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Full Length Article

Simultaneously Testing Deoxynivalenol, T2 Toxin and Ochratoxin A in Wheat with a Colloidal Gold Test Strip and Matrix Solid-Phase Dispersion Cleaning

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Abstract

Deoxynivalenol (DON, also called as Vomitoxin), T2 toxin and Ochratoxin A (OTA) are three fungal toxins commonly found in corn or wheat. Chromatography and Mass spectrometer are main analysis methods for testing these mycotoxins. But these methods are time consuming, difficult to operate, and high cost on human resources and economy. Immune Colloidal Gold Assay (ICGA) is a rapid testing method suitable for on-spot testing. Unfortunately, the main reason for ICGA difficult to test these three toxins simultaneously is the optimization of the testing condition and sample preparation procedures. In this study, by utilizing three different coating antigens with different concentration, three test lines were set (T_1, T_2, T_3), for detecting DON, T2 toxin and OTA, respectively. The Test Line disappears when the concentration reach 1 mg/kg (for DON), 1 mg/kg (for T2) and 5 μg/kg (for OTA), separately. We also developed a sample preparation method by using MSPD for extraction and cleaning of the three toxins above for the simultaneously ICGA. That makes it easier for detecting toxins in wheat and for assurance of the food that can meet official limit of residue restrictions of China. The method could obviously improve the people's food safety and human health in real practice of agro-products' processing and purchasing. © 2017 Friends Science Publishers

Keywords: Deoxynivalenol; T2 toxin; Ochratoxin A; Colloidal gold; Immunoassay; Matrix solid-phase dispersion cleaning

Introduction

Deoxynivalenol (DON, also called as Vomitoxin) is one of mycotoxins produced by *Fusarium* (*F. graminearum* and *F. culmorum*). It is very common in the wheats affected by fungals, together with the other two mycotoxins T2 toxin (T2) and Ochratoxin A (OTA) (Nesic *et al.*, 2014). DON could affect the mammals' breeding ratio and growth rate. T2 and OTA are also two mycotoxins produced by *Fusarium*, *Aspergillus* and *Penicillium* in crops such as wheat and maize. The former mainly affects blood, liver, kidney, pancreas muscle and lymph cell function, the latter two mainly affect animals' liver and kidney, and has teratogenic effects (Sobrova *et al.*, 2010). The wheats, affected by fungal, always contained the three toxins which exceeded the Maximum Limit of Residues (MRL) of China or other countries. They may cause not only the health damage to consumers but also economic loss (Pestka and Smolinski, 2005). In China, the MRL of DON, T2 toxin and OTA are 1 mg/kg, 1 mg/kg and 5 μg/kg, respectively (USDA Foreign Agricultural Service, 2014).

Now, the mainstream of testing methods for the three toxins are Thin Layer Chromatography (TLC) (Gimeno, 1979), High Performance Liquid Chromatography (HPLC) (Frisvad and Thrane, 1987; Kong *et al*., 2014), LC-MS/MS (Lattanzio *et al*., 2011) and UPLC-MS/MS (Arroyo-Manzanares *et al*., 2015) etc. Though, TLC method is easy to operate, it is not cost-effective, is time consuming and has low sensitivity. HPLC, GC and their tender MS methods are the main stream testing methods with good accuracy and sensitivity. However, these methods need expensive instruments and skillful operators. Thus, they were not easy to carry out in China, especially in rural areas. In recent years, the immunoassays including ELISA (Sinhar *et al*., 1995; Zhang *et al*., 2011) and Immune Colloidal Gold Assay (ICGA) (Xu *et al*., 2010) with low cost, high sensitivity and rapidity for operators have been developed. However, the immunoassays of testing multi-targets simultaneously need to be developed (Martos *et al*., 2013; Lattanzio and Nivarlet, 2017). The major obstacle in this direction is however, difficulty of preparing samples to unify for different targets in immunoassays. Recently, the new sample preparation method of Matrix Solid-Phase Dispersion (MSPD) has opened a window for us to solve this problem (Li *et al*., 2016; Martínez-Domínguez *et al*., 2016).

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In the present work, we developed a sample preparation method by using MSPD for extraction and cleaning of the three toxins above in wheat samples for the ICGA by using the coated immobile antigen of the three toxins to draw three testing lines, for DON, T2 toxin and OTA immuno-assay. This method can fulfill the requirements of the stakeholders in wheat purchasing, storage and processing in order to reduce the economic losses and health risks for consumers.

