Nanoscale

PAPER

View Article Online
View Journal | View Issue

Cite this: *Nanoscale*, 2016, **8**, 5245

A gold nanoparticle-based semi-quantitative and quantitative ultrasensitive paper sensor for the detection of twenty mycotoxins†

Dezhao Kong,[a,b] Liqiang Liu,[a,b] Shanshan Song,[a,b] Steven Suryoprabowo,[a,b] Aike Li,*[c] Hua Kuang,[a,b] Libing Wang[a,b] and Chuanlai Xu*[a,b]

Received 24th December 2015,
Accepted 4th February 2016

DOI: 10.1039/c5nr09171c

www.rsc.org/nanoscale

A semi-quantitative and quantitative multi-immunochromatographic (ICA) strip detection assay was developed for the simultaneous detection of twenty types of mycotoxins from five classes, including zearalenones (ZEAs), deoxynivalenols (DONs), T-2 toxins (T-2s), aflatoxins (AFs), and fumonisins (FBs), in cereal food samples. Sensitive and specific monoclonal antibodies were selected for this assay. The semi-quantitative results were obtained within 20 min by the naked eye, with visual limits of detection for ZEAs, DONs, T-2s, AFs and FBs of 0.1–0.5, 2.5–250, 0.5–1, 0.25–1 and 2.5–10 μg kg^{-1}, and cut-off values of 0.25–1, 5–500, 1–10, 0.5–2.5 and 5–25 μg kg^{-1}, respectively. The quantitative results were obtained using a hand-held strip scan reader, with the calculated limits of detection for ZEAs, DONs, T-2s, AFs and FBs of 0.04–0.17, 0.06–49, 0.15–0.22, 0.056–0.49 and 0.53–1.05 μg kg^{-1}, respectively. The analytical results of spiked samples were in accordance with the accurate content in the simultaneous detection analysis. This newly developed ICA strip assay is suitable for the on-site detection and rapid initial screening of mycotoxins in cereal samples, facilitating both semi-quantitative and quantitative determination.

1 Introduction

Mycotoxins are toxic secondary metabolites produced by specific fungi. To date, nearly 300 known types of mycotoxins have been identified and mainly cause grain contamination.[1] All crops may be infected by fungi during the production process, including field cultivation, storage and grain processing, leading to contamination by mycotoxins.[2–4] The most common harmful mycotoxins are aflatoxins (AFs), ochratoxin A (OTA), deoxynivalenol (DON), zearalenone (ZEA), T-2 toxin (T-2) and fumonisins (FBs), and the main toxigenic fungi isolated from food and feed are *Aspergillus*, *Penicillium* and *Fusarium*.[5] Due to their worldwide prevalence and pernicious effects, mycotoxins not only cause grain contamination leading to significant wastage, but are also carcinogens, teratogens and mutagens, therefore presenting a serious threat to human health and survival.[6–8] Consequently, strict national standards for the limits of mycotoxin levels are set by many countries to ensure food safety and the protection of human health.

Various detection techniques have been developed to monitor mycotoxins. The currently used instrumental methods are those with the highest sensitivity for the detection of mycotoxin contamination in food and feed, and include high-performance liquid chromatography (HPLC),[9] gas chromatography-mass spectrometry (GC-MS)[10,11] and high-performance liquid fluorescence detection (HPLC-FLD).[12] In addition to these expensive and time-consuming methods, which require complex pretreatment procedures, the rapid, specific and highly sensitive immunoassays are widely used for mycotoxin detection due to their simplicity and practicability. These methods include the enzyme-linked immunosorbent assay (ELISA),[13] chemiluminescent enzyme-linked immunosorbent assay (CL-ELISA),[14] time-resolved fluoroimmunoassay (TRFIA),[15] immunoaffinity columns (IAC),[16] immunochromatographic strips (ICA),[17] surface plasmon resonance[18] and other methods.[19,20]

As food and feed may be contaminated with multiple mycotoxins,[7,21] a single detection method is insufficient to meet the current requirements. The *Fusarium* toxins (B-trichothecenes which contain DONs, T-2 toxins, ZEAs, and FBs) are the most frequent and co-occur in contaminated feed samples. AFs are toxic substances and widely emerge in various crops world-

[a]*State Key Lab of Food Science and Technology, Jiangnan University, Wuxi, JiangSu 214122, People's Republic of China. E-mail: xcl@jiangnan.edu.cn; Tel: +0510-85329076*
[b]*International Joint Research Laboratory for Biointerface and Biodetection, and School of Food Science and Technology, Jiangnan University, Wuxi, JiangSu 214122, People's Republic of China*
[c]*Cereals & Oils Nutrition Research Group, Academy of Science & Technology of State Administration of Grain, Beijing 100037, People's Republic of China. E-mail: lak@chinagrain.org*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c5nr09171c

Table 1 Advances in the simultaneous detection of mycotoxins

Detection method	Target analyte	Mycotoxin kind	Limit of detection (μg kg^{-1})	Ref.
LC/APCI-TOF-MS	Fusarium mycotoxins and Aspergillus mycotoxins	13	0.1–6.1	Tanaka *et al.*[22]
FRET	FB and OTA	2	FB: 0.1; OTA: 0.02	Wu *et al.*[20]
Membrane-based immunoassay	AFB1 and OTA	2	AFB1: 2; OTA: 10	Saha *et al.*[42]
Immunochip	AFB1, AFM1, DON, OTA, T-2 and ZEA	6	AFB1: 0.04–1.69, AFM1: 0.45–3.90, DON: 20.20–69.23, OTA: 35.68–363.18, T-2: 0.11–1.81, and ZEA: 0.08–7.47	Wang *et al.*[23]
ICA	AFM1	1	4	Zhang *et al.*[29]
ICA	ZEA	1	1–50	Liu *et al.*[30]
ICA	ZEA and FB1	2	ZEA: 6; FB1: 50	Wang *et al.*[43]
ICA	DON	1	300	Liu *et al.*[31]
ICA	DON, FB1, ZEA, T-2, HT-2	5	DON: 1400; FB1: 3200; ZEA: 280; T-2/HT-2: 400	Lattanzio *et al.*[25]
ICA	AFB1, OTA and ZEA	3	AFB1: 0.25; OTA: 0.5; ZEA: 1	Li *et al.*[32]
ICA	AFs, ZEAs and DONs	3	AFB1: 1; ZEA: 50; DON: 60	Song *et al.*[44]

wide. Thus, the development of new methods for the simultaneous detection of multiple mycotoxins is urgently needed (Table 1). A liquid chromatography/time-of-flight mass spectrometry method (HPLC/APCI-TOF-MS) was successfully developed for the detection of 13 mycotoxins in one sample.[22] Immunochip was also effective for the detection of multiple mycotoxins.[23] The tandem immunoassay column and multiplex dipstick immunoassay have been used for the detection of multiple compounds.[24,25]

