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A double-film screening card for rapid detection of organophosphate and carbamate pesticide residues by one step in vegetables and fruits

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ABSTRACT

A double-film screening card, composed of an enzyme (acetylcholinesterase, AChE) and its substrate (indoxyl acetate) films, was developed for simple and rapid screening of organophosphate and carbamate pesticide residues. On the card, indoxyl acetate is decomposed by AChE to produce a blue-green compound. Compared with the speed of inhibition of AChE activity by pesticides, The color developing speed is relatively slow. When a sample solution with pesticides is dropped in the test window, this reaction of AChE is efficiently and rapidly inhibited to a degree depending on the level of pesticide. Any remaining active AChE would hydrolyze indoxyl acetate to show color. Qualitative and quantitative detection of pesticide residues could be achieved based on the color intensity. The selected suitable carrier films containing the enzyme and substrate were hydrophilic materials that were capable of adsorbing and releasing the full doze of enzyme or substrate. The optimum materials for containing AChE and indoxyl acetate on the card were glass fiber RB65 and polyester fiber VL78, respectively. The optimum quantities of AChE and indoxyl acetate per film in the test window were 15 μL of 3500 U/mL and 10 μL of 100 mM, respectively. The maximum color intensity was reached at room temperature in 15 min. The limits of detection were up to 0.05 μg/mL for phoxim, 0.1 μg/mL for acephate, 0.5 μg/mL for malathion, 0.5 μg/mL for omethoate, 0.04 μg/mL for carbofuran and 0.09 μg/mL for aldicarb. The card showed good reproducibility and high sensitivity when applied to real food samples.

1. Introduction

Along with the social progress and the improvement of people's living standard, food safety has attracted more and more attention in the world. In agriculture, numerous agrichemicals are used to protect crops and seeds before and after harvesting (Mańkowski, Pudelko, Kolodziej, & Karas, 2015). Using pesticides also plays a major role in the improvement of agricultural production and in the control of many disease vectors in the area of public health in China (Zhang et al., 2016). But pesticides, chemicals designed to treat pests such as insects, have been associated with adverse food safety, ecological environment and human health (Liu et al., 2016; Schwartz, Glascoe, Torres, Ramos, & Delgado, 2015; Han, Currell, & Cao, 2016; Blair, Ritz, Wesseling, & Beane Freeman, 2015). Food, particularly vegetables and fruits, need to be tested for the presence of harmful pesticide residues before consumption. According to the Environmental Protection Agency, there are currently more than 865 registered pesticides (Wanwimolruk, Kanchanamayoon, Phopin, & Prachayasittiku, 2015). Many international organizations and countries have established maximum residue limits or tolerances to protect consumer's health and also the environment (Ambrus & Yang, 2016; Lozowicka et al., 2014; Skretteberg et al., 2015.

Recently, considerable progress has occurred in the analytical methods available for the determination of pesticide residues. (Wong et al., 2010; Fillâtre et al., 2011; Chen, Chen, Feng, & Li, 2009; Meng, Wei, Ren, Ren, & Tang, 2013). However, those methods for the determination of pesticide residues in food are mainly based on gas chromatography, liquid chromatography or enzyme-linked immunoassay (Hu et al., 2015; Sivaperumal, Anand, & Riddhi, 2015; Chen et al., 2015). Although these methods are sensitive and accurate, they have some disadvantages, such as the requirement for sophisticated equipment, skilled operators, and time-consuming sample preparation steps. Rapid, inexpensive, simple, and sensible techniques for pesticides have been developed, including nanoparticles (Zheng et al., 2015; López_Marzo et al., 2013; Alkasir, Ganesana, Won, & Stanciu, 2010), surface enhance raman scattering (Chen et al., 2016), silica-hydrogel hybrid

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microbeads (Wang et al., 2014). However, the operation of those methods are usually complicated and expensive, preventing their extended application in the routine screening and detection.

Recently, some methods based on visual determination of pesticide residues have been established. One most important issue is to enhance the color resolution so as to improve the accuracy. Previous result on visual acuity shows that the rot cells of human retina have a peak sensitivity to the blue-green (Hunt, 2004). Several methods were proposed based on the principle of AChE hydrolysis indoxyl acetate to a blue-green color, but these methods still need multiple-step operations. (Guo, Zhang, Cai, Shen, & Zhu, 2013; Han et al., 2012). Here, we proposed a simple and fast double-film rapid screening card for determination of pesticide residues by one step. The card was composed of two films, AChE and indoxyl acetate films. Compared with previous study, the films we choose are special hydrophilic films that are capable of adsorbing and releasing the full dise of enzyme or substrate. (Homaei, Sariri, Vianello, & Stevanato, 2013; Jesionowski, Zdarta, & Krajewska, 2014). When a sample solution with pesticides is dropped in the test window, the AChE is efficiently and rapidly inhibited to a degree depending on the level of pesticide. Any remaining active AChE would hydrolyze indoxyl acetate to show color. The amount of pesticide residue could be qualitatively or quantitatively measured based on the color intensity. We also optimized this system of card in sensitivity, stability, reproducibility, and used it in the detection of real food samples.