Materials and Methods

Samples

Blank wheat samples (Zhongmai No. 9, harvested from experimental base of China Agricultural University) were generously granted by China Agriculture University. Four bunches of wheat samples (two bunches were Sumai No. 6, collected from south area of Jiangsu Province; two bunches were Xumai 27, collected from north area of Jiangsu Province) were provided by Agricultural Academy of Jiangsu Province.

Chemical Reagent

Methanol (AR grade), was from Shanghai Chemical reagents production. And other chemical reagents (AR grade), were purchased from Beijing Chemical Reagents Plant. Deionized water was prepared by MillQ (U.S.A). Bovine serum albumin (BSA) was produced by Siama Co. (U.S.A.). C18 came from Aijieer Co. (Tianjin, China).

Instruments

MK3 Microplate Reader: Labsystems, Finland. MS2 vortex mixer: Germany IKA production. Sprinkler and scraper machine, cutting machine and test paper related materials were purchased from the Shanghai Jinbiao Science and Technology Co., Ltd., China.

Methods

Preparation of antigens: DON toxins, T2 toxins and Ochratoxin haptens were purchased from Beijing Weikanghuihua Technology Co., Ltd. and Beijing Houshengzhengde Technology Co., Ltd. Preparation of coupling antigen (coated original) using activated ester method. 0.01 mmol toxin hapten, 0.021 mmol N-Hydroxysuccinimide (NHS, 2.41 mg) and 0.021 mmol N'-(ethylcarbonimidoyl)-N, N-dimethylpropane-1, 3-diamine monohydrochloride (EDC, 4 mg) were dissolved in 200 μL DMF. The reaction was stirred overnight and then added dropwise to a solution of 2 mL of 50 mM K_2HPO_4 (pH=9.3) with 10 mg bovine serum albumin (BSA). And the mixture reacted overnight at 4℃ and finally dialyzed by using PBS for three days to have the antigen. After the concentration measured, sodium azide was added in the solution as fresh keeping agent and stored at -20ºC.

Preparation of 30 nm colloidal gold particles: Gold nanoparticles with diameters of 30 nm were used in this experiment and synthesized (Ju *et al*., 2010). 2 mL 1% hydrochloric acid ($HAuCl_4$) solution was added into 200 mL of boiling ddH_2O quickly. After 1 min, 3.6 mL 1% trisodium citrate ($Na_3C_6H_5O_7$ $2H_2O$) was quickly added into the mixture. The mixed solution was continuously heated, with the color turning from yellow to black, blue, and then red. The whole process was about 15 min until the solution color was stable. Then the heat source was removed to make the solution cool naturally. Certain amount of water was added to fulfill volume of solution to 200 mL as original.

Determination of the optimum labeling pH: Eight centrifuge tubes were prepared, and added with colloidal gold solution 5 mL per tube. The pH value was adjusted with potassium carbonate (K_2CO_3, 0.1 M) to 3.1, 4.2, 5.0, 6.0, 7.2, 8.2, 9.0 and 10.2, respectively. The solution was divided into three 1 mL centrifuge tubes to study the optimum pH of DON, T2 toxin and OTA antibodies, respectively. After laid for 10 min at room temperature, 60 μL of the antibody with concentration of 0.2 mg/mL was added into each tube. After shaking, the mixture was laid at room temperature for 2 h. The color change was observed during the process. The minimum pH value for a stable color was selected as the optimum pH value (Park, 1989).

Amount of monoclonal antibody optimization: The three toxin antibodies involved in this study are monoclonal antibodies belonging to immunoglobulin IgG. The concentration of the protein was adjusted to 100 μg/mL to 30 μg/mL by UV spectrophotometry. After adding 1 mL K_2CO_3 (0.1 M) to adjust colloidal gold solution to pH 8.2, the final concentration of 1% ($m \cdot v^{-1}$) sodium chloride (NaCl) was added to each tube, vortexed and laid for 2 h and observed.