The rapid, simple, and low-cost ICA assay plays a vital role in the detection of analytes in many food analyses.[26–28] Especially in the detection of mycotoxins, many methods for the detection of single mycotoxins have been developed.[29–31] To date, with the development of the ICA method, some simultaneous detections of two or three types of mycotoxins have been achieved using ICA.[32]

In the present study, a multi-ICA assay based on the antibody–antigen reaction was developed for the rapid and simultaneous detection of mycotoxins. Twenty types of mycotoxins belonging to five classes were detected using this method with both semi-quantitative and quantitative results. Cereal samples were selected for the reliability compliance test. This technique facilitated the simultaneous semi-quantitative screening of each mycotoxin in cereals and the quantitative results were obtained using a hand-held strip scan reader to determine the color intensity of the strips.

2 Materials and methods

2.1 Reagents and instruments

ZEA (zearalenone), alpha-ZAL (alpha-zearalanol), beta-ZAL (beta-zearalanol), alpha-ZOL (alpha-zearalenol), beta-ZOL (beta-zearalenol), zearalanone, DON (deoxynivalenol), 3-AC-DON (3-acetyldeoxynivalenol), 15-AC-DON, NIV (nivalenol), T-2 Toxin, HT-2 Toxin, AFB1 (aflatoxin B1), AFB2, AFG1, AFG2, AFM1, FB1 (Fumonisin B1), FB2, and FB3 were obtained from J&K Scientific Ltd (Shanghai, China). Goat anti-mouse immunoglobulin (IgG) antibody was purchased from Jackson ImmunoResearch Laboratories (PA, USA), and bovine serum albumin (BSA) was from Sigma-Aldrich (St Louis, MO, USA). Other reagents and chemicals were obtained from the National Pharmaceutical Group Chemical Reagent Co., Ltd (Shanghai, China). Cereal samples were purchased from a local supermarket.

Nitrocellulose (NC) high flow-plus membranes (Pura-bind RP) were obtained from Whatman-Xinhua Filter Paper Co. (Hangzhou, China). The sample pad (glass fiber membrane, CB-SB08), polyvinylchloride backing material and absorption pad (SX18) were supplied by Goldbio Tech., Co. (Shanghai, China).

Conjugated coating antigens (ZEA-CMO-BSA, DON-BSA, T-2-HS-BSA, AFB1-CMO-BSA and FB1-GA-BSA) and specific monoclonal antibodies (anti-ZEA mAb, anti-DON mAb, anti-T-2 mAb, anti-AFB1 mAb and anti-FB1 mAb) were produced in our laboratory.

All buffer solutions were prepared with ultrapure water purified using the Milli-Q Synthesis system (Millipore Co., Bedford, MA, USA). AirJet Quanti 3000^{TM} and BioJet Quanti 3000^{TM} were used as the dispensers (XinqidianGene-Technology Co., Ltd, Beijing, China), and the strip cutting model CM 4000 was employed (Gene, Shanghai, China). The hand-held strip scan reader was provided by Huaan Magnech Bio-Tech Co., Ltd (Beijing, China).

2.2 Synthesis of coating antigens

ZEA was initially derived using carboxymethoxylamine (CMO) for conjugation with a protein and then ZEA-CMO-BSA was synthesized by the active ester method.[33,34] DON-BSA was synthesized using the previously reported carbodiimide technique with modifications.[35] For the T-2 toxin, the hydroxyl group was converted to carboxyl by initially reacting with succinic anhydride. Then T-2-HS (T-2-hemisuccinate) was conjugated to BSA using the active ester method.[36] AFB1 was also initially derived using CMO and conjugated with BSA using the active ester method similar to the method for ZEA synthesis.[37] FB1-GA-BSA was prepared using the glutaraldehyde method.[38]

All five antigens were characterized by the sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE) method.

2.3 Characterization of mAbs

An indirect competitive ELISA (Ic-ELISA) for the detection of each analyte was developed, which was similar to conventional protocols:[39] The 96-well microplate was coated (100 μL per well) with a coating antigen at 37 °C for 2 h, washed, and blocked with 2% gelatin in carbonate buffer (CB) at 37 °C for 2 h. After washing, 50 μL mAb and 50 μL analyte in 0.01 M PBS were added to each well, followed by incubation at 37 °C for 30 min. Next, 50 μL horseradish peroxidase (HRP)-labeled goat anti-mouse IgG was added to each well and incubated at 37 °C for 30 min. After three washes, 100 μL 3,3′,5,5′-tetramethylbenzidine (TMB) substrate was added and incubated at 37 °C for 15 min. The enzymatic reaction was terminated with 50 μL sulfuric acid (2 M) per well, and the results were read using a microplate reader at 450 nm.

The 50% inhibition concentration (IC_{50}) value of each mAbs was determined using Ic-ELISA, and the specificity was investigated by cross-reactivity (CR). CR values were calculated using the following formula:

$$\mathrm{CR}\,(\%) = (\mathrm{IC}_{50}\text{of analyte}/\mathrm{IC}_{50}\text{of competition analogue}) \times 100\%$$

2.4 Preparation of gold nanoparticle-labeled mAbs

Colloidal gold nanoparticles (GNPs) were prepared in our laboratory as described previously.[40] Briefly, 200 mL of 0.1 g L^{-1} chloroauric acid was boiled under constant stirring (100 g) and rapidly mixed with 8.0 mL of 1% trisodium citrate (w/v) at 300 °C. The mixture was stirred for 10 min until the color turned from yellow to wine-red. After boiling for a further 5 min, the solution was allowed to cool at room temperature under constant stirring and stored at 4 °C before use.

