant No. P-RB502). The authors also extend their appreciation to the research team at Biogenes Technologies Sdn. Bhd. for their valuable technical insights and contributions to the development of the MARDI*sense* platform.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Ethics Statement

All animal procedures were reviewed and approved by the MARDI Animal Ethics Committee (Approval No. 20230622/R/MAEC00139) and conducted in accordance with relevant institutional and national guidelines for the care and use of laboratory animals.

References

- Ascoli, C. A., & Aggeler, B. (2018). Overlooked benefits of using polyclonal antibodies. *Biotechniques*, 65(3), 127–136. <https://doi.org/10.2144/btn-2018-0065>
- Aymard, C., Kanso, H., Serrano, M. J., Pagán, R., Noguer, T., & Istamboulie, G. (2022). Development of a new dual electrochemical immunosensor for a rapid and sensitive detection of enrofloxacin in meat samples. *Food Chemistry*, 370, 131016. <https://doi.org/10.1016/j.foodchem.2021.131016>
- Azri, F. A., Selamat, J., Sukor, R., Yusof, N. A., Raston, N. H. A., Eissa, S., Zourob, M. & Chinnappan, R. (2021). Determination of minimal sequence for zearalenone aptamer by computational docking and application on an indirect competitive electrochemical aptasensor. *Analytical and Bioanalytical Chemistry*, 413(15), 3861–3872. <https://doi.org/10.1007/s00216-021-03336-1>
- Bianchi, V., Boni, A., Bassoli, M., Giannetto, M., Fortunati, S., Careri, M., & De Munari, I. (2021). IoT and biosensors: A smart portable potentiostat with advanced cloud-enabled features. *IEEE Access*, 9, 141544–141554. <https://doi.org/10.1109/ACCESS.2021.3120022>
- Curulli, A. (2021). Electrochemical biosensors in food safety: Challenges and perspectives. *Molecules*, 26(10), 2940. <https://doi.org/10.3390/molecules26102940>
- European Commission. (2006). Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. *Official Journal of the European Union*, L 70, 12–34.
- Food and Agriculture Organization of the United Nations (FAO). (2024). Agricultural production statistics. FAO Knowledge Repository. <https://openknowledge.fao.org>
- Forzato, C., Vida, V., & Berti, F. (2020). Biosensors and sensing systems for rapid analysis of phenolic compounds from plants: A comprehensive review. *Biosensors*, 10(9), 105. <https://doi.org/10.3390/bios10090105>
- Foubert, A., Beloglazova, N. V., Hedström, M., & De Saeger, S. (2019). Antibody immobilization strategy for the development of a capacitive immunosensor detecting zearalenone. *Talanta*, 191, 202–208. <https://doi.org/10.1016/j.talanta.2018.08.062>
- Fox, P. F., & Brodtkorb, A. (2008). The casein micelle: Historical aspects, current concepts and significance. *International Dairy Journal*, 18(7), 677–684. <https://doi.org/10.1016/j.idairyj.2008.03.002>
- Fumagalli, F., Ottoboni, M., Pinotti, L., & Cheli, F. (2021). Integrated mycotoxin management system in the feed supply chain: Innovative approaches. *Toxins*, 13(8), 572. <https://doi.org/10.3390/toxins13080572>
- Goud, K. Y., Kumar, V. S., Hayat, A., Gobi, K. V., Song, H., Kim, K. H., & Marty, J. L. (2019). A highly sensitive electrochemical immunosensor for zearalenone using screen-printed disposable electrodes. *Journal of Electroanalytical Chemistry*, 832, 336–342. <https://doi.org/10.1016/j.jelechem.2018.10.058>
- Gruber-Dorninger, C., Jenkins, T., & Schatzmayr, G. (2019). Global mycotoxin occurrence in feed: A ten-year survey. *Toxins*, 11(7), 375. <https://doi.org/10.3390/toxins11070375>

