

Application of nanozymes in food safety testing

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Abstract. Nanozymes have advantages such as high stability, low cost, large-scale production, function design and cutting, and easy modification, so they have great application prospects in food safety testing. This paper mainly summarizes the testing principle and application of nanozymes in food safety testing, including pesticide and veterinary drug residues, pathogenic bacteria and other substances. The challenges faced by the future development of nanozymes in food safety testing was also discussed.

Keywords: nanozyme; food safety testing; food safety.

1. Introduction

Nanozymes represent a novel class of nanomaterials that possess catalytic properties similar to those found in natural enzymes. In comparison to natural enzymes, they exhibit enhanced stability and can be produced at a lower cost on a larger scale. Additionally, their functions can be tailored through design modifications with ease. Nanozymes have garnered significant attention across various fields including sensing analysis, biomedical science, antibacterial treatment, and environmental protection ^[1]. As such, nanozymes have emerged as strong contenders and potential substitutes for natural enzymes. The catalytic process employed by nanozymes is distinct from that observed in natural enzymes, and its activity is closely associated with factors such as size, surface lattice, surface modification, and composition ^[2].

With the advancement of The Times, there has been a significant expansion in the variety of food, production technology, and raw materials. Consequently, this has led to increased complexity and difficulty in conducting food testing. The utilization of nanozymes in food testing offers several advantages such as cost savings, reduced reaction time, and enhanced detection sensitivity. Moreover, it addresses the limitations associated with traditional detection methods including laborious pretreatment steps, expensive equipment requirements, dependence on skilled professionals, and lengthy detection cycles ^[2]. Additionally, nanozymes exhibit excellent capabilities for degrading organic pollutants, toxic substances, harmful compounds, and heavy metal ions present in food. They enable rapid, effective, and accurate food analysis. For instance, the integration of nanozymes into a sensor allows for the swift identification of toxic substances within minutes ^[3]. This study primarily focuses on exploring how nanozymes can be applied to detect pesticide residues, veterinary drug residues, pathogens, and other relevant substances.

2. Detection of pesticide and veterinary drug residues

Nanozymes facilitate specific chemical reactions, resulting in observable indications such as alterations in color, ultraviolet absorption values, or electrical signals. This enables the detection of pesticide and veterinary drug residues.

GUAN^[4] et al. based on the liposome reactor constructed an enzyme electrochemical biosensor for rapid detection of organophosphorus pesticide residues with multi-layer nanozymes membrane modified electrode core. The average particle size of the enzyme liposome microreactor was $(7.26 \pm 0.75) \mu\text{m}$. The optimum number of multilayer enzyme membrane of modified electrode is 6 layers. Using dichlorvos as an organophosphorus pesticide model, the peak current value of the sensor showed a good linear relationship with the inhibition rate of acetylcholinesterase in the linear range of $0.25 \sim 1.75 \mu\text{mol/L}$ and $2.00 \sim 10.00 \mu\text{mol/L}$, respectively. The lowest detection limit is

(0.72 ± 0.065) $\mu\text{g/L}$ (signal-to-noise ratio is 3). GCE-(ALB/SiO₂)₆ constructed by layer upon layer self-assembly technology shows good sensitivity, accuracy, selectivity and repeatability against residues of dichlorvos.

ZHU^[5] et al. produced a colorimetric nanozymes sensor array based on heteroatom-doped graphene for the detection of aromatic pesticides. The peroxidase simulation activity of the nanozymes is related to its active sites. The activity is reduced with the adsorption of different pesticides on graphene. Based on this principle, five pesticides (lactamine, fluoroxy, methansulfuron, flurane and dithiourea) ranging from $5\mu\text{M}$ to $500\mu\text{M}$ were successfully distinguished by sensor array. The method successfully validated the practical application of the sensor array: identification of pesticides in soil samples. In addition, the feasibility of the identification of two mixed pesticides and pesticides in different proportions in real samples was also verified.

WANG^[6] et al. simulated peroxidase activity based on gold nanoparticles (AuNPs) and target-induced aptamer replacement to achieve an enzyme-free, ultra-sensitive electrochemical detection of kanamycin residues. AuNPs showed the activity of simulated peroxidase. In the absence of kanamycin, the active sites of AuNPs were blocked for aptamers were adsorbed on the surface, resulting in a reduction in its peroxidase activity. When kanamycin binds to the adsorbed aptamer with high affinity, the AuNPs surface was exposed, and the peroxidase activity was restored. Then AuNPs catalyzed the reaction of hydrogen peroxide with reduced thiosine to produce thiosine oxide. The kanamycin concentration is related to the reduction peak of thiosine in differential pulse voltammetry (DPV). The method was directly used to detect kanamycin in standard solution and honey samples, respectively. The method does not need to modify the electrode, and has obvious specificity and good reproducibility. In addition, the strategy can be used as a general strategy for the preparation of aptamers.