Preparation and purification of gold marked antibody: Gold marked antibodies were prepared and purified following Hu *et al.* (2010). K_2CO_3 (0.1 $mol \cdot L^{-1}$) was used to adjust the colloidal gold solution to pH 8.2. At room temperature, 180 μg of monoclonal antibody was dropwise added in under vigorous stirring. After stirring for 45 min, 2.5 mL of 5% ($m \cdot v^{-1}$) fetal bovine serum (BSA) was added and stirred for 5 min and centrifuged at 1500 rpm for 15 min at 4ºC. The supernatant was taken and washed with 0.45 μm sterile membrane filter. The filtered supernatant was centrifuged at 12,000 rpm for 30 min at 4ºC. The upper layer of supernatant was discarded and the precipitate obtained by centrifugation was washed with PBS containing 0.5% PEG 2000 (pH=7.2, 5 mM), then suspended and centrifuged again at speed of 12,000 rpm for 30 min at 4ºC. The precipitation was suspended by a borate buffer (pH=8.2) with 1% BSA ($m \cdot v^{-1}$) complex solution, and put into 4ºC refrigerator for storage.

Gold marked antibody, immobilized antigen, rabbit anti-mouse IgG spray: The antibody binding pad was treated by pretreat liquids (pH 7.4 PBS with 2% BSA, 2% sucrose, and 2.5% Tween-20 and 0.3% PVP-10). Then, the

Gold marked antibodies were fixed on binding pad (Oliver, 2010). The three antigens and rabbit anti-mouse IgG were drawn on the NC membrane, as three T-lines (T_1, T_2, T_3) and the control lines. The NC membranes were laid at 37ºC for 20–30 min. The criteria were that if the red band appears at the C line of the test strip, the detection is valid; if red bands appear as T_1, T_2, T_3 lines indicates that the test result is negative; if T_1 does not appear as red band, means DON toxin detection positive; if T_2 does not appear as red band, indicates that T_2 toxin test positive; T_3 does not appear as red band, means OTA detection positive. The disappearing of red C line indicates that the test is failure.

Fabrication of the immunological test strips: The test strip was constructed by two layers of absorbent glass fiber, one layer nitrocellulose membrane, filter paper and white plastic pad. The absorbent filter paper, NC film coated with a fixed antigen (as test line) and rabbit anti-mouse IgG (control line), glass fiber (gold marked antibody) were fixed on the white plastic plate from top to bottom in turn, and cut into strips as 0.4 cm×5 cm, sealed into the aluminum foil bag with desiccant, and laid at the 4ºC for storage (Chan *et al*., 2016).

Sample preparation and real sample testing: The sample preparation procedure is described as Fig. 1. Ten gram wheat sample were milled with 5 g C_{18} and 5 g $MgSO_4$ efficiently for 15 min, and then the mixture was transferred into a column, and washed by using PBS pH=7.4 and methanol with the volume of 5 mL, and after the solid phase dispersive extraction, the elution liquid was tested with immuno-assay strips.

Results

Preparation of Colloidal Gold Solution

The color of colloidal gold solution should be a transparent wine red. The maximum absorption wavelength was scanned by a full-wavelength grating spectrophotometer. It was found that there was a maximum absorption peak near 530 nm with a maximum OD of 1.2.

Determination of Optimum pH

From the experiment above, it was determined that the optimum pH was 8, which was similar to that of most of colloidal gold marked antibodies.

Determination of the Minimum Marker Protein

As operated with the conditions in Table 1, the protein amount was optimized. The effect shown in Fig. 2 indicates that the final experimental results determined that the minimum protein dosage is of 250 μL (0.125 μg). 50 mL of H_2O_2 (2.8 M) and 50 mL of $FeSO_4$ (0.10 M) were added simultaneously to flasks (500 mL) containing 200 mL of a sterile PVA solution (5.0%, $wt \cdot v^{-1}$) (Chan *et al*., 2016).

Binding Pad and Sample Pad Pretreatment

The pH 7.4 PBS with 2% BSA, 2% sucrose, and 2.5% Tween-20 and 0.3% PVP-10, was used as the binding pad and sample pad treatment solution.

Preparation of Colloidal Gold Test Paper for Immuno Chromatography

The preparation of the multi-residue colloidal gold test strip got two more test lines than the traditional colloidal gold test strip. The concentration of T_1, T_2 and T_3 antigen for drawing the three test lines was 0.03 mg/mL, 0.10 mg/mL and 0.02 mg/mL respectively, and the concentration for control line was 0.3 mg/mL.

