Biosensors and Bioelectronics 64 (2015) 633–638

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Disposable and reliable electrochemical magnetoimmunosensor for Fumonisins simplified determination in maize-based foodstuffs

Adrián Jodra, Miguel Ángel López, Alberto Escarpa *

Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering, University of Alcalá, Ctra. Madrid-Barcelona, Km. 33,600, E-28871 Alcalá de Henares, Madrid, Spain

ARTICLE INFO

Article history:
Received 3 July 2014
Received in revised form
3 September 2014
Accepted 22 September 2014
Available online 28 September 2014

Keywords:
Mycotoxin
Fumonisin
Magnetoimmunosensor
CSPE
Electrochemical detection

ABSTRACT

An electrochemical magnetoimmunosensor involving magnetic beads and disposable carbon screen-printed electrode (CSPE) for Fumonosins (FB1, FB2 and FB3) has been developed and evaluated through a certified reference material (CRM) and beer samples. Once the immunochemical reactions took place on the magnetic beads solution, they were confined on the surface of CSPE, where electrochemical detection is achieved through the addition of suitable substrate and mediator for enzymatic tracer (Horseradish peroxidase – HRP). A remarkable detection limit of 0.33 μg L^{-1}, outstanding repeatability and reproducibility ($RSD_{intraday}$ of 5.6% and 2.9%; $RSD_{interday}$ of 6.9% and 6.0%; both for 0 and 5 μg L^{-1} FB1 respectively), and excellent accuracy with recovery rate of 85–96% showed the suggested approach to be a very suitable screening tool for the analysis of Fumonisin B1 and B2 in food samples.

A simultaneous simplified calibration and analysis protocol allows a fast and reliable determination of Fumonisin in beer samples with recovery rate of 87–105%. This strategy enhanced the analytical merits of immunosensor approach towards truly disposable tools for food-safety monitoring.

1. Introduction

Nowadays, one of the most important areas in food analysis is the so called food safety. Usually defined as the area related to "*all those hazards, whether chronic or acute, that may make food injurious to the health of the consumer*" (Joint FAO/WHO, 2003) has produced a highly concern in contemporary society and research scene. Mycotoxins, secondary metabolites produced by different filamentous fungi, with a high impact related to health and economical aspects, are key analytes for monitoring in the food safety area. Included within this group, Fumonisins are a class of mycotoxins produced by certain species of *Fusaria* in foods under specific temperature and humidity conditions.

At least twenty eight different Fumonisins have been described and classified in three groups (A, B and C) based in structural similarities. Fumonisin B1 (FB1), Fumonisin B2 (FB2) and Fumonisin B3 (FB3) are the most common and dangerous analogs (Fig. S1). FB1 usually constitute about 70% of the total Fumonisins content found in naturally contaminated foods and feeds (Krska et al., 2007). Isolated in 1988 (Bezuidenhout et al., 1988), the Fumonisins have determined to cause leukoencephalomalacia in horses (Marasas et al., 1988), pulmonary edema in pigs (Harrison et al., 1990), nephrotoxicity and liver cancer in rats (Gelderblom et al., 1988). Also in humans, they can produce liver, kidney and esophageal cancer (Dyoshizawa et al., 1994; Scott, 2012; Sydenham et al., 1990) being considered as category 2B carcinogen by U.S. Environmental Protection Agency and International Agency for Research on Cancer (IARC).

Natural occurrence of Fumonisins has been mainly described in maize, maize-based food and feedstuffs. However, isolation of *Fusarium* species has been reported in other grains such as barley, wheat, rice, peanut, pistachio, as well as raisin, fig and other human foods like fruits and milk (Waskiewicz et al., 2012; Yang et al., 2012). Also beer, the oldest alcoholic beverage and the cereal-based product worldwide consumed, has been contaminated of Fumonisins (Rubert et al., 2013; Torres et al., 1998). Produced by brewing and fermentation of starches, mainly derived from cereals grains, such as barley, wheat, maize and rice; beer is a benefit beverage that can be part of a healthy diet. In spite of the fact that the main raw material for beer production is barley, brewing factories often replace a portion of barley by adjuncts, like maize grits, being a frequently way of Fumonisins contamination (Stewart, 1995). By other hand, beer is a complex matrix containing water, carbohydrates, protein substances, mineral salts and alcohol. The presence of alcohol as a fermentation sub-product can strongly influence the extraction of mycotoxins in this matrix.