The conjugation steps were described in a recent report by our group.[41] Briefly, colloidal GNPs were adjusted to a concentration of 1 nM. Next, 10 mL GNP solution was obtained and the pH value was adjusted with 0.1 M K_2CO_3. Subsequently, purified mAbs in 400 μL 0.01 M PBS were added dropwise into the GNP solution and incubated at room temperature for 50 min. Then, 1 mL of 0.5% BSA (w/v) was added dropwise into the solution and stirred for 2 h to block GNPs and stabilize the labeled mAb. The solution was centrifuged twice at 7000*g* for 30 min each time to remove unconjugated mAbs and BSA. The resulting GNP-mAb precipitate was washed three times with 0.01 M PBS (containing 5% sucrose, 1% BSA, and 0.5% PEG 6000), and re-suspended in 1 mL of resuspending solution (0.01 M PBS containing 0.02% NaN_3, 1% BSA, 0.5% PEG 6000, and 5% sucrose) and stored at 4 °C.

The pH value of the colloidal GNP solution and the dosage of each purified mAb were optimized. Each conjugate was prepared in a similar manner under individually optimized conditions.

2.5 Preparation of the multi-ICA strip

The composition of the multi-ICA strip is shown in Fig. 1A. The NC membrane was fixed in the middle of the PVC backing card, and the sample and absorption pads were fixed on both ends of the PVC card with a 2 mm overlap. The sample pad was first immersed in 0.01 M PBS (containing 1% BSA and 0.2% Tween-20) and dried at room temperature for 4 h before use. Goat anti-mouse IgG was sprayed onto the NC membrane at 1 μL cm^{-1} with a membrane dispenser (XinqidianGene-Technology Co., Ltd, Beijing, China) and formed the control line (C line). Five different coating antigens were sprayed onto the NC membrane to form the five test lines (TL-1, 2, 3, 4, 5). The NC membrane was dried at 37 °C for 30 min and stored in a desiccator.

2.6 Principle of multi-ICA strip detection

The multi-ICA strip system was developed based on a competitive reaction between the free mycotoxin contained in the samples and the fixed coating antigens sprayed on the NC membrane binding to GNP-labeled mAbs.

For the sample assay, a 150 μL sample solution was mixed with 50 μL GNP-mAb solution and reacted at room temperature for 5 min to allow complete conjugation with the mycotoxin (if present). The mixed solution was then added to the sample pad of the strip, and migrated toward the absorption pad. The results were obtained after 15 min.

In the negative sample without mycotoxin, GNP-mAb bound to the coating antigens was fixed on the five T lines and goat anti-mouse IgG antibody was fixed on the C line, forming six red lines (Fig. 1B). In the positive sample which contained mycotoxins, GNP-mAb conjugated with the corresponding mycotoxin first, forming the GNP-mAb–mycotoxin complex, leading to lower GNP-mAb binding to the corresponding antigens fixed on the five T lines. Thus, the red lines formed on the five T lines were lighter in color. On increasing the mycotoxin concentration, the color of the T line disappeared (Fig. 1C). Therefore, the color intensity of the T line was inversely proportional to the concentration of mycotoxin in the sample.

The color intensities of the T lines were read with a handheld strip scan reader and the T/T_0 value (the ratio of optical density of the T line in the positive samples to that in the negative sample) was obtained. The concentration of each analyte in the samples was calculated based on the calibration curve (T/T_0 value as the ordinate and log(mycotoxin concentration) as the abscissa).

The C line should always emerge; otherwise, the procedure was incorrectly performed or the strip was poorly assembled.

2.7 Calibration curve, sensitivity and specificity of the multi-ICA strip in mycotoxin detection in cereal samples

The selected cereal was confirmed using HPLC and was proved to be a blank sample. The sample preparation steps were as follows: 5.0 g infant cereal was extracted with 25 mL methanol/water (70 : 30, v/v) containing 5 g NaCl, subjected to velocity

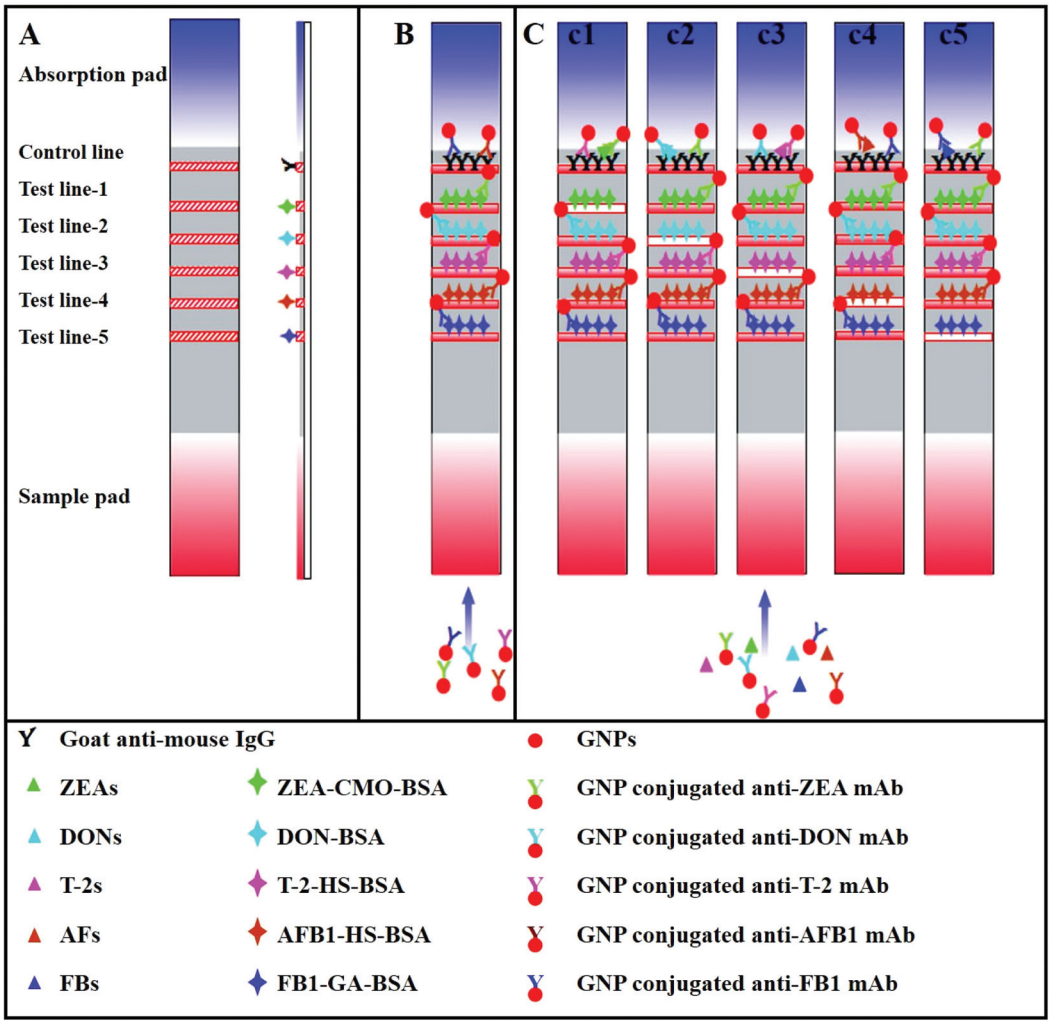

Fig. 1 (A) Composition of the multiplex immunochromatographic strip. (B) Strip detection with a negative sample. (C) Strip detection with a positive sample. c1, ZEAs contained only; c2, DONs contained only; c3, T-2s Toxin contained only; c4, AFs contained only; c5, FBs contained only.

