Determination of deoxynivalenol in grain sorghum by chemiluminescence enzyme immunoassay

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Abstract. A chemiluminescence enzyme-linked immunosorbent assay (CLEIA) with enhanced chemiluminescent was developed for the detection of deoxynivalenol (DON) in grain sorghum. Assay conditions, including concentrations of antibody and enzyme conjugate, Na^+ (NaCl), organic solvent **(**methyl alcohol**)** and pH were investigated. The optimized CLEIA allowed the DON detection in a linear working range of 0.588-26.783 ng mL^{-1} with the IC_{50} value of 3.968ng mL^{-1} and a limit of detection (LOD) of 0.311 ng mL^{-1}. The CLEIA was one time more sensitive than the colorimetric ELISA by using the same antibody and HRP-conjugate. A series of spiked grain sorghum were detected by the method. The recovery rate ranged from 71.68% -96.56%, and the coefficient variation (CV) ranged from 5.93% -13%.

Introduction

Deoxynivalenol (DON) is produced by certain *Fusarium fungu*s. It occurs more seriously in barley, wheat, corn, and oats but slightly in rye, sorghum and rice [1-3]. DON exhibited serious toxic effects to animals and human beings, such as feed refusal, weight loss, cardiotoxicity, teratogenicity, immunotoxicity and apoptosis in vitro without significant dose–effect relationship [1]. To protect human and animal safety, many countries established the limits of DON in cereals, the limit standard of DON in China is 1 mg kg^{-1} [4].

To control the contamination of DON in cereals, various analytical methods for the determination of DON in foods and feeds have been developed, which includes high performance liquid chromatography [5-7], and chromatography tandem mass spectrometry [8-9]. Although these methods are accurate and sensitive, they are time consuming and not suitable for rapid screening. The immunoassay assay was proved to be a good alternative method because of its simple, rapid, and sensitive characteristics with wide linear range. Then the immunoassay assay [10-14] for the detection of DON in food has been developed. This paper established luminol-hydrogen peroxide chemiluminescence system to detect DON in sorghum by indirect competitive chemiluminescent enzyme immunoassay.

Materials and methods

Reagents

Keyhole Limpet Hemocyani (KLH), DON and goat anti-mouse IgG-HRP was purchased from Sigama (St. Louis, USA). The anti-DON monoclonal antibody was obtained from our own laboratory. The chemiluminescence substrate solution was purchased from Helisence (Shanghai, China). Samples were purchased from a local market

Buffers and Solutions

0.01M phosphate-buffered solution (PBS, pH 7.4). 5% (w/v) skimmed milk solution. PBST, 0.01M phosphate-buffered solution (PBS, pH7.4) with 0.05% Tween-20 (v/v).

Apparatus

Luminoskan ascent (Thermo, USA), Wellwash versa (Thermo scientific), 96-well white polystyrene plates (Costar). Pipettes from Eppendorf Co, Ltd. were used in all experiments.

Incubator.

CLEIA procedure

The micro-plates were coated with 120μL of DON-KLH solution per well diluted in 0.01M PBS for 2.5 h at 37˚C. The plates were washed with PBST 5 times then blocked with 5% skimmed milk 320μL was added to each well for 3.5 h at 37˚C. After the plates have been washed 4 times, 50μL antibody dilution solution and 50μL DON standard solution or sample solution was added. The plate was incubated at 37˚C for 45 min then washed 5 times. Subsequently, 100μL 1:3000 (v/v) goat anti-mouse IgG-HRP dilution solution was added with incubation 45 min at 37˚C. At last, 100μL chemiluminescence substrate solution was added. The results were express in relative light units (RLU).

CLEIA optimization

The concentration of ionic strength, pH, and organic solvent **(**methyl alcohol**)** were optimized. Competitive curves were created using 0.001M PBS solutions containing series concentrations of Na^+ (NaCl), and pH values, methyl alcohol to evaluate the effects of ionic strength, pH, and the solvent, respectively.

Sample extraction and spiking

The DON standard was added to each sorghum sample (1 g), Then 5 mL of acetonitrile–H_2O (85: 15, v/v) added to sample for extraction. Then the mixture was votered 30 min and centrifuged at 10000 rpm for 15 min 4℃. Then, the supernatant was dyed under N_2 in 45˚C. After that the resident dissolved in super water.

Results and Discussion

Optimization of the antigen concentration and antibody dilution

Considered the RLU and, the optimization of antigen is 8μg L^{-1}.The antibody concentration was too low, led to the RLU was too small and all was blocked. So choose the dilution of antibody is from1:6000 to 1:48000. The CLEIA showed the highest RLU max/IC_{50} when the antibody dilution was 1:6000.