- Guo, K. (2024). Design and fabrication of a molecularly imprinted electrochemical sensor with high sensitivity for zearalenone assessment in maize. *International Journal of Electrochemical Science*, 19(6), 100612. <https://doi.org/10.1016/j.ijoes.2024.100612>
- Jiang, X., Mu, Z., Wang, J., Zhou, J., & Bai, L. (2024). A novel sandwich-type electrochemical immunosensor for sensitive detection of zearalenone using NG/PDDA/HNTs and Ti-MOF-KB composites for signal amplification. *Food Chemistry*, 436, 137704. <https://doi.org/10.1016/j.foodchem.2023.137704>
- Jusoh, N. S., Said, N. A. M., Salam, F., Awaludin, N., Kadir, A. A. A., & Ismail, M. R. (2022). An integrated handheld biosensing device with IoT system for in-situ and rapid detection of multi-mycotoxins. *2022 4th International Conference on Smart Sensors and Application (ICSSA)*, 11–16. IEEE. <https://doi.org/10.1109/ICSSA54161.2022.9870938>
- Li, R., Wen, Y., Wang, F., & He, P. (2021). Recent advances in immunoassays and biosensors for mycotoxins detection in feedstuffs and foods. *Journal of Animal Science and Biotechnology*, 12(1), 108. <https://doi.org/10.1186/s40104-021-00629-4>
- McDougal, T. (2025, March 9). Persistently high mycotoxin co-contamination levels pose ongoing challenges. <https://www.dairyglobal.net/health-and-nutrition/persistently-high-mycotoxin-co-contamination-levels-poses-ongoing-challenges>
- Meliana, C., Liu, J., Show, P. L., & Low, S. S. (2024). Biosensor in smart food traceability system for food safety and security. *Bioengineered*, 15(1). <https://doi.org/10.1080/21655979.2024.2310908>
- Nji, Q. N., & Mwanza, M. (2024). Three-year multi-mycotoxin analysis of South African commercial maize from three provinces. *Frontiers in Fungal Biology*, 5, 1426782. <https://doi.org/10.3389/ffunb.2024.1426782>
- Radi, A. E., Eissa, A., & Wahdan, T. (2020). Molecularly imprinted impedimetric sensor for determination of mycotoxin zearalenone. *Electroanalysis*, 32(8), 1788–1794. <https://doi.org/10.1002/elan.201900528>
- Rai, A., Das, M., & Tripathi, A. (2020). Occurrence and toxicity of a fusarium mycotoxin, zearalenone. *Critical Reviews in Food Science and Nutrition*, 60(16), 2710–2729. <https://doi.org/10.1080/10408398.2019.1655388>
- R-Biopharm AG. (2023). RIDASCREEN®FAST Zearalenon: Competitive enzyme immunoassay for the quantitative analysis of zearalenone in cereals and feed (Art. No. R5502) [Product specifications and instructions for use]. R-Biopharm AG. <https://food.r-biopharm.com/products/ridascreenfast-zearalenon/>
- Rizzotto, F., Khalife, M., Hou, Y., Chaix, C., Lagarde, F., Scaramozzino, N., & Vidic, J. (2023). Recent advances in electrochemical biosensors for food control. *Micromachines*, 14(7), 1412. <https://doi.org/10.3390/mi14071412>
- Robin, P., & Gerber-Lemaire, S. (2023). Design and preparation of sensing surfaces for capacitive biodetection. *Biosensors*, 13(1), 17. <https://doi.org/10.3390/bios13010017>
- Said, N. A. M., Awaludin, N., Ismail, M. R., Razali, H., Masdek, E. M., Zulkepli, S. A., & Ramli, S. N. M. (2025). Polyclonal Antibodies against Zearalenone: Production, Characterisation, and Application in Food Safety Biosensors. *Pertanika Proceedings* 1 (7): 133–137 (2025).
- Seaton, B. T., Hill, D. F., Cowen, S. L., & Heien, M. L. (2020). Mitigating the effects of electrode biofouling-induced impedance for improved long-term electrochemical measurements in vivo. *Analytical Chemistry*, 92(9), 6334–6340. <https://doi.org/10.1021/acs.analchem.9b05194>
- Siva Sangu, S., Chandra Bose Gopinath, S., Abdul Shukur, M. F., & Mohamed Saheed, M. S. (2022). An electrochemical approach for ultrasensitive detection of zearalenone in commodity using disposable screen-printed electrode coated with MXene/chitosan film. *BioNanoScience*, 12(3), 814–823. <https://doi.org/10.1007/s12668-022-00984-0>
- Stejskal, J., & Gilbert, R. G. (2002). Polyaniline. Preparation of a conducting polymer (IUPAC technical report). *Pure and Applied Chemistry*, 74(5), 857–867. <https://doi.org/10.1351/pac200274050857>
- Thongrussamee, T., Kuzmina, N. S., Shim, W. B., Jiratpong, T., Eremin, S. A., Intraseek, J., & Chung, D. H. (2008). Monoclonal-based enzyme-linked immunosorbent assay for the detection of zearalenone in cereals. *Food Additives and Contaminants*, 25(8), 997–1006. <https://doi.org/10.1080/02652030701823159>