Sample Testing

Sample toxin extraction and purification: In the experiment, three pieces of 5 g wheat (with adding 1 mg/kg DON, 1 mg/kg T2 and 5 μg/kg OTA, respectively) were used as real samples. Chloroform: ethyl acetate: methanol: water= 2: 1: 1: 1 were mixed as the sample extraction liquid. The samples were milled effectively, and added 10 mL of extraction agent, fully shaken 1 min, followed by 5000 rpm centrifugation for 3 min. Supernatant (2 mL) were taken and blown to dry with nitrogen. After that, 1 mL of the sample testing solution (methanol 20% dissolved in 1 mM PBS pH=7.4) were added for testing on strips.

The results of Fig. 3 showed that the prepared colloidal gold test strips can detect the wheat samples containing vomiting toxins, T2 toxins and OTA, as the same amount as national residual limit values (elimination line). Five minutes time is enough for giving the results. The method is simple, and can be used as on-site rapid detection. The results' stability test shows that visual observation results can be maintained for 20 min.

Actual sample detection: The actual sample detection was carried out (Fig. 4) by using the samples of Jiangsu (two samples, No. 1 and No. 2), Henan (two samples, No. 3 and No. 4) collected in June 2016 (provided by Jiangsu Academy of Agricultural Sciences). The results showed that one sample of Jiangsu and one sample of Henan had positive result of DON (No. 2, No. 4). ELISA kit (provided by Beijing Weikanghuihua Technology Co., Ltd. as a gift) was used for verification of the results. The data of testing result are shown in Table 2. It also can be observed that some parts of the wheat samples No. 2 and No. 4 with pink color, which showed a character of infection of *Fusarium graminearum*. It means that the result of colloidal gold test is consistent with ELISA kit and sensory analysis, indicating that the result is correct.

Discussion

In recent years, due to the global climate change, the

Table 1: Best antibody amount for AuNPs binding study

Characteristics	1	2	3	4	5	6
AuNPs (mL)	0.2	0.2	0.2	0.2	0.2	0.2
Antibodies (5 μg/mL)	0.05	0.10	0.15	0.20	0.25	0.30
10% NaCl (mL)	0.02	0.02	0.02	0.02	0.02	0.02

Table 2: Real Samples testing result with the method of colloidal gold test strip and ELISA kit

Name of mycotoxins	Testing Methods	Samples			
		No.1	No.2	No.3	No.4
DON	Colloidal Gold Test	-	+	-	+
	ELISA Kit (μg/kg)	ND	64.49	ND	78.77
T2-toxin	Colloidal Gold Test	-	-	-	-
	ELISA Kit (μg/kg)	ND	ND	ND	ND
OTA	Colloidal Gold Test	-	-	-	-
	ELISA Kit (μg/kg)	ND	ND	ND	ND

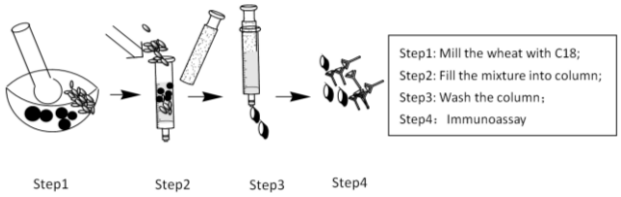

Fig. 1: Matrix solid-phase dispersion extraction and cleaning for Deoxynivalenol, T2 toxin and Ochratoxin A

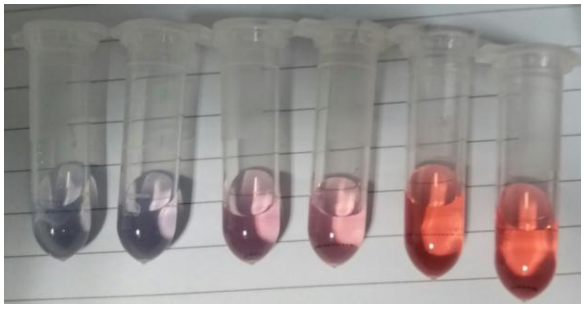

Fig. 2: Optimization of antibody amount for AuNPs binding study

endemic areas of the red mildew disease are changing constantly, and the risk of wheat-red mildew disease break out in our country's wheat cultivation area is growing higher and higher (Groves *et al*., 1999). Thus, the mycotoxins are becoming large potential threats as chemical hazards in peoples' main food necessitating new user friendly mycotoxin detection methods for scientists working in agriculture and food science (Bondy and Pestka, 2000).