Due to the adverse effects in animals and humans, the European Union has established maximum residue limits (MRLs)

* Corresponding author. Tel.: +34 91 885 49 95.
E-mail address: alberto.escarpa@uah.es (A. Escarpa).

http://dx.doi.org/10.1016/j.bios.2014.09.054
0956-5663/

of Fumonisins (as the sum of FB1 + FB2) in maize-derived foodstuff, ranging from 200 μg Kg^{-1} for baby food to 4000 μg Kg^{-1} for raw maize (Commission Regulation No. 856/2005, European Commission, 2005; Commission Regulation No. 1881/2006, European Commission, 2006; Commission Regulation No. 1126/2007, European Commission, 2007).

Analytical methods and research related to Fumonisins have been recently reviewed (Berthiller et al., 2014; Gelderblom and Marasas, 2012; Krska et al., 2007, 2008; Maragos and Busman, 2010; Prieto-Simón et al., 2007; Scott, 2012; Shephard et al., 2013; World Health Organization, 2006). Due to the absence of a good chromophore in the Fumonisin structure, most widely used methods for Fumonisins detection are based on LC–MS (Devreese et al., 2012; Ediage et al., 2012b; Li et al., 2012; Pietri and Bertuzzi, 2012; Shephard et al., 2011; Song et al., 2013; Yang and Wu, 2012), derivatisation with fluorescence labels (LC-FLD) (Abrunhosa et al., 2011; Kong et al., 2012; Muscarella et al., 2011; Ndube et al., 2011; Solfrizzo et al., 2011) and those usually involve in the interaction with selective binding materials (antibodies, aptamers or synthetic materials) (Berthiller et al., 2014; Goryacheva et al., 2009; Prieto-Simón and Campàs, 2009; Vidal et al., 2013). Over the last years, and compared to LC-FLD a pronounced shift towards the use of LC–MS, particularly in the context of multitoxin methods has occurred. These methods require expensive equipment, complicated and time-consuming clean-up procedures and skilled operators that make them unsuitable methods for monitoring purposes. Since contamination by Fumonisins can occur at any stage of the food chain (e.g., on field, at harvest, during storage and transportation) frequent analyzes are required to promptly detect any contamination, and reducing risks for the consumer. In this sense, immunoassays using a variety of formats (ELISAs, LFDs, biosensors) continue to be an active area of research. In recent years, even enzyme-linked immunosorbent assays for single Fumonisin analyte (Liu et al., 2013; Sheng et al., 2012; Wang et al., 2014) as well as multimycotoxin assays such as lateral flow devices (Lattanzio et al., 2012; Wang et al., 2013; Yan-Song et al., 2012) flow through immunoassay (Ediage et al., 2012a) membrane-based dot assay (He et al., 2012) chip-based microarray (Oswald et al., 2013; Wang et al., 2011) and enconding microespheras multiplexing assays (Czeh et al., 2012; Deng et al., 2013; Peters et al., 2013) have been widely used in agricultural and food-safety area. Biosensors and especially electrochemical biosensors have received special focus in modern analysis because of their technical simplicity, sensitivity, low cost and possibility of decentralization in field analysis. Excellent reviews are available on general biosensors (Campas et al., 2009; Logrieco et al., 2005; Maragos and Busman, 2010; Prieto-Simón et al., 2007; van der Gaag et al., 2003) and electrochemical biosensors (Hervás et al., 2012; Palchetti and Mascini, 2008; Vidal et al., 2013; Viswanathan et al., 2009) for determining mycotoxin. In Fumonisins analysis, although some optical biosensors have been reported (Mirasoli et al., 2012; Mullett et al., 1998; Wu et al., 2012), to our knowledge only one electrochemical immunosensors have been described in the literature (Kadir and Tothill, 2010).

