ACS OMEGA

Article

Cite This: ACS Omega 2019, 4, 12803−12807

http://pubs.acs.org/journal/acsodf