This study used a series of mycotoxins specific antibodies to manufacture the fast testing kit to test the DON, T2 toxin and OTA, with a rapid sample pretreatment technique, to overcome the limitations of high cost, time consuming, and complexity for operation of instrument analysis. The detection results indicate that this method is reliable, rapid and sensitive with simple operation. Colloidal

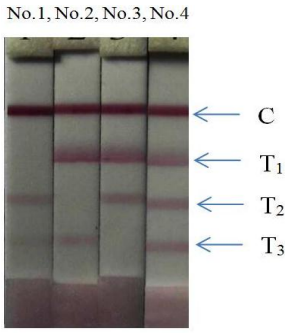

Fig. 3: Testing result for multi-residues of mycotoxins by ICGA

No.1 is the wheat sample with adding DON (1 mg/kg); No. 2 is the sample with adding T2 toxin (1 mg/kg); No. 3 is the sample with adding OTA (5 μg/kg); No. 4 is the blank sample

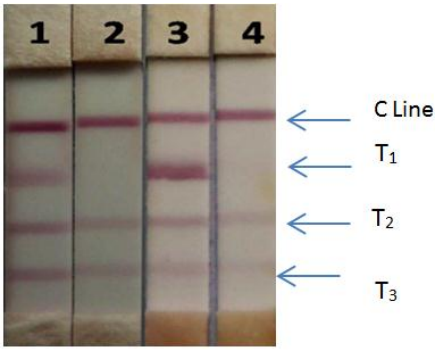

Fig. 4: Real sample testing result for multi-residue colloidal gold test strip

Samples of Jiangsu (2 samples, No. 1 and No. 2), Henan (2 samples, No. 3 and No. 4)

Gold Immuno chromatography is a unique immunological detection technique that appeared in the 1980s with characteristics of being simple, rapid and specific (Leuvering *et al*., 1980; Goryacheva, 2016a). The only limitation with this method was its use/application for determination of only one component of mycotoxin. The current modified method of preparing new multi-toxin detection strips reported here does not have the limitation mentioned above. The technology of ICGA can be applied with a reasonable reliability in the grain purchase, storage and transportation in a certain range (Goryacheva, 2016b). The new ICGA strips must be more popular and save more benefit and health for consumers (Liu *et al*., 2016).

Conclusion

The method developed in this study is user friendly for detecting toxins in wheat and for assurance of the food that can meet official limit of residue restrictions of China. The method could obviously improve the people's food safety and human health in real practice of agro-products' processing and purchasing.

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References

Arroyo-Manzanares, N., J.F. Huertas-Pérez and L. Gámiz-Gracia, 2015. Simple and efficient methodology to determine mycotoxins in cereal syrups. *Food Chem.*, 177: 274–279

Bondy, G.S. and J.J. Pestka, 2000. Immunomodulation by fungal toxins. *J. Toxicol. Environ. Hlth. Part B*, 3: 109–143

Chan, K.W., C. Lo, C.S. Chu, L.T. Chin, Y.T. Wang and W.C. Yang, 2016. Development of a colloidal gold-based immunochromatographic test strip for detection of cetacean myoglobin. *J. Vis. Exp.*, 113: e53433

Frisvad, J.C. and U. Thrane, 1987. Standardized high-performance liquid chromatography of 182 mycotoxins and other fungal metabolites based on alkylphenone retention indices and UV-VIS spectra (diodearray detection). *J. Chromatogr.*, 404: 195–214

Gimeno, A., 1979. Thin layer chromatographic determination of aflatoxins, ochratoxins, sterigmatocystin, zearalenone, citrinin, T-2 toxin, diacetoxyscirpenol, penicillic acid, patulin, and penitrem. *J. Assoc. Off. Anal. Chem.*, 62: 579–585

Goryacheva, I.Y., 2016a. Rapid tests progress through the years. *Compr. Anal. Chem.*, 72: 5–32

Goryacheva, I.Y., 2016b. Formats of Rapid Immunotests—Current-Day Formats, perspectives, pros and cons. *Compr. Anal. Chem.*, 72: 33–78

Groves, F.D., L. Zhang and Y.S. Chang, 1999. Fusarium mycotoxins in corn and corn products in a high-risk area for gastric cancer in shandong province, China. *J. AOAC Int.*, 82: 657–662

Hu, R., J.L. Cai and Jia-Yuan Xu, 2010. Preparation of HPV16 Immune Colloidal Gold Diagnostic Strip. *J. Chongqing Univ. Technol.*, 2: 34–39