ZHANG^[7] et al. prepared a double-signal amplification electrochemical sensor based on a heteropolyacid anion (NH₄)₅ PV₈ Mo₄ O₄₀ (NPV₈ Mo) and transition metal zirconia (zirconia) nanocomposite for simultaneous detection of clenbuterol (CLB) and Ractobamide (RAC). This is the first time to use zirconia as POM carrier to prepare electrochemical sensors. Zirconia is a P-type semiconductor with good adsorption capacity as a multifunctional catalyst^[9]. It can not only provide an active surface oxygen species generated by a three-dimensional oxidation stage, but also increase the electrochemically active surface area, thereby making better use of the catalytic site and controllable pore size of the catalyst^[10]. The results of electrochemical measurement show that NPV₈ Mo/ZrO₂/GCE has high stability, fast response, good conductivity and electrocatalytic activity, enhances the peak current, and negatively changes the oxidation potential of CLB and RAC. The method was used to determine CLB and RAC in pork samples with recoveries ranging from 94.4 to 102.3%. These results indicate that it has a good application prospect and food safety in the extensive monitoring of β 2-adrenergic agonists in meat products.

3. Detection of pathogenic bacteria

By altering the surface of nanozymes, it is possible to confer them with the ability to specifically bind with pathogenic bacteria. Upon interaction with pathogenic bacteria, the catalytic activity of nanozymes undergoes a change that can be analyzed through corresponding detection methods in order to facilitate the identification and quantification of such pathogens.

LUO^[8] et al. developed a photoelectrochemical (PEC) sensor for detection of *Staphylococcus aureus* (*S. aureus*). The PEC sensor is composed with Cu-C₃N₄-tio₂ heterostructure as the photoactive material, and a single layer of Cu-C₃N₄ nanomases as the signal amplifier. In the presence of *S. aureus*, Cu-C₃N₄@Apt and *S. aureus* are specifically anchored to the surface of the photoelectrode. Cu-C₃N₄@Apt nanozymes catalyze the oxidation of 4-chloro-1-naphthol (4-CN), resulting in a significant reduction in photocurrent. Based on this amplification strategy, the *Staphylococcus aureus* detection range of the constructed PEC sensor is between 10-108 CFU/mL,

and the detection limit (LOD) is as low as 3.40 CFU/mL. In addition, the PEC sensor was able to detect *Staphylococcus aureus* in added orange juice and milk.

JIANG^[9] et al. have successfully developed a new immunochromatographic assay (ICA) for detection of *E. coli* O157: H7. Sandwich type immune response was performed on ICA and Pt-Au bimetallic nanoparticles (NPs) were accumulated in the experimental area. The amplification strategy is based on Pt-Au bimetallic NPs, which has a high peroxidase activity against 3, 3', 5, 5'-tetramethyl benzidine and produce characteristic color bands, so that the targets can be visually detected without instruments. The innovation of this approach lies in visualizing and quantifying target pathogens by detecting color intensity. Because Pt-AuNPs have good peroxidase activity, they emit a strong visible color intensity for visual observation and quantification using commercial analyzers. Compared with traditional gold colloids test strips, the sensitivity is more than 1,000 times higher. The method can be developed into a general signal amplification technique for the detection of foodborne pathogens and protein biomarkers.

WANG^[10] et al. proposed a method for rapid detection of *Escherichia coli* O157: H7 based on the nanenzyme-bacteria-antibody sandwich model. The functional nanozymes of man-PB nanoparticles has the dual function of recognition and signal indication, which can effectively capture target pathogens with affinity to *E. coli* O157: H7 flagellum. The signal readout with the sensor has three formats: qualitative detection by visual readout, quantitative analysis of signal strength on the T-line or colorimetric signal after the catalytic reaction. The detection quantification range of target pathogens with this LFIA is 102-102cfu-108mL⁻¹ and the detection limit is 102cfumL⁻¹. The platform has good sensitivity and selectivity, and has successfully detected targets in complex substrates. man-PB can provide targeted binding to target bacteria with two quantitative formats by binding to the FimH protein in the flagella of target bacteria. This pattern of target-binding can more fully expose antigenic determinants on the bacterial surface, improve effective recognition and detection sensitivity. In addition, the manufacturing process of the sensors is fast, economical, simple and labor-saving.