This work explores for the first time, the use of disposable immunosensing surfaces on carbon screen-printed electrodes using magnetic beads coupled to ELISA method for determination of Fumonisins (FB1 + FB2) in different food matrices. The use of magnetic beads improves the performance of the immunological reaction due to an increase in the surface area, as well as the assay kinetics are achieved more rapidly since the beads are in suspension and the probability that antibody-coated magnetic beads meet the analyte is very high while keeping the solution under stirring. Furthermore, the magnetic beads can easily be manipulated through the use of permanent magnets, and the matrix effect is also minimized due to improved washing and separation steps which allows the analysis to be made without any pre-enrichment, purification, or pre-treatment steps. Besides, the use of magnetic beads as affinity reaction support implies that electrode surface is only used for transduction step avoiding its shielding by deposition of the antibodies which can cause hindrance of the electron transfer and reducing electrochemical signal.

In order to obtain a simple and disposable analytical tool for fast, accurate and reliable determination of Fumonisins *in-field* analysis, a strategy based on simultaneous performing of simplified calibration and analysis protocol on the screen-printed immunosensing surfaces was accomplished in this work.

2. Materials and methods

2.1. Reagents and solutions

Fumonisin B1 (FB1), bovine serum albumin (BSA), hydrogen peroxide (30%), hydroquinone (HQN), benzoquinone (BQN) and Tween 20 were purchased from Sigma-Aldrich (Madrid, Spain). Monoclonal antibody anti-Fumonisin B1 and the enzyme tracer FB1 conjugated to horseradish peroxidase (HRP) were supplied by Soft Flow Hungary Ltd. (Pécs, Hungary). Fumonisin B2 and B3 (FB2 and FB3) were purchased from Romer Labs (Tulln, Austria). Standard Fumonisin B1, B2 and B3 solutions were prepared daily by dilution of stock solution (50 μg mL^{-1} in acetonitrile:water, 50:50). All other reagents were of the highest available grade.

All buffer solutions were prepared with MiliQ water. Phosphate buffer saline (PBS) was modified with Tween 20 and BSA. The composition of the PBS solution was phosphate buffer 10 mmol L^{-1}, pH 7.4, with 0.8% (w/v) NaCl. In other solutions, 0.05% (v/v) Tween 20 and 2.5% (w/v) BSA (blocking buffer) or 0.1% (w/v) BSA (dilution buffer) were also added.

2.2. Materials

Superparamagenetic polymer beads with protein-G covalently coupled to the surface (Dynabeads® Protein G) were supplied by Life Technologies (Oslo, Norway).

Screen-printed electrodes (DS110) based on carbon working, counter, and silver pseudo-reference electrodes were provided by Dropsens (Oviedo, Spain). The working electrode diameter was 4 mm. The magnetic holder (DRP-MAGNET) used for holding magnetic beads on the surface of the working electrode was supplied by Dropsens (Oviedo, Spain).

2.3. Samples

Fumonisins maize certified reference material ([FB1] = 2.0 ± 0.4 mg Kg^{-1}; [FB2] = 0.5 ± 0.2 mg Kg^{-1}; [FB3] = 0.2 ± 0.1 mg Kg^{-1}) was purchased from Pribolab® (Singapore). Beer samples were purchased from a local retail store (Madrid, Spain).

2.4. Equipment

Amperometric measurements were performed with a multipotenciost *μSTAT8000* and the software DropView 8400 from Dropsens (Oviedo, Spain), and magnetic stirrer *Microstirrer* from Velp Scientifica (Usmate, Italy).

The competitive curves were analyzed with a four parameter logistic equation using the proper software GraphPad Prism 5.

2.5. Immunoassay procedure

The immunoanalytical methodology was developed on the basis of the competition scheme where mycotoxin FB1 and its