mixing for 2 min, and filtered with quantitative filter paper. Next, 1 mL extract was diluted with 1 mL water and then a 150 μL sample solution was obtained as the matrix for detection.

The standard curve for each mycotoxin was established by spiking cereal samples with different concentrations of mycotoxins. The T/T_0 value of each concentration for each mycotoxin detected was obtained using a hand-held strip scan reader and used to produce a standard curve corresponding to each analyte.

The sensitivity of the multi-ICA strip was evaluated by determining a series of mycotoxins spiked in blank cereal samples. For semi-quantitative evaluation, the visual limit of detection (vLOD) value was defined as the lowest concentration which resulted in the test line color intensity being significantly weaker than that of the negative control line. The cut-off value was the threshold of concentration that resulted in the disappearance of the test line. For a quantitative evaluation, the calculated limit of detection (cLOD) was defined as the concentration with 10% inhibition (IC_{10}) of the signal (T/T_0) and the IC_{90} value was defined as the maximum detection value.

The specificity was determined by testing with other mycotoxins. All twenty types of mycotoxins (ZEA, alpha-ZAL, beta-ZAL, alpha-ZOL, beta-ZOL, zearalanone, DON, 3-AC-DON, 15-AC-DON, NIV, T-2 Toxin, HT-2 Toxin, AFB1, AFB2, AFG1, AFG2, AFM1, FB1, FB2, and FB3) were tested individually at a high concentration.

ZEA, DON, T-2, AFB1 and FB1 were chosen to represent the five classes of mycotoxins and were mixed for the simultaneous detection analysis. Each test was repeated ten times at each concentration.

3 Results and discussion

3.1 Characterization of coating antigens and mAbs

Coating antigens and mAbs were the main factors affecting the multi-ICA assay system.

Different coating antigens (ZEA-CMO-BSA, DON-BSA, T-2-HS-BSA, AFB1-CMO-BSA and FB1-GA-BSA) were characterized by SDS-PAGE, as shown in Fig. S1.† The offset of the position between coating antigens and the carrier protein confirmed the success of synthesis in each case. The conjugation rate was calculated using the Bio-gel image analysis system (Table S1†).

The IC_{50} and CR values of each mAb were determined using Ic-ELISA, as shown in Table S2.† There was no intersection between different classes of mycotoxins, and the chemical structures of the different mycotoxins are shown in Fig. S2.†

3.2 Preparation of GNP-labeled mAbs

GNPs with a negative charge on the surface can quickly and steadily adsorb positively charged polymer materials and remain biologically active. GNPs with a diameter of 15 nm were selected for further experiments in this study.

The conjugation capacity of GNPs to proteins depends on the pH value of the reaction system, which affects the net protein charge. The optimal pH value for conjugation was between 7.0 and 9.0, which was slightly higher than the isoelectric point (PI) of the mAb, making it easier to conjugate with GNPs and more stable. K_2CO_3 (0.1 M) was used to optimize the pH value of the reaction system. Under optimal conditions, the dose of 0.1 M K_2CO_3 for each conjugated system (10 mL) was 60, 60, 40, 40, and 40 μL for anti-ZEA, anti-DON, anti-T-2, anti-AFB1 and anti-FB1 mAb, respectively. The final pH value for each conjugated system (10 mL) was 9.0, 9.0, 8.5, 8.5 and 8.5 for anti-ZEA, anti-DON, anti-T-2, anti-AFB1 and anti-FB1 mAb, respectively.

The mAb dose also influenced the preparation of conjugates. The minimum amount of mAb required to form a stable conjugate was optimal for application. A lower dose of mAb would lead to a lower sensitivity of the GNP-mAb, and a higher dose was wasteful and may even cause conjugate aggregation and seriously affect the conjugation activity. The optimal dose of mAb for each conjugated system (10 mL) was 100, 80, 100, 80 and 100 μg for anti-ZEA, anti-DON, anti-T-2, anti-AFB1 and anti-FB1 mAb, respectively. Transmission electron microscopy (TEM) images of GNPs and GNP-mAb, UV-Vis spectroscopy, and the dynamic light scattering (DLS) diameter are shown in Fig. S3.†

3.3 Optimization of the multi-ICA strip

As the sensitivity of the multi-ICA strips was dependent on the color intensities of the T and C lines, the performance of the strips was mainly affected by the concentrations of GNP-mAbs and coating antigens (containing goat anti-mouse IgG antibody).

GNP-mAb solutions for each mAb type under individually optimized conditions were mixed at optimal volumetric proportions of 5, 7, 10, 7 and 10 μL for anti-ZEA, anti-DON, anti-T-2, anti-AFB1 and anti-FB1 mAbs, and these mixtures were finally adjusted to 50 μL with a resuspending solution.

For the test lines, the optimal concentration for each coating antigen was 0.5, 1, 0.5, 1 and 0.3 mg mL^{-1} for ZEA-CMO-BSA, DON-BSA, T-2-HS-BSA, AFB1-CMO-BSA and FB1-GA-BSA, respectively. The goat anti-mouse IgG antibody concentration was 0.5 mg mL^{-1}.

The color intensity of the C and T lines was confirmed in each test with negative samples, which resulted in stable detection conditions and a reliable calibration curve for the semi-quantitative and quantitative assays.

3.4 Analytical characteristics of the multi-ICA strip

Spiked cereal samples were used to eliminate matrix interference.

All twenty types of mycotoxins at different concentrations were tested using the multi-ICA strip. Each test was repeated ten times.

The results of each analyte are shown in Fig. 2. For a semi-quantitative evaluation, the vLOD value and cut-off value for each mycotoxin in the individual tests were 0.25, 0.25, 0.1, 1, 1, 0.5 and 0.5, 0.5, 0.25, 2.5, 2.5, 1 μg kg^{-1} for ZEAs (ZEA, alpha-ZAL, beta-ZAL, alpha-ZOL, beta-ZOL and zearalanone, Fig. 2A); 10, 2.5, 50, 250 μg kg^{-1} and 50, 5, 250, 500 μg kg^{-1} for DONs (DON, 3-AC-DON, 15-AC-DON and NIV, Fig. 2B); 0.5, 1 μg kg^{-1} and 1, 10 μg kg^{-1} for T-2s (T-2 Toxin and HT-2 Toxin, Fig. 2C); 0.25, 0.5, 0.5, 1, 0.25 μg kg^{-1} and 0.5, 1, 1, 2.5, 0.5 μg kg^{-1} for AFs (AFB1, AFB2, AFG1, AFG2 and AFM1, Fig. 2D); 2.5, 10, 2.5 μg kg^{-1} and 5, 25, 5 μg kg^{-1} for FBs (FB1, FB2 and FB3, Fig. 2E), respectively. Standard curves for each mycotoxin in the individual tests were developed and are shown in Fig. 3. For a quantitative evaluation, the cLOD value of each mycotoxin in the individual tests was 0.04, 0.05, 0.06, 0.17, 0.16, 0.13 μg kg^{-1} for ZEAs (ZEA, alpha-ZAL, beta-ZAL, alpha-ZOL, beta-ZOL and zearalanone); 2.43, 0.06, 11.4, 49 μg kg^{-1} for DONs (DON, 3-AC-DON, 15-AC-DON and NIV); 0.15, 0.22 μg kg^{-1} for T-2s (T-2 Toxin and HT-2 Toxin); 0.056, 0.14, 0.14, 0.49, 0.035 μg kg^{-1} for AFs (AFB1, AFB2, AFG1, AFG2 and AFM1); 0.53, 1.05, 0.53 μg kg^{-1} for FBs (FB1, FB2 and FB3), respectively.

The results in Fig. 2 also indicated that each T line only detected the corresponding mycotoxins. There was no interference between other classes of mycotoxins even at a high concentration of the interferent based on the specificity of the mAbs.

For the simultaneous detection analysis based on the multi-ICA strips, cereal samples were spiked with different concentrations of each mycotoxin and the results are shown in Fig. 4.

The results shown in Fig. 4A indicated that even in the simultaneous detection analysis of different mycotoxins from different classes, the developed multi-ICA strips still had a high sensitivity and specificity. The semi-quantitative detection results are shown in Table 2, the multi-ICA strip sensor performed well based on the analysis of the vLOD values and cut-off values of 0.25, 10, 0.5, 0.25, 2.5 μg kg^{-1} and 0.5, 50, 1, 0.5, 5 μg kg^{-1} for ZEA, DON, T-2 Toxin, AFB1 and FB1, respectively, similar to the results in the individual tests.

The T/T_0 values of each test line in the simultaneous detection analysis of the five different mycotoxins are shown in