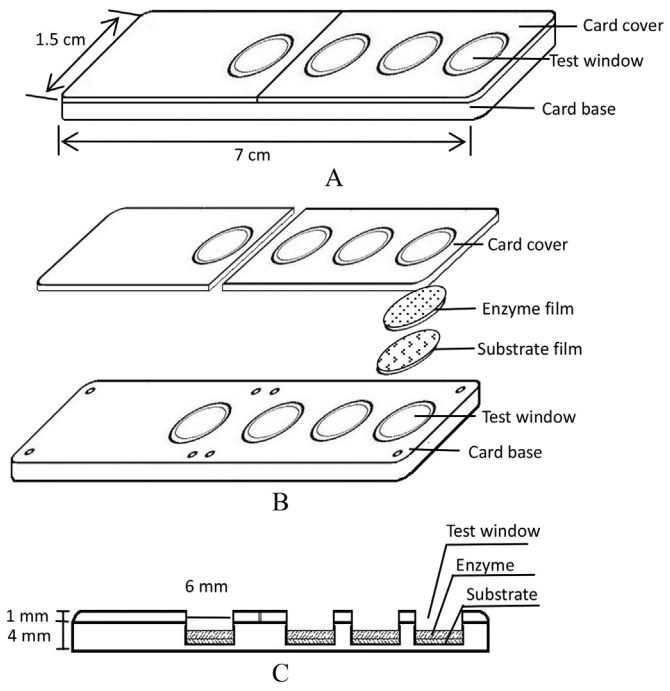

Fig. 1. Schematic diagram of the whole double-film visual screening card. (A) Outlook. (B) Decomposition diagram. (C) Longitudinal section.

2. Material and methods

2.1. Material

AChE from *Drosophila melanogaster* was purchased from Beibei biotech. Co., Ltd. (Zhengzhou, China). Indoxyl acetate, acetylthiocholine chloride, 5,5'-dithiobis-2-nitrobenzoic acid and other general chemicals were purchased from Sigma-Aldrich China (Shanghai, China). Polyester fiber (PF), glass fiber (GF) and absorbent paper (AP) were purchased from Shanghai Kinbio Tech. Co., Ltd (Shanghai, China). Test cards were made of acrylonitrile butadiene styrene plastic and customized as Fig. 1. Ultra-pure water was prepared using a Milli-Q reagent water system (Millipore, Billerica, MA).

Pesticide analytical standards were purchased from the National Information Center for Certified Reference Materials (Beijing, China). Individual pesticide stock solutions (100 μg/mL) were prepared in acetone and stored at −20 °C in the darkness. To prepare working solutions, the sample solutions were spiked to the desired concentrations. Cabbage, lettuce and apple samples were purchased from a local supermarket (Zhengzhou, China), and were pre-checked with Ellman's method to confirm the absence of the target compound, and stored in the dark at < 4 °C until required for analysis (Ellman, Courtney, Andres, & Featherstone, 1961).

2.2. Preparation of the card

GF, PF and AP (Table 1) were cut into small circle pieces (6 mm diameter) to fit the size of test window of the card. AChE was dissolved in phosphate buffer, and indoxyl acetate in methanol. Solutions with AChE and indoxyl acetate were sprayed on different film pieces. Three kinds of drying modes was used for enzyme film: air drying at 37 °C for 20 min, air drying at 4 °C for 2 h, and freeze-drying for 20 min. The substrate film was air dried at room temperature for 10 min.

2.3. Optimization of detection system

The recovery rates of AChE and substrate were used to select the optimum film materials for these substances. AChE activity was detected by Ellman's method (Ellman et al., 1961), while the amount of substrate was measured by spectrophotometry (Villatte, Bachman, Hussein, & Schmid, 2001). The optimum loading of AChE on the film was determined according to its recovery rate and inhibition, and the optimum substrate loading was determined according to the final color intensity of the test window. Cards were incubated at 4 °C, 25 °C, and 37 °C, and observed at 5, 10, 15, and

Table 1
Properties of different types of films.

Types	Weight (g/m^2)	Thickness (mm)	Absorbent quantity (g/m^2)	Absorbent speed (S/4 cm)
GF-KB50	33–48	0.20–0.28	200–400	60–160
GF-RB65	70–85	0.25–0.35	500–700	55–120
GF-CB08	75–110	0.32–0.48	550–800	>160
GF-SB06	55–75	0.24–0.36	400–600	75–160
PF-DL42	85–110	0.37–0.53	480–680	20–80
PF-VL78	70–95	0.18–0.26	170–270	10–40
PF-VL98	90–115	0.25–0.35	220–320	15–40
PF-KL43	65–80	0.3–0.4	340–450	90–125
AP-SX18	100–130	0.3–0.4	300–450	7–15