ZHANG^[11] et al. studied a continuous cascade nanozymes biosensor based on monazidine (PMA), loop-mediated isothermal amplification (LAMP) and nanozymes bands. The *ompA* gene of ES was detected by FITC and Bio-modified primer LAMP. The survival state and death state of ES were distinguished by LAMP combined with PMA. Then, using ferric oxide as a nanozymes probe, MNP-based immunochromatographic bands (nanozymes bands) are further used to amplify the signal, allowing visual detection and quantification via a band reader. LAMP products are sandwiched between anti-FITC and anti-BIO, and the accumulation of ferric tetroxide magnetism makes visual detection of ES possible. Compared to previously reported technologies, the nanozymes biosensor has an improved detection limit of 10 CFU/mL, and the entire operation process is much faster (within 1 hour) and simpler (without specialized facilities). Therefore, the developed continuous cascade nanozymes biosensor provides a fast, ultra-sensitive and simple tool for the field detection of live ES. In addition, the biosensor enables quantitative analysis of viable ES in infant powders through visual detection of simple sample pretreatment.

4. Detection of other substances

Nanozymes possess the capabilities of various REDOX enzymes, including peroxidase (POD), oxidase (OXD), catalase (CAT), and superoxide dismutase (SOD). They exhibit a distinctive catalytic mechanism that allows for substrate oxidation without requiring additional H₂O₂ or O₂, thereby facilitating the detection of other substances.

TAO^[12] designed and assembled a mesoporous silica modified magnetic nanoparticle (Fe₃O₄@SiO₂) with intrinsic peroxide-like (POD) catalytic activity. Mesoporous SiO₂ can ensure the flexible size, good dispersion and stability of the nanozymes. By combining catalytic oxidation-induced color development with cholesterol oxidase, Fe₃O₄@SiO₂ can be used for visual colorimetric determination of cholesterol with a linear range of 10-300 μM and a detection limit as

low as 7.12 μM . The method has been successfully applied to the determination of cholesterol content in milk and egg yolk samples. It has the characteristics of low cost, accuracy, high efficiency and strong specificity.

GANGANBOINA^[13] et al. introduced v2o5 nanosheets (NS) as a new type of nanozymes with biomimetic oxidase activity to rapidly detect glutathione. By means of ultrasonic assisted removal of bulk vanadium pentoxide, 5-6 layers of vanadium pentoxide NS with the size of 300-600nm were successfully prepared with N, N-dimethylformamide as intercalation reagent. The sensing mechanism involves the oxidation of 3, 3', 5, 5'-tetramethylbenzylamine (TMB) by vanadium pentoxide NS, while increasing the absorption strength. Upon the introduction of glutathione, the oxidized TMB is converted back to its original form, resulting in a simultaneous reduction in peak strength at 650 nm. The sensor has a good linear response to glutathione in the range of 10-500nM, with a detection limit of 2.4 nM. In addition, the o2o5-TMB sensor has good selectivity and high specificity, making the detection of glutathione suitable for diluted human serum samples.

XIONG^[14] et al. demonstrated a simple and rapid in situ partial oxidation synthesis strategy to manufacture mixed valence Ce-MOF (MVCM), which exhibits intrinsic oxidase-like activity. In addition, based on the excellent catalytic activity of MVCM, a colorimetric method for the detection of biothiols in serum samples with high throughput was developed.

ZHAO^[15] et al. developed a novel colorimetric aptamer sensor (aptamer) for the detection of streptomycin (STR) based on the peroxisase-like activity of gold nanoparticles (AuNPs) and their interaction with the Strap ligand complex. Further studies also validated the suitability of the proposed adapter in milk samples, suggesting that it may have great potential applications in practical STR detection in food in the future.

5. Conclusion

By modifying the size, shape, and structure of nanozymes, as well as surface adjustments to functional groups, incorporating hybrid components or activators, and implementing reasonable design modifications, researchers have successfully developed new nanozymes with precise catalytic sites and activities. These novel nanozymes offer numerous advantages for food testing applications including portability, versatility in application range, simplicity in operation, recyclability, and stability. However, challenges still exist when it comes to utilizing nanozymes in food testing. Firstly, nanozymes can be unstable in solvents and tend to aggregate; although surface modification can enhance their stability to some extent, it does occupy a portion of the active sites on the surface of nanozymes. Secondly, the sensitivity of food testing based on nanozymes needs continuous improvement. Additionally, colorimetric sensors using nanozymes have met specific detection requirements for risk factors in food, yet there are still unresolved issues within these detection systems. Current research primarily focuses on liquid samples such as milk or fruit juice drinks, rather than solid food samples. More efforts should be invested into exploring this field. Compared to HPLC, gas chromatography, and ion chromatography, detection platforms based on nanozymes restrict themselves to detecting single target substances rather than multiple substances within complex systems. There is also a gap between laboratory-based applications of nanozymes detection and practical implementation. Further research should prioritize the development and commercialization of test strips, microfluidic chips, and kits that utilize nanozymes^[16].

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