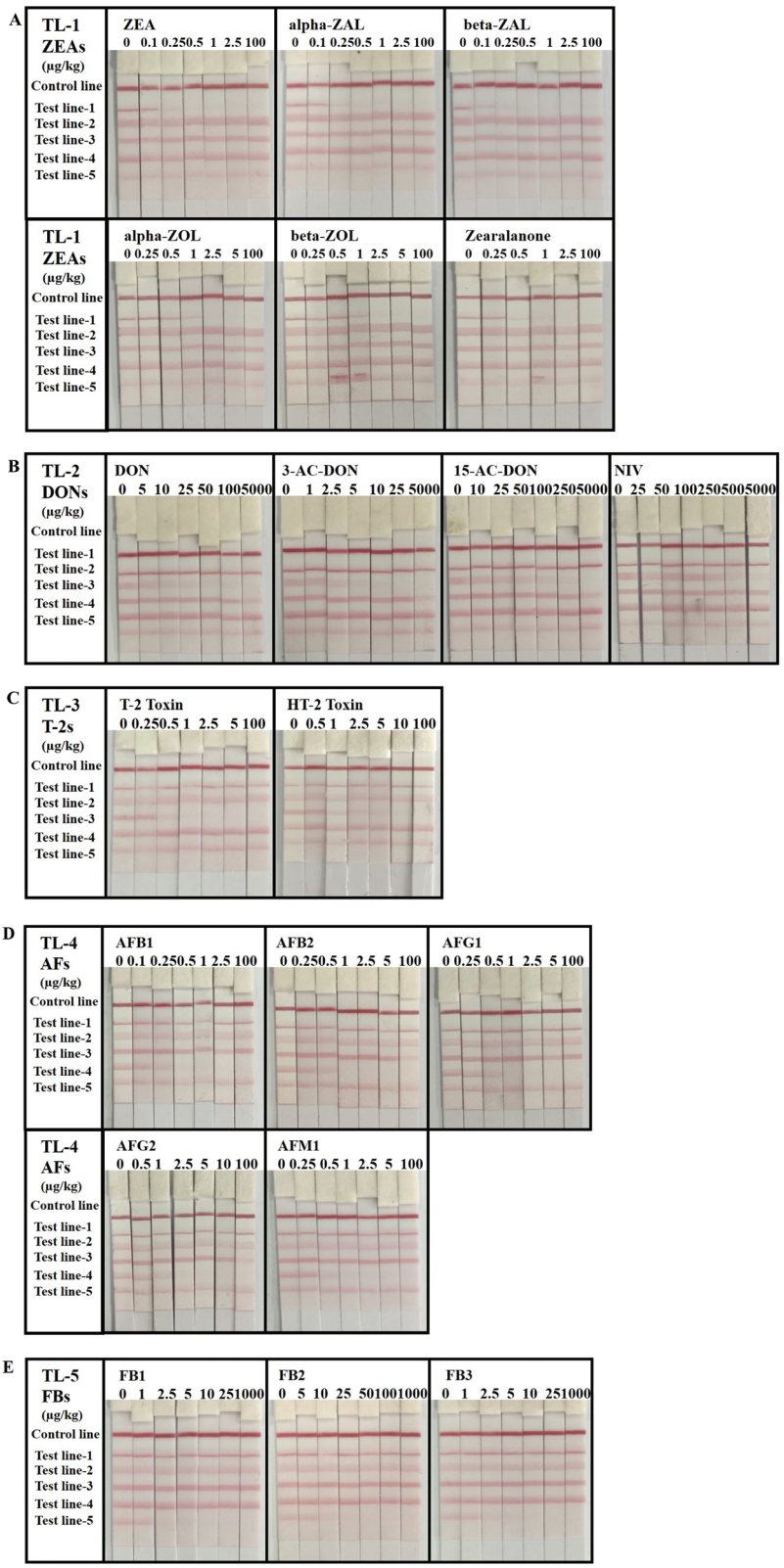

Fig. 2 The individual test of twenty types of mycotoxins belonging to five classes with multi-ICA assay. (A) ZEAs: ZEA, alpha-ZAL, beta-ZAL, alpha-ZOL, beta-ZOL and zearalanone; (B) DONs: DON, 3-AC-DON, 15-AC-DON and NIV; (C) T-2s: T-2 Toxin and HT-2 Toxin; (D) AFs: AFB1, AFB2, AFG1, AFG2 and AFM1; (E) FBs: FB1, FB2, FB3.

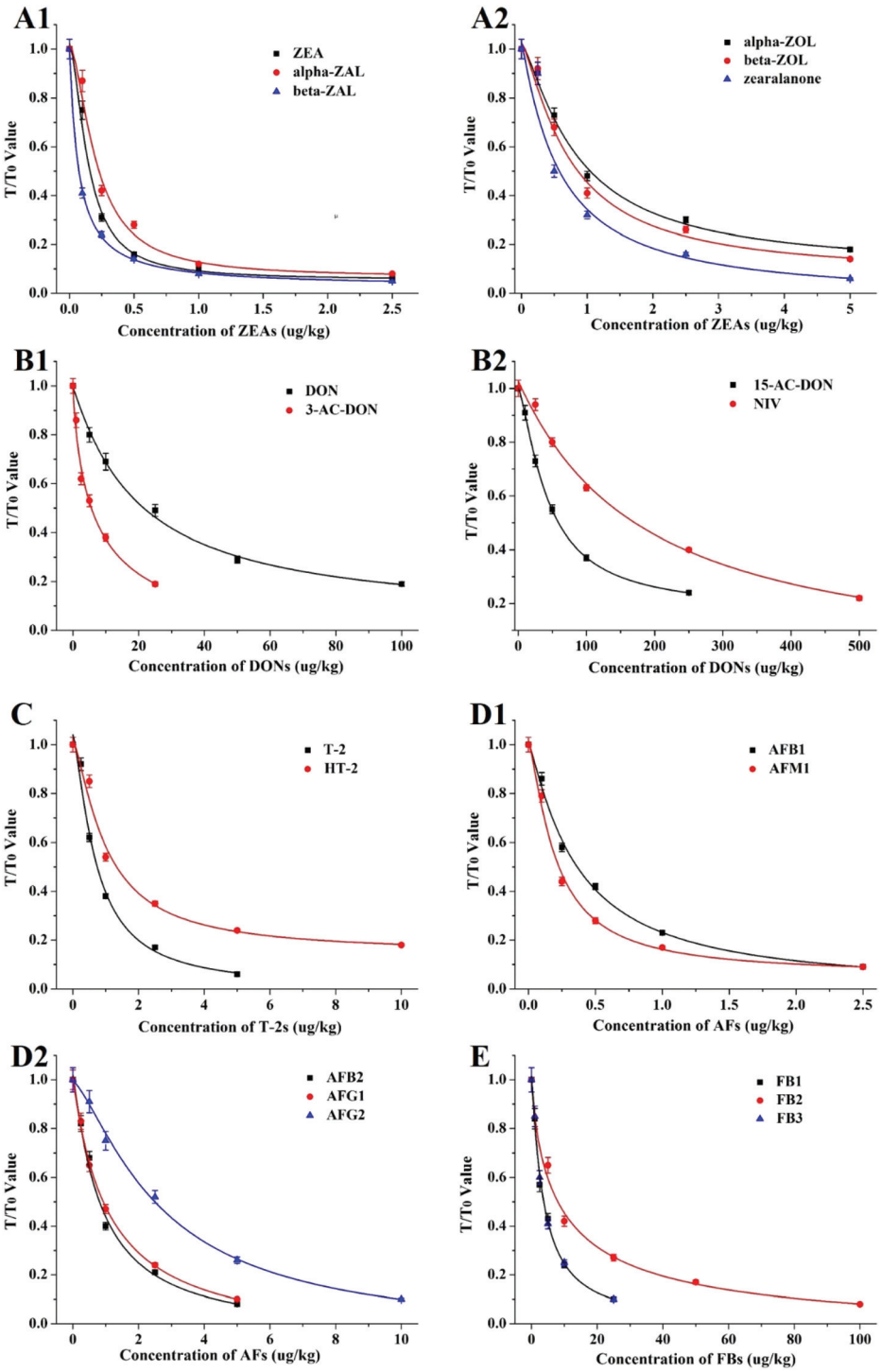

Fig. 3 The standard curve for each individual test of twenty types of mycotoxins with multi-ICA assay. (A1) ZEA, alpha-ZAL and beta-ZAL; (A2) alpha-ZOL, beta-ZOL and zearalanone; (B1) DON and 3-AC-DON; (B2) 15-AC-DON and NIV; (C) T-2 Toxin and HT-2 Toxin; (D1) AFB1 and AFM1; (D2) AFB2, AFG1 and AFG2; (E) FB1, FB2 and FB3.

Fig. 4B. The quantitative detection and recovery validation experiments were based on these T/T_0 values. Each mycotoxin was tested at three different spiked concentrations within a linear range using the multi-ICA strip. The results are shown in Table 3 and indicated good stability and application value with recoveries ranging from 79.8% to 120% coincident with the spiked data.

Therefore, this newly developed multi-ICA strip not only facilitated the detection of mycotoxins in both the semi-quantitative and quantitative methods in a short time period with

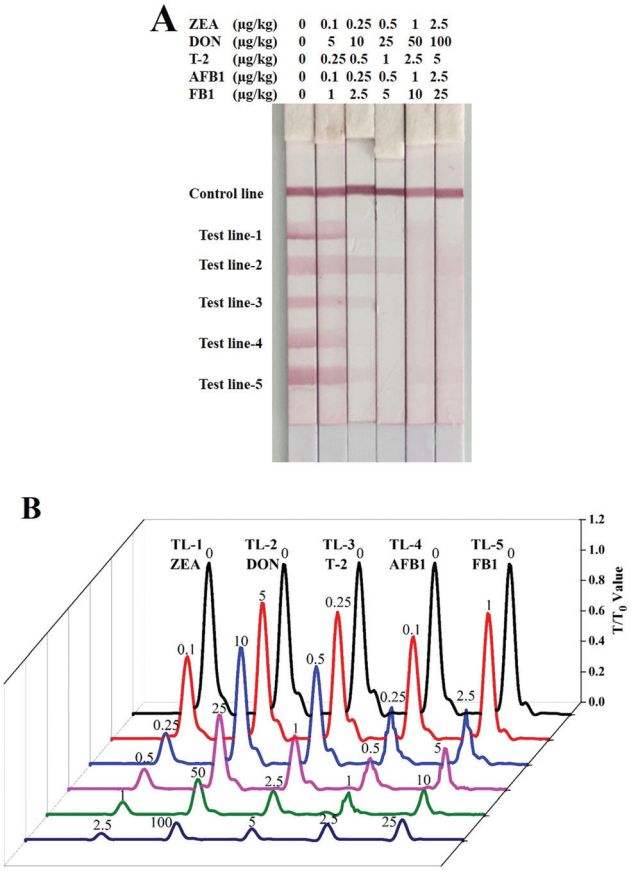

Fig. 4 (A) The simultaneous detection analysis based on the multi-ICA strips with five different mycotoxins; (B) the T/T_0 values of each of the test lines (TL-1,2,3,4,5) in simultaneous detection analysis with five different mycotoxins.

sensitive results, but can also be used for the detection of both individual mycotoxins and multiple mycotoxins within one sample.

Compared with other simultaneous detection methods, our system showed strong advantages with a simpler pretreatment process, higher sensitivity, and multiple mycotoxin detection. Our newly developed assay is suitable for field analysis and rapid monitoring of samples.

4. Conclusions

In this study, a multi-ICA strip system was developed, which simultaneously and sensitively detected twenty types of mycotoxins belonging to five classes and included ZEA, alpha-ZAL, beta-ZAL, alpha-ZOL, beta-ZOL, zearalanone, DON, 3-AC-DON, 15-AC-DON, NIV, T-2 Toxin, HT-2 Toxin, AFB1, AFB2, AFG1, AFG2, AFM1, FB1, FB2, and FB3. The whole detection process took 20 min in total, and had the advantages of simplicity, rapidity, sensitivity, specificity, cost-effectiveness and being non-instrumental. The test results of the spiked samples showed that both the semi-quantitative and quantitative results were reliable. In addition, stable results were obtained for both the detection of individual and multiple mycotoxins. This method was successfully used for the detection of mycotoxins in cereal samples.

Acknowledgements

This work is financially supported by the Key Programs from MOST (2012YQ090194), and grants from the Natural Science

Table 2 The semi-quantitative detection results in the simultaneous detection by a multi-ICA strip. (n = 10, the results were judged by four different individuals)

	Semi-quantitative detection results by visual (n = 10)				
ZEA/DON/T-2/AFB1/FB1 Concentration (μg kg^{-1})	T Line-1	T Line-2	T Line-3	T Line-4	T Line-5
0/0/0/0/0	−[a]	−	−	−	−
0.1/5/0.25/0.1/1	±[b]	−	−	±	−
0.25/10/0.5/0.25/2.5	±	±	±	±	±
0.5/25/1.0/0.5/5	+[c]	±	+	+	+
1/50/2.5/1/10	+	+	+	+	+
2.5/100/5/2.5/25	+	+	+	+	+

[a] Negative result. The test line is obviously observed. [b] Weakly positive result. A light test line is observed. [c] Positive result. No test line is observed.

Table 3 The quantitative results and recovery analysis of the spiked samples in the simultaneous detection by a multi-ICA strip

	Quantitative detection results by using a strip scan reader (n = 10)				
ZEA/DON/T-2/AFB1/FB1 concentration (μg kg^{-1})	T Line-1	T Line-2	T Line-3	T Line-4	T Line-5
0/0/0/0/0	ND[a]	ND	ND	ND	ND
0.2/10/0.4/0.1/2	0.16 ± 0.011[b]	7.98 ± 0.61	0.39 ± 0.033	0.12 ± 0.009	1.97 ± 0.11
0.4/20/0.8/0.2/4	0.39 ± 0.016	22.77 ± 1.21	0.88 ± 0.047	0.24 ± 0.031	4.21 ± 0.19
0.8/40/1.6/0.4/8	0.68 ± 0.044	43.5 ± 0.71	1.32 ± 0.13	0.46 ± 0.062	8.4 ± 0.68

[a] Not detected. [b] Mean value ± SD.

Foundation of Jiangsu Province, MOF and MOE (BK20150138, BK20140003, CSE11N1310, 201513006, BE2013613, BE2013611).

References

1 E. M. Binder, L. M. Tan, L. J. Chin, J. Handl and J. Richard, *Anim. Feed Sci. Technol.*, 2007, **137**, 265–282.
2 K. R. N. Reddy, B. Salleh, B. Saad, H. K. Abbas, C. A. Abel and W. T. Shier, *Toxin Rev.*, 2010, **29**, 137–137.
3 W. L. Bryden, *Anim. Feed Sci. Technol.*, 2012, **173**, 134–158.
4 J. W. Park, E. K. Kim, D. H. Shon and Y. B. Kim, *Food Addit. Contam.*, 2002, **19**, 1073–1080.
5 M. Kokkonen, M. Jestoi and A. Rizzo, *Int. J. Food Microbiol.*, 2005, **99**, 207–214.
6 E. E. Creppy, *Toxicol. Lett.*, 2002, **127**, 19–28.
7 A. C. Mak, S. J. Osterfeld, H. Yu, S. X. Wang, R. W. Davis, O. A. Jejelowo and N. Pourmand, *Biosens. Bioelectron.*, 2010, **25**, 1635–1639.
8 R. Ghali, K. Hmaissia-Khlifa, H. Ghorbel, K. Maaroufi and A. Hedili, *Food Control*, 2008, **19**, 921–924.
9 W. S. Khayoon, B. Saad, C. B. Yan, N. H. Hashim, A. S. M. Ali, M. I. Salleh and B. Salleh, *Food Chem.*, 2010, **118**, 882–886.
10 B. Warth, A. Parich, C. Bueschl, D. Schoefbeck, N. K. N. Neumann, B. Kluger, K. Schuster, R. Krska, G. Adam, M. Lemmens and R. Schuhmacher, *Metabolomics*, 2015, **11**, 722–738.
11 S. C. Cunha and J. O. Fernandes, *Food Chem. Toxicol.*, 2012, **50**, 1019–1026.
12 A. Visconti, V. M. T. Lattanzio, M. Pascale and M. Haidukowski, *J. Chromatogr. A*, 2005, **1075**, 151–158.
13 I. Kaniou-Grigoriadou, A. Eleftheriadou, T. Mouratidou and P. Katikou, *Food Control*, 2005, **16**, 257–261.
14 M. M. Vdovenko, C. C. Lu, F. Y. Yu and I. Y. Sakharov, *Food Chem.*, 2014, **158**, 310–314.
15 Z. W. Zhang, D. Wang, J. Li, Q. Zhang and P. W. Li, *Anal. Methods*, 2015, **7**, 2822–2829.
16 M. Uchigashima, M. Saigusa, H. Yamashita, S. Miyake, K. Fujita, M. Nakajima and M. Nishijima, *J. Agric. Food Chem.*, 2009, **57**, 8728–8734.
17 A. Y. Kolosova, S. De Saeger, L. Sibanda, R. Verheijen and C. Van Peteghem, *Anal. Bioanal. Chem.*, 2007, **389**, 2103–2107.
18 D. Dorokhin, W. Haasnoot, M. C. R. Franssen, H. Zuilhof and M. W. F. Nielen, *Anal. Bioanal. Chem.*, 2011, **400**, 3005–3011.
19 A. Pal, D. Acharya, D. Saha and T. K. Dhar, *Anal. Chem.*, 2004, **76**, 4237–4240.
20 S. J. Wu, N. Duan, X. Y. Ma, Y. Xia, H. G. Wang, Z. P. Wang and Q. Zhang, *Anal. Chem.*, 2012, **84**, 6263–6270.
21 J. W. Bennett and M. Klich, *Clin. Microbiol. Rev.*, 2003, **16**, 497–516.
22 H. Tanaka, M. Takino, Y. Sugita-Konishi and T. Tanaka, *Rapid Commun. Mass Spectrom.*, 2006, **20**, 1422–1428.
23 Y. Wang, N. Liu, B. A. Ning, M. Liu, Z. Lv, Z. Y. Sun, Y. Peng, C. C. Chen, J. W. Li and Z. X. Gao, *Biosens. Bioelectron.*, 2012, **34**, 44–50.
24 I. Y. Goryacheva, S. De Saeger, B. Delmulle, M. Lobeau, S. A. Eremin, I. Barna-Vetro and C. Van Peteghem, *Anal. Chim. Acta*, 2007, **590**, 118–124.
25 V. M. T. Lattanzio, N. Nivarlet, V. Lippolis, S. Della Gatta, A. C. Huet, P. Delahaut, B. Granier and A. Visconti, *Anal. Chim. Acta*, 2012, **718**, 99–108.
26 N. Xu, L. Xu, W. Ma, L. Liu, H. Kuang and C. Xu, *Food Agric. Immunol.*, 2015, **26**, 635–644.
27 W. Wang, M. Feng, D. Kong, L. Liu, S. Song and C. Xu, *Food Agric. Immunol.*, 2015, **26**, 738–745.
28 B. B. Dzantiev, N. A. Byzova, A. E. Urusov and A. V. Zherdev, *TrAC, Trends Anal. Chem.*, 2014, **55**, 81–93.
29 D. H. Zhang, P. W. Li, Q. Zhang, Y. Yang, W. Zhang, D. Guan and X. X. Ding, *Anal. Methods*, 2012, **4**, 3307–3313.
30 G. Liu, Z. Han, D. Nie, J. H. Yang, Z. H. Zhao, J. B. Zhang, H. P. Li, Y. C. Liao, S. Q. Song, S. De Saeger and A. B. Wu, *Food Control*, 2012, **27**, 200–205.
31 J. Liu, S. Zanardi, S. Powers and M. Suman, *Food Control*, 2012, **26**, 88–91.
32 X. Li, P. W. Li, Q. Zhang, R. Li, W. Zhang, Z. W. Zhang, X. X. Ding and X. Q. Tang, *Biosens. Bioelectron.*, 2013, **49**, 426–432.
33 D. Thouvenot and R. F. Morfin, *Appl. Environ. Microbiol.*, 1983, **45**, 16–23.
34 Z. K. Li, S. S. Song, L. G. Xu, H. Kuang, S. D. Guo and C. L. Xu, *Sensors*, 2013, **13**, 8155–8169.
35 C. M. Maragos and S. P. McCormick, *Food Agric. Immunol.*, 2000, **12**, 181–192.
36 H. Xiao, J. R. Clarke, R. R. Marquardt and A. A. Frohlich, *J. Agric. Food Chem.*, 1995, **43**, 2092–2097.
37 F. S. Chu and I. Ueno, *Appl. Environ. Microbiol.*, 1977, **33**, 1125–1128.
38 Y. Wang, C. H. He, H. Zheng and H. B. Zhang, *Int. J. Mol. Sci.*, 2012, **13**, 84–96.
39 C. F. Peng, Y. W. Chen, W. Chen, C. L. Xu, J. M. Kim and Z. Y. Jin, *Food Chem.*, 2008, **109**, 647–653.
40 F. X. Sun, L. Q. Liu, W. W. Ma, C. L. Xu, L. B. Wang and H. Kuang, *Int. J. Food Sci. Technol.*, 2012, **47**, 1505–1510.
41 M. Feng, D. Z. Kong, W. B. Wang, L. Q. Liu, S. S. Song and C. L. Xu, *Sensors*, 2015, **15**, 4291–4301.
42 D. Saha, D. Acharya, D. Roy, D. Shrestha and T. K. Dhar, *Anal. Chim. Acta*, 2007, **584**, 343–349.
43 Y. K. Wang, Y. B. Shi, Q. Zou, J. H. Sun, Z. F. Chen, H. A. Wang, S. Q. Li and Y. X. Yan, *Food Control*, 2013, **31**, 180–188.
44 S. Q. Song, N. Liu, Z. Y. Zhao, E. N. Ediage, S. L. Wu, C. P. Sun, S. De Saeger and A. B. Wu, *Anal. Chem.*, 2014, **86**, 4995–5001.