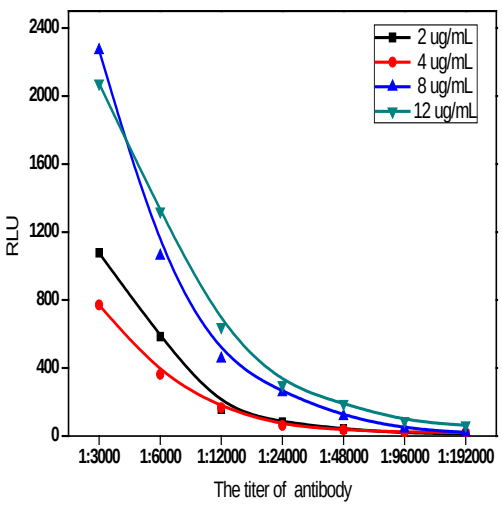

Fig.1. The concentration effect of DON-KLH on the DON immunoassay

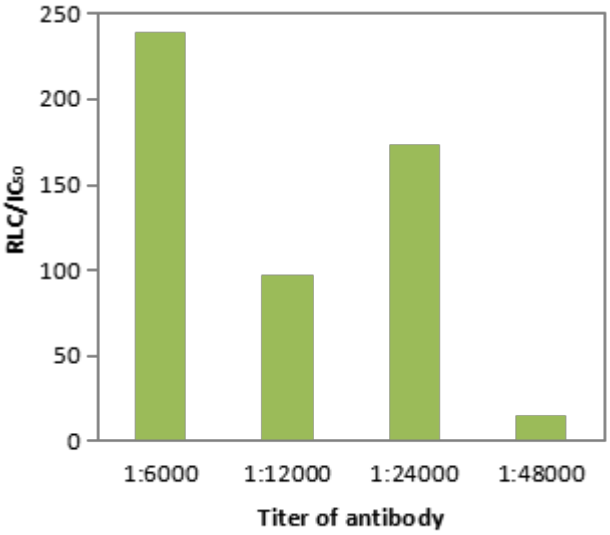

Fig.2 The effect of antibody dilution ratio on CLEIA

Optimization of CLEIA

The RLU_{max}/IC_{50} ratio was used as a parameter to judge the impact of factors. The Na^+ concentration was evaluated from 0.4 mol L^{-1} to 1.6 mol L^{-1}. The highest RLU $_{max}/IC_{50}$ was obtained at 1.2mol L-1 Na^+. The pH value was optimized from 4.4 to 8.4. At pH 6.4, the CLEIA showed the highest RLU $_{max}/IC_{50}$ and the lowest LOD. The methyl alcohol concentration was evaluated from 0% to 20%, affecting the CLEIA performance dramatically. The CLEIA showed the

highest RLU_{max}/IC_{50} when the methyl alcohol concentration was 0%. The result was showed in Table 1, the optimum parameters were1.2 mol L^{-1} Na^{+}, pH6.4 and 0% methyl alcohol for CLEIA.

Table 1. Effect of ionic strength, pH and organic solvent value on CLEIA

		CLEIA		
		$IC_{50}(\mu gL^{-1})$	RLU_{max}/IC_{50}	R^2
Na^{+}(mol L^{-1})	0.4	4.77	93	0.983
	0.8	4.69	103	0.992
	1.2	2.54	181	0.990
	1.6	5.30	141	0.991
pH value	5.4	3.39	223	0.967
	6.4	1.28	508	0.974
	7.4	2.01	380	0.971
	8.4	4.46	153	0.992
methyl alcohol(%,V/V)	0%	4.70	879	0.984
	5%	6.85	588	0.985
	10%	8.25	419	0.991
	15%	8.36	409	0.981
	20%	12.34	239	0.991

Establishment of the Standard Curve

The CLEIA was established under optimal conditions.Fig.3 shows the standard curve for DON immunoassay based on CLEIA. From the calibration curves, we can calculate the IC_{50} was 3. 968 ng mL^{-1} and IC_{10} was 0.311 ng mL^{-1}. The linear working range determined was 0.588-26.783 ng mL^{-1}.

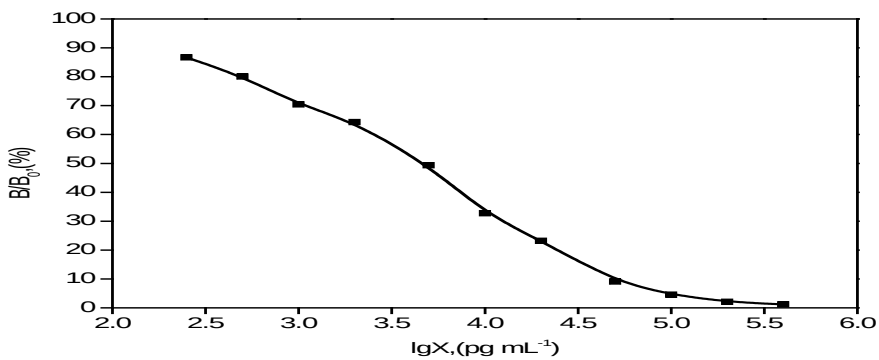

Fig. 3 Standard curve of CLEIA procedure for DON

Recovery test of Spiked Samples

Sorghum samples were spiked with DON at concentration of 120, 400 and 800 ng g^{-1} respectively, and then they were analyzed by using the optimized CLEIA. Mean recovery values ranged from 71.68%-96.56% with coefficient variation (CV) less than13 %. (Table. 2)

Table 2 The recovery of determination of DON in sorghum

Sample	Analyte	Spiked level (ng g^{-1})	Mean recovery (%)	CV(%)
Sorghum	DON	120	71.68	13
		400	96.56	7.88
		800	83.64	5.93

Conclusions

In this study, CLEIA method for the detection of DON in sorghum was established. It was performed with wide linear range and lower limit of detection. This developed CLEIA method

could be a suitable tool for rapid screening of DON in sorghum samples.

Acknowledgements

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