- Umapathi, R., Ghoreishian, S. M., Rani, G. M., Cho, Y., & Huh, Y. S. (2022). Emerging trends in the development of electrochemical devices for the on-site detection of food contaminants. *ECS Sensors Plus*, 1(4), 044601. <https://doi.org/10.1149/2754-2726/ac9d4a>
- United States Department of Agriculture Foreign Agricultural Service (USDA-FAS). (2024). World Agricultural Production: Grain Markets and Trade. USDA FAS Report. <https://www.fas.usda.gov/data/production/0440000>
- Urraca, J. L., Benito-Peña, E., Pérez-Conde, C., Moreno-Bondi, M. C., & Pestka, J. J. (2005). Analysis of zearalenone in cereal and swine feed samples using an automated flow-through immunosensor. *Journal of Agricultural and Food Chemistry*, 53(9), 3338–3344. <https://doi.org/10.1021/jf048092p>
- USDA Foreign Agricultural Service. (2023). Malaysia: Grain and Feed Annual. GAIN Report No. MY2023-0001. United States Department of Agriculture.
- Wan Syahidah, H., Suhaimi, D., Hartini, Y., & Nurulhayati, A. B. (2021). Screening of fungal contamination of *Aspergillus* and *Fusarium* mycotoxin in grain corn from Peninsular Malaysia. In M. S. Bastami, M. Muhammad, M. A. M. Marzuki, Y. S. Ting, N. H. M. Zairi, M. H. Ahmad, M. M. M. Ajat, A. Q. Sazili, M. A. M. Noor, N. F. M. Sohaimi, & M. A. Azman (Eds.), *Proceedings of the 40th Malaysian Society of Animal Production Annual Conference 2021* (pp. 157–159).
- Wang, K., Lin, X., Zhang, M., Li, Y., Luo, C., & Wu, J. (2022). Review of electrochemical biosensors for food safety detection. *Biosensors*, 12(11), 959. <https://doi.org/10.3390/bios12110959>
- Wang, X., Luo, Y., Huang, K., & Cheng, N. (2022). Biosensor for agriculture and food safety: Recent advances and future perspectives. *Advanced Agrochem*, 1(1), 3–6. <https://doi.org/10.1016/j.aac.2022.08.002>
- Wang, Y., Wang, X., Zhang, H., Jiang, J., & Fotina, H. (2021). Synthesis of zearalenone immunogen and comparative analysis of antibody characteristics. *International Journal of Analytical Chemistry*, 2021(1), 7109383. <https://doi.org/10.1155/2021/7109383>
- Yagati, A. K., Chavan, S. G., Baek, C., Lee, M. H., & Min, J. (2018). Label-free impedance sensing of aflatoxin B1 with polyaniline nanofibers/Au nanoparticle electrode array. *Sensors*, 18(5), 1320. <https://doi.org/10.3390/s18051320>
- Yazid, S. N. E., Ng, W. J., Selamat, J., Ismail, S. I., & Samsudin, N. I. P. (2021). Diversity and toxigenicity of mycobiota in grain corn: A case study at pioneer grain corn plantations in Terengganu, Malaysia. *Agriculture*, 11(3), 237. <https://doi.org/10.3390/agriculture11030237>
- Zambry, N. S., Prathaban, S. N., Ibrahim, F., Thiha, A., Karajacob, A. S., Jamaluddin, N. F., Syed Omar, S.F., Shariffuddin, I.I., Bustam, A. & Tay, S. T. (2025). A label-free electrochemical immunosensor on gold-printed electrode for the electrochemical detection of C-reactive protein from blood samples. *Microchimica Acta*, 192(7), 437. <https://doi.org/10.1007/s00604-025-07252-4>
- Zeng, Y., Camarada, M. B., Lu, X., Tang, K., Li, W., Qiu, D., Wen, Y., Wu, G, Luo, Q. & Bai, L. (2022). Detection and electrocatalytic mechanism of zearalenone using nanohybrid sensor based on copper-based metal-organic framework/magnetic Fe₃O₄-graphene oxide modified electrode. *Food Chemistry*, 370, 131024. <https://doi.org/10.1016/j.foodchem.2021.131024>
- Zhang, J., Wang, B., Xu, B., Cheng, G., & Dong, S. (2000). Amperometric quantification of polar organic solvents based on a tyrosinase biosensor. *Analytical Chemistry*, 72(15), 3455–3460. <https://doi.org/10.1021/ac000373i>
- Zinedine, A., Soriano, J. M., Moltó, J. C., & Manes, J. (2007). Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: an oestrogenic mycotoxin. *Food and Chemical Toxicology*, 45(1), 1–18. <https://doi.org/10.1016/j.fct.2006.07.030>
- Ziyatdinova, G., Guss, E., & Yakupova, E. (2021). Electrochemical sensors based on the electropolymerized natural phenolic antioxidants and their analytical application. *Sensors*, 21(24), 8385. <https://doi.org/10.3390/s21248385>