Ju, Y., H.J. Hao, G.H. Xiong, H.R. Geng, Y.L. Zheng, J. Wang, Y. Cao, Y.H. Yang, X.H. Cai and Y.Q. Jiang, 2010. Development of colloidal gold-based immunochromatographic assay for rapid detection of *Streptococcus suis* serotype 2. *Vet. Immunol. Immunopathol.*, 133: 207–211

Kong, W., R. Wei and A.F. Logrieco, 2014. Occurrence of toxigenic fungi and determination of mycotoxins by HPLC-FLD in functional foods and spices in China markets. *Food Chem.*, 146: 320–326

Lattanzio, V.M., S.D. Gatta, M. Suman and A. Visconti, 2011. Development and in-house validation of a robust and sensitive solid-phase extraction liquid chromatography/tandem mass spectrometry method for the quantitative determination of aflatoxins B1, B2, G1, G2, ochratoxin A, deoxynivalenol, zearalenone, T-2 and HT-2 toxins in cereal-based foods. *Rapid. Commun. Mass Spectrom.*, 25: 1869–1880

Lattanzio, V.M. and N. Nivarlet, 2017. Multiplex Dipstick Immunoassay for Semiquantitative Determination of *Fusarium Mycotoxins* in Oat. *Oat: Methods Protocols*, 1536: 137–142

Leuvering, J.H., P.J.H.M. Thal, M.V.D. Waart and A.H.W.M. Schuurs, 1980. Soil particle immunoassay (SPIA). *J. Immunoassay*, 1: 77–91

Li, M., W. Kong, Y. Li, H. Liu, Q. Liu, X. Dou and M. Yang, 2016. High-throughput determination of multi-mycotoxins in Chinese yam and related products by ultra fast liquid chromatography coupled with tandem mass spectrometry after one-step extraction. *J. Chromatogr. B*, 1022: 118–125

Liu, B.H., K.C. Chu and F.Y. Yu, 2016. Novel monoclonal antibody-based sensitive enzyme-linked immunosorbent assay and rapid immune chromatographic strip for detecting aflatoxin M1 in milk. *Food Cont.*, 66: 1–7

Martínez-Domínguez, G., R. Romero-González, F.J. Arrebola and A.G. Frenich, 2016. Multi-class determination of pesticides and mycotoxins in isoflavones supplements obtained from soy by liquid chromatography coupled to Orbitrap high resolution mass spectrometry. *Food Cont.*, 59: 218–224

Martos, P.A., W. Thompson and G.J. Diaz, 2013. *Cheaper and more Efficient Method of Testing Grains for Multiple Fungal Toxins Simultaneously*. URL: http://hdl.handle.net/10214/6154

Nesic, K., S. Ivanovic and V. Nesic, 2014. Fusarial toxins: secondary metabolites of Fusarium fungi. *Rev. Environ. Contam. Toxicol.*, 228: 101–120

Oliver, C., 2010. Conjugation of colloidal gold to proteins. *Methods Mol. Biol.*, 588: 369-373

Park, K., 1989. Factors affecting efficiency of colloidal gold staining: pH-dependent stability of protein-gold conjugates. *Scanning Microscopy Supplement*, 3: 15

Pestka, J.J. and T.A. Smolinski, 2005. Deoxynivalenol: Toxicology and Potential Effects on Humans. *J. Toxicol. Environ. Hlth.* Part B, 8: 39–69

Sinhar, C.L., M.E. Savard and R. Lau, 1995. Production of monoclonal antibodies for the specific detection of deoxynivalenol and 15-acetyldeoxynivalenol by ELISA. *J. Agric. Food Chem.*, 43: 1740–1744

Sobrova, P., V. Adam, A. Vasatkova, M. Beklova, L. Zeman and R. Kizek, 2010. Deoxynivalenol and its toxicity. *Interdiscipl. Toxicol.*, 3: 94–99

USDA Foreign Agricultural Service, 2014. *China's Maximum Levels for Mycotoxins in Foods*. National Food Saf. Standard: GB 2761–2012

Xu, Y., Z.B. Huang and Q.H. He, 2010. Development of an immune chromatographic strip test for the rapid detection of deoxynivalenol in wheat and maize. *Food Chem.*, 119: 834–839

Zhang, A., Y. Ma and L. Feng, 2011. Development of a sensitive competitive indirect ELISA method for determination of ochratoxin A levels in cereals originating from Nanjing, China. *Food Cont.*, 22: 1723–1728

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