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Using of Tyramine Signal Amplification to Improve the Sensitivity of ELISA for Aflatoxin B_1 in Edible Oil Samples

Yuanyuan Zhang[1] · Ming Li[1] · Yin Cui[1] · Xia Hong[1] · Daolin Du[1]

Abstract

Tyramine signal amplification (TSA), the excellent signal amplification strategy, has the potential to improve the sensitivities of analytes analysis. In this work, the sensitivity of enzyme-linked immunosorbent assays (ELISA) has significantly improved by coupling TSA system and further biotin-streptavidin (BSA) system. Thus, a sensitive TSA-ELISA based on TSA was developed for detecting aflatoxin B_1 (AFB_1) in edible oil samples. Under optimal conditions, the limit of detection (LOD, IC_{10}) and the half-maximal inhibition concentration (IC_{50}) of the TSA-ELISA were 0.004 and 0.039 ng/mL for AFB_1, respectively. The developed TSA-ELISA for AFB_1 has an 11-fold improved LOD value and 6-fold improved IC_{50} value when compared with ELISA. The cross-reactivities of the TSA-ELISA with its analogues were negligible (< 3.48%), which indicated high specificity. The spiked recoveries were 81.4 to 118.8% with relative standard deviations (RSDs) of 3.8 to 9.0% for AFB_1 in edible oil samples. Furthermore, the results of TSA-ELISA correlated well with those obtained by HPLC-fluorescence detector. The proposed TSA-ELISA was a satisfactory tool for sensitive, inexpensive, high-throughput, and alternative detection of AFB_1 in edible oil samples. This study could provide the strategy for improving the sensitivity of ELISA with simple and practical approach, which has significant popularizing value and application prospect.

Keywords Aflatoxin B_1 · Tyramine signal amplification · Biotin-streptavidin · ELISA · Edible oil samples

Introduction

Aflatoxins, the highly toxic secondary metabolites produced by a number of different fungi, are present in a wide range of food and feed commodities and are assumed significant because of their deleterious effects on human beings, livestock, and poultry (Rossi et al. 2012; Xie et al. 2017; Yao et al. 2017). Aflatoxins are very stable to physical and chemical stresses and cannot be removed by industrial processing (Li et al. 2016b); therefore, carryover of aflatoxins and its metabolites to raw and processed foods can occur and increase human exposure. Being identified as the usually predominant of aflatoxins and the most toxic, aflatoxin B_1 (AFB_1) has been established the maximum limits

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(MLs) by various government agencies (Zhang et al. 2017; Xie et al. 2017). The European Commission has regulated at 2 μg/kg for AFB_1 in groundnuts, nuts, dried fruits, and cereal as the rigorous legal limit (Chen et al. 2017; European Commission 2006). In China, the AFB_1 in rice and edible oil has been limited below 10 μg/kg and below 5 μg/kg in infant foods (Ministry of Health of China 2017).

The most effective control measure depends on a rigorous program of monitoring the food and feed-producing chain using sensitive and reliable analytical methods in order to minimize health risks. Chromatographic methods have been largely developed for AFB_1 determination, such as HPLC-fluorescence detector (FLD) (Golge et al. 2016; Chen et al. 2017) and HPLC-MS/MS (Hickert et al. 2015; Fan et al. 2015; Zhang et al. 2016). Based on the immunochemical assays, enzyme-linked immunosorbent assays (ELISA) (Rossi et al. 2012) as well as gold immunochromatographic assay (Chen et al. 2016) have been used for detection of AFB_1 in different specimens. The chromatographic methods are standardized, high precision and sensitivity, but time-consuming, expensive, and unsuitable for screening purposes. Immunoassays have been demonstrated to be simple, rapid, sensitive, cost-effective,

and suitable for high-throughput screening analyses in monitoring programs (Liu et al. 2009; Liu et al. 2013; Silva et al. 2014; Kong et al. 2017; Li et al. 2017).

To acquire a high sensitivity and a low detection limit, a variety of colorimetric (Lai et al. 2017; Yu et al. 2017), electrochemical (Lv et al. 2014), fluorescence resonance energy transfer (FRET) (Ko et al. 2015), and surface-enhanced Raman scattering (SERS) (Lu et al. 2014; Li et al. 2016a) immunosensors for AFB$_1$ detection also have been established. However, most new types of immunoassays were based on the using of rare signal identifications and special materials, whose instrument and operation were diffusion limited. Therefore, it is highly urgent to develop an efficient, sensitive, and simple signal amplification strategy for AFB$_1$ detection based on the classical ELISA (Xu et al. 2015; Kong et al. 2016).

The tyramine signal amplification (TSA) system and biotin-streptavidin (BSA) system were two potential and valuable approaches for achieving signal amplification and improving sensitivity (Yuan et al. 2014). These two systems have been used separately or together for developing analytical method, which could solve the limitation of the labeling HRP amount on target proteins and have gained perfect results (Fang et al. 2015). With the help of the TSA technology, a large amount of tyramine could combine with target proteins at the present of HRP and H$_2$O$_2$, and this was the essential step in signal amplification (Liu et al. 2010). The biotinylated tyramine, which was conjugated of biotin with the aforementioned tyramine, can bind to streptavidin through the high affinity and specificity. Then, a large amount of the HRP, which have conjugated with streptavidin, could be reflected by the substrate catalyzes and chromogenic reaction (Li et al. 2013; Kong et al. 2017a). Hence, higher sensitive analysis could be achieved easily using TSA system coupled with ELISA (TSA-ELISA).

The present work aims to use TSA strategy to improve the sensitivity of ELISA for AFB$_1$ present in edible oil samples (Fig. 1). For this purpose, the ELISA and TSA-ELISA based on monoclonal antibody (McAb) for detection of AFB$_1$ were developed and reported. The TSA-ELISA has shown improved sensitivity and high specificity for detecting AFB$_1$. The matrix effects of edible oil samples for TSA-ELISA have been

evaluated. Moreover, the TSA-ELISA has been applied to detect AFB$_1$ in edible oil samples and confirmed by HPLC-FLD.

Materials and Methods

Reagents and Equipments

Analytical standards of AFB$_1$, their analogues (AFB$_2$, AFG$_1$, AFG$_2$, AFM$_1$), and goat anti-mouse immunoglobulin horseradish peroxidase (GAM-IgG-HRP) were purchased from Sigma Chemical Co. (St. Louis, USA). Commercial coating antigen and immunogen of AFB$_1$ were obtained from Wuxi Determine Bio-Tech Co., Ltd. (Wuxi, China). Ovalbumin (OVA), 3′,5,5′-tetramethyl benzidine (TMB), H$_2$O$_2$, polyoxyethylene sorbitan monolaurate (Tween-20), and other chemical reagents were all purchased from Aladdin (Shanghai, China). BLAB/c female mice were obtained from the Center of Comparative Medicine of Yangzhou University (Yangzhou, China). All animals used in this study and animal experiments were approved by the Committee of Laboratory Animal Management of Jiangsu Province. The license number was SYXK (SU) 2007-0005.

Carbonate-buffered saline buffer (CBS, 0.05 mol/L, pH 9.6), phosphate-buffered saline (PBS, 0.01 mol/L, pH 7.4), and phosphate-buffered saline containing 0.05% Tween-20 (PBST) were prepared and stored in our laboratory. The concentrated solution of tyramine conjugated with biotinylated (T-B solution) and streptavidin conjugated with HRP (SA-HRP) was purchased from Beijing Biodragon Immunotechnologies Co., Ltd. (Beijing, China). The T-B solution was diluted with 0.01 mol/L PB buffer containing 0.15 mol/L NaCl and 0.02% H$_2$O$_2$ (PB-H$_2$O$_2$ buffer). The TMB solution contained 0.4 mmol/L TMB and 3 mmol/L H$_2$O$_2$ in citrate buffer (pH 5.0).

Ultraviolet absorbance was obtained on a Nanodrop-1000 spectrophotometer (Thermo, USA). The Jet Biofil 96-well transparent microplates were provided by Suzhou Kechuang Biotechnology Co., Ltd (Suzhou, China). Milli-Q purified water was obtained from a Milli-Q purification system (Bedford, MA, USA). Immunoassays were detected using

Fig. 1 Schematic illustration of the TSA-ELISA for detecting AFB$_1$
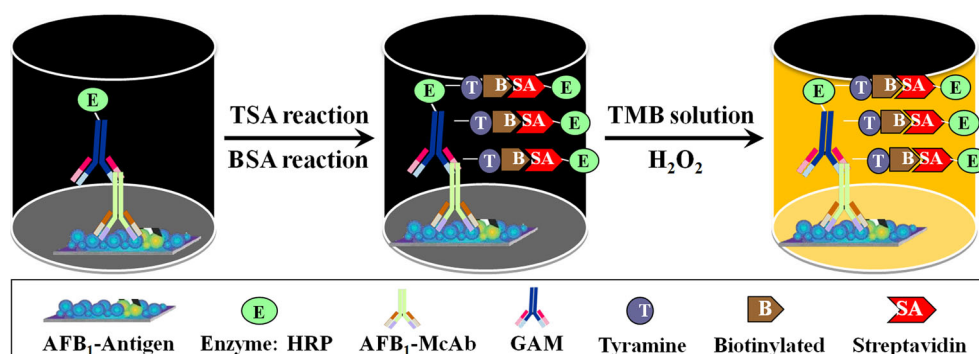
an Infinite M1000 Pro microtiter plate reader (Tecan, Switzerland). Washing steps were carried out using a Detie HBS-4009 Automatic Washer (Nanjing, China). The centrifugation was performed on a Neofuge 18R Centrifuge (Hong Kong, China). The results of TSA-ELISA were validated with an Aglient 1260 HPLC equipped with fluorescence detector (Wilmington, DE, USA).

Preparation of Monoclonal Antibody

The anti-AFB_1 McAb was prepared according to the classic hybridoma technology (Wang et al. 2011). Firstly, BALB/c mice (6 weeks old) were immunized with the immunogen by intraperitoneal injection. The mouse showing the highest anti-AFB_1 activity was selected to be spleen donors. Secondly, the hybridoma cells were acquired by fusion of the spleen cells isolated from the selected mouse with SP2/0 myeloma cells. Hybridoma cells secreting highly specific and sensitive antibodies were selected with the limiting dilution method and expanded. Finally, the selected clones were used for McAb production by ascite growth. The ascites fluids were collected and purified by saturated ammonium sulfate precipitation (Kong et al. 2017b), and purified McAb was identified subtype and stored at − 20 °C.

Procedures of Immunoassays

Microplates were coated with the coating antigen of AFB_1 (100 μL/well, in CBS) overnight at 4 °C. The plates were washed five times with PBST and blocked by incubating with 1% OVA in PBS (200 μL/well) for 45 min at 37 °C. After another washing step, either the samples or the standard serial concentrations of AFB_1 in PBS containing methanol (50 μL/well) were added, followed by addition of the optimal McAb dilution (50 μL/well, in PBS) together for 1 h at 37 °C. Following a further washing, the GAM-IgG-HRP dilution (100 μL/well, in PBS) was dispensed into each well and incubated for 1 h at 37 °C. Then, the plates were washed again.

For ELISA The TMB solution (100 μL/well) was added to the plates. Then, the reaction was stopped with 2 mol/L sulfuric acid (50 μL/well) after 15 min at 37 °C of incubation, and the absorbance was measured at 450 nm.

For TSA-ELISA The schematic diagram of the TSA-ELISA procedures for determination of AFB_1 is shown in Fig. 1. The T-B solution dilution (100 μL/well, in PB-H_2O_2 buffer) was dispensed and incubated for 15 min at 37 °C. Following another signal amplification reaction of BSA system, the peroxidase activity was revealed and the absorbance was measured.

Standard Curves A series of AFB_1 standards were prepared by diluting the AFB_1 standards in PBS containing methanol.

Determinations were carried out in triplicate, and the mean values of B/B_0 (B: the absorbance signal with analytes; B_0: the absorbance signal absence of analytes) were plotted against the logarithm of the analyte concentration to obtain the competitive curves. The half-maximal inhibition concentration (IC_{50}) and limit of detection (LOD, IC_{10}) were obtained from a four-parameter logistic equation of the sigmoidal curves using Origin Pro 7.0 software.

Optimization of Experimental Parameters The experimental parameters (concentrations of the coating antigens, McAb, GAM-IgG-HRP, T-B solution, SA-HRP and ionic strength, contents of organic solvent and pH) were studied to improve the sensitivity of immunoassays. The solutions with series concentrations of analytes and varied experimental parameters were tested. The B_0/IC_{50} ratio and the IC_{50} were used as primary criterions to evaluate immunoassay performances; the highest ratio of B_0/IC_{50} and the lowest of IC_{50} were the most desirable.

Cross-Reactivities The specificity of the TSA-ELISA was evaluated by testing the cross-reactivity (CR) of antibodies with the analogues of AFB_1 under optimized conditions. The CR values were calculated as follows:

$$CR\% = (IC_{50} \text{of analyte}/IC_{50} \text{of analogue}) \times 100$$

Analysis of Spiked Samples by the TSA-ELISA

The edible oil samples that had been certified as free of AFB_1 were used for the matrix effect and recovery studies. The edible oil samples (5 g) were spiked with AFB_1 at 1, 2, 5, and 10 ng/g and stored at room temperature for 2 h to allow drug-matrix interaction. Next, the extraction solution (20 mL of methanol-PBS, v/v, 3:2, and 20 mL of n-hexane) was added. The tubes were shaken with a vortex mixer for 20 min and then let stand for 30 min. The below solution were filtered and then diluted and adjusted to the optimized working solution condition prior to TSA-ELISA.

Each analysis was performed in triplicate. The recoveries and relative standard deviations (RSDs) were conducted to evaluate the accuracy and precision of the TSA-ELISA system.

Analysis of Matrix Effects on the Sensitivity

The extracted edible oil samples were analyzed by a series of dilutions with PBS (containing 5% methanol). The matrix effects were determined by comparing AFB_1 standard curves prepared in matrix extract and those standard curves prepared in PBS solution of matrix free.

Evaluation of the TSA-ELISA with HPLC-FLD

To test the effectiveness of the developed TSA-ELISA, the edible oil samples were separately analyzed using the TSA-ELISA and HPLC-FLD. For HPLC-FLD, the samples were detected according to the national standard method of China (Ministry of Health of China 2008, 2016) with modifications. The edible oil samples extracted as described above were adjusted to about pH 6.0–7.0 and then cleaned up and concentrated through the AflaTest immunoaffinity columns (Vicam, USA). AFB_1 was eluted with 1 mL methanol and dried under nitrogen. After deriving with trifluoroacetic acid in *n*-hexane and drying under nitrogen, the residue was dissolved in mobile phase and filtered through a membrane filter (0.22 μm). Then, 100 μL supernatant was injected into the HPLC-FLD for analysis.

The HPLC-FLD was performed on an Eclipse XDB2-C18 column (250 mm × 4.6 mm × 5 μm) using a mixture of water, methanol, and acetonitrile (11:4:5, *v/v*) as the mobile phase at a flow rate of 1.0 mL/min at 35 °C. The excitation wavelength and detection wavelength were set as 355 and 430 nm, respectively.

Results and Discussion

Preparation and Identification of McAb

After the steps of immunization, cell fusion, hybridoma selection, and cloning, the hybridoma cell $4D_9$ was selected for subsequent McAb production and immunoassays. From determination with the Mouse Monoclonal Antibody Isotyping kit (Sigma, USA), the McAb belonged to the IgG3 subclass with a kappa light chain. The affinity constant of the McAb was 6.59×10^7 L/mol according to the methodology of Beatty et al. (1987). Based on the ELISA, the optimal IC_{50} value of the McAb was 0.245 ng/mL and the LOD value was 0.044 ng/mL. In addition, the CR of the McAb was negligible with analogues of AFB_1 (4.02% for AFM_1, 3.64% for AFG_1, 0.65% for AFB_2 and < 0.05% for AFG_2), which indicated the high specificity of the McAb for AFB_1.

Optimization of Immunoassay Conditions

As shown in Table 1, the parameters for ELISA and TSA-ELISA were optimized. The dilution multiple of coating antigen (4.2 mg/mL of initial concentration) and McAb (6.1 mg/mL of initial concentration) were firstly optimized base on the checkerboard titration (Sun et al. 2010; Kong et al. 2017c). The optimal dilution multiples of coating antigen were 1:12000 for ELISA and 1:17000 dilution for TSA-ELISA. The optimal dilution multiples of McAb were 1:6000 and 1:10000 for ELISA and TSA-ELISA, respectively. And

Table 1 Optimization of the parameters for immunoassays

Factors	ELISA	TSA-ELISA
Coating antigen	1:12000	1:17000
McAb	1:6000	1:10000
GAM-IgG-HRP	1:8000	1:12000
T-B solution	/	1:350
SA-HRP	/	1:38000
Methanol (*v/v*, %)	10	5
Na^+ (mol/L)	0.4	0.4
pH value	7.4	7.4

1:8000 and 1:12000 of GAM-IgG-HRP were used for ELISA and TSA-ELISA. The developed TSA-ELISA has shown less use of coating antigen, McAb, and GAM-IgG-HRP compared with ELISA, which accorded with the principle of more economical.

Organic solvent, ionic strength, and pH were investigated to optimize the immunoassays, and the optimal results are also summarized in the Table 1. The methanol was selected to improve solubility of analytes and evaluate its effect on the immunoassays. The values of B_0/IC_{50} tended to decrease with the increase of methanol, and the IC_{50} values showed drastic increase above 10 and 5% methanol for ELISA and TSA-ELISA, respectively. The change of Na^+ concentration from 0.1 to 0.6 mol/L influenced the immunoassays dramatically. The highest B_0/IC_{50} and lowest IC_{50} were all acquired at 0.4 mol/L Na^+ for ELISA and TSA-ELISA. In addition, the pH did not have a notable effect on the sensitivity of the immunoassays. On the basis of these results, 10 and 5% methanol were chosen as the optimal ELISA and TSA-ELISA, respectively. At the same time, 0.4 mol/L Na^+ and pH 7.4 were chosen as the optimal ELISA and TSA-ELISA.

Sensitivities

The calibration curves of AFB_1 by immunoassays were constructed under the optimum conditions. The graph between percent binding (% B/B_0) and the logarithm of concentration of AFB_1 (ng/mL) was plotted (Fig. 2). The ELISA for AFB_1 was shown to have an IC_{50} of 0.245 ng/mL, a LOD of 0.044 ng/mL, and a linear range (IC_{10}-IC_{90}) of 0.044–1.35 ng/mL. The TSA-ELISA showed higher sensitivity, with the LOD value, IC_{50} value, and linear range of 0.004, 0.039, and 0.004–0.414 ng/mL, respectively.

With the LOD of ELISA and TSA-ELISA below the MLs of AFB_1, the sensitivity of the developed immunoassays can meet the requirements for detecting AFB_1. Through the use of new labeled techniques and signal amplification strategies, the sensitivities of immunoassays could be improved, which were popular requirement and desirable in the detection of harmful substances. In this study, the sensitivity of ELISA has
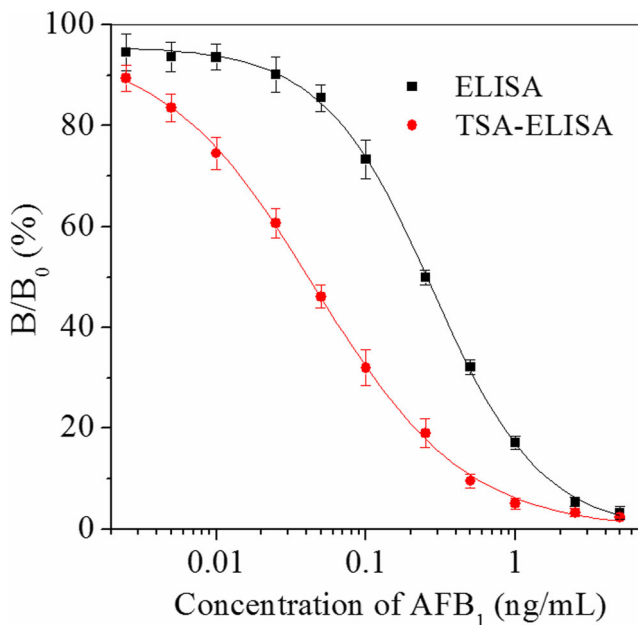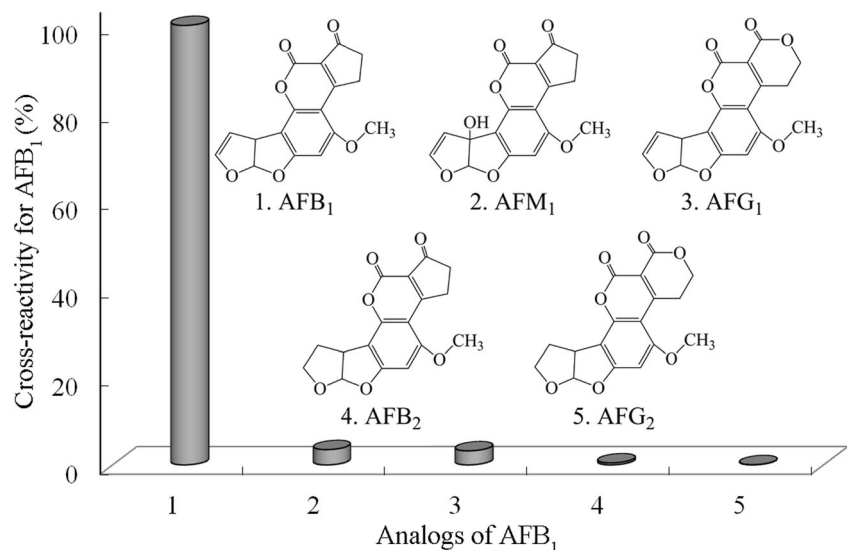
Fig. 2 Immunoassay calibration curves for AFB$_1$

displayed a significant improvement by coupling TSA system and BSA system, and the TSA-ELISA was developed for detecting AFB$_1$. Without special instruments and expensive reagents, the TSA-ELISA could conduct more sensitive determination of AFB$_1$ on the basis of ELISA after quick and easy procedure. More specifically, the developed TSA-ELISA for AFB$_1$ has an 11-fold improved LOD value and 6-fold improved IC$_{50}$ value when compared with ELISA. In comparison of the reported instrument-based detection methods and immunoassays for AFB$_1$, the developed TSA-ELISA possessed high performance on sensitivity, which should be feasible and worth of widely use. Therefore, the proposed TSA-ELISA format was selected for further research and application in detection of AFB$_1$ in edible oil samples.

Without the use of special instruments and expensive reagents, the higher sensitivity of TSA-ELISA could be achieved after adding two simple steps of reactions (about adding 30 min). It benefited from contributions of excellent signal amplification of TSA and BSA systems; the sensitivity of proposed TSA-ELISA showed significant improvement when compared with traditional ELISA. At the same time, the less use of antigen and antibody in TSA-ELISA than ELISA has caused the lower detectable concentration of AFB$_1$ and higher sensitivity. In addition, the TSA-ELISA helps avoiding the complicated sample pre-treatment process of chromatographic methods and reducing the potential harm and pollution for inspectors, because of its high sensitivity, so that AFB$_1$ extractions from spiked specimens can be diluted to a low concentration to be measured.

Specificity

The TSA-ELISA showed negligible CRs with analogues (Fig. 3). In some reports, the different levels of CRs had been found between AFB$_1$ and its analogues (Zhang et al. 2009; Xiao et al. 2011; Li et al. 2012). In this TSA-ELISA study, the CRs of AFB$_1$ for AFM$_1$, AFG$_1$, and AFB$_2$ were 3.48, 3.19, and 0.45%, respectively. Meanwhile, the CR was lower than 0.04% for AFG$_2$. This result was similar to the result of high specificity of the McAb, which obtained using ELISA for AFB$_1$. Therefore, the negligible CRs between AFB$_1$ and its analogues guaranteed the development of TSA-ELISA for specific determination AFB$_1$.

Matrix Effects

Matrix effects are one of the most common challenges in performing immunoassays on complex samples. In this study, the dilution method has been used to minimize the matrix effects of samples, which has been agreed as an easiest and
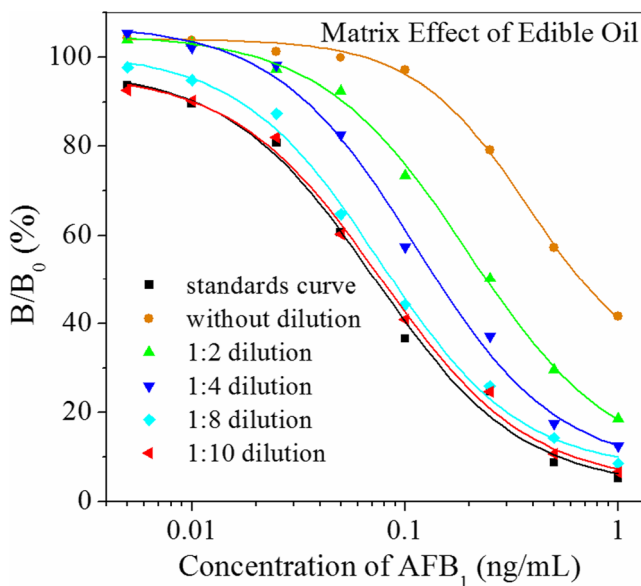
Fig. 3 Cross-reactivity of AFB$_1$ toward their analogues in TSA-ELISA

Fig. 4 The matrix effects of edible oil samples on the sensitivity of the TSA-ELISA for detecting AFB$_1$

Fig. 5 Correlation between the TSA-ELISA and the HPLC-FLD for detecting AFB$_1$ in edible oil samples ($n = 3$)

most immediate way. The matrix effect of edible oil samples on the sensitivity of the TSA-ELISA are shown in Fig. 4. With the increasing of the dilution multiples, the matrix effects on the sensitivity were reduced. The matrix interference could be negligible when the matrixes were 1:10 dilutions for edible oil samples. The schemes of dilution were applied for subsequent experiments.

Accuracy and Precision

As illustrated in Table 2, the recoveries of AFB$_1$ for TSA-ELISA ranged from 81.4 to 118.8% with the RSDs between 3.8 and 9.0%. These results indicated that the accuracy and precision of the developed TSA-ELISA were satisfactory for the qualitative and quantitative determinations of AFB$_1$ in edible oil samples.

Correlation of the TSA-ELISA and HPLC-FLD

The referential method of HPLC-FLD gave largely consistent results with the TSA-ELISA. And the results are presented in Fig. 5; good correlations between the TSA-ELISA (x) and HPLC-FLD (y) were obtained ($y = 0.8195x + 0.3235$, $R^2 = 0.9898$), which further demonstrated that the results of TSA-ELISA detection were reliable.

Analysis of Authentic Samples Using TSA-ELISA and HPLC-FLD

The naturally contaminated edible oil samples were analyzed using the proposed TSA-ELISA and confirmed by HPLC-FLD. The TSA-ELISA found that 11 out of 18 samples were tested AFB$_1$ positive, which ranged from 0.031

Table 2 Accuracy and precision of AFB$_1$ in edible oil samples by TSA-ELISA

Sample	Spiked (ng/g, $n = 3$)	Dilution times	Mean recovery ± SD (%)	RSD (%)
Luhua 5S peanut oil	1	10	94.5 ± 4.3	4.6
	2		109.5 ± 9.9	9.0
	5		85.6 ± 6.9	8.1
	10		118.1 ± 6.1	5.2
Arowana edible oil	1	10	83.6 ± 3.6	4.3
	2		100.2 ± 5.6	5.6
	5		96.6 ± 5.7	5.9
	10		105.5 ± 4.9	4.6
Bellina extra virgin olive oil	1	10	90.2 ± 7.3	8.1
	2		81.4 ± 3.1	3.8
	5		82.0 ± 4.3	5.2
	10		118.8 ± 5.9	5.0

Table 3 Analysis of AFB$_1$ in authentic samples by TSA-ELISA and HPLC-FLD

Sample	Mean ± SD (ng/g, $n = 3$)		Sample	Mean ± SD (ng/g, $n = 3$)	
	TSA-ELISA	HPLC-FLD		TSA-ELISA	HPLC-FLD
1	0.18 ± 0.017	0.182 ± 0.026	10	0.27 ± 0.054	0.31 ± 0.023
2	1.15 ± 0.149	1.24 ± 0.083	11	4.73 ± 0.377	4.32 ± 0.137
3	0.67 ± 0.130	0.64 ± 0.056	12	ND	ND
4	0.72 ± 0.035	0.81 ± 0.016	13	ND	ND
5	ND	ND	14	0.110 ± 0.013	0.104 ± 0.009
6	ND	ND	15	ND	ND
7	0.048 ± 0.005	0.052 ± 0.006	16	0.45 ± 0.044	0.48 ± 0.042
8	0.53 ± 0.039	0.51 ± 0.092	17	ND	ND
9	ND	ND	18	0.031 ± 0.008	0.027 ± 0.0006

ND not detected

to 4.73 ng/g (Table 3). The subsequent HPLC-FLD gave largely consistent results as with TSA-ELISA, where the positive results ranged from 0.027 to 4.32 ng/g. Therefore, the proposed TSA-ELISA demonstrated good practical applicability in authentic samples.

Conclusions

In summary, a sensitive TSA-ELISA for determination of AFB$_1$ was successfully developed by coupling TSA system and BSA system with ELISA. After adding two simple steps of reactions, the sensitivity of the TSA-ELISA has improved appreciably compared with ELISA. The accuracy and precision of the TSA-ELISA met the requirements of AFB$_1$ analysis. It is noteworthy that the study of edible oil samples were conducted both TSA-ELISA and HPLC-FLD to demonstrate the reliability of TSA-ELISA in AFB$_1$ assessment. Moreover, the developed TSA-ELISA was ideally suited as more economical (less use of antigen and antibody). The developed TSA-ELISA provided a sensitivity and economic method for large-scale screening and monitoring AFB$_1$ in edible oil samples. In the future studies, the TSA-ELISA should be expandable to assay more analytes and more different matrix samples; thus, the determination of harmful substances will be more sensitive, inexpensive, and alternative.

Compliance with Ethical Standards

All animals used in this study and animal experiments were approved by the Committee of Laboratory Animal Management of Jiangsu Province. The license number was SYXK (SU) 2007-0005.

Ethical Approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of Interest Yuanyuan Zhang declares that she has no conflict of interest. Ming Li declares that he has no conflict of interest. Yin Cui declares that she has no conflict of interest. Xia Hong declares that she has no conflict of interest. Daolin Du declares that he has no conflict of interest.

Informed Consent Not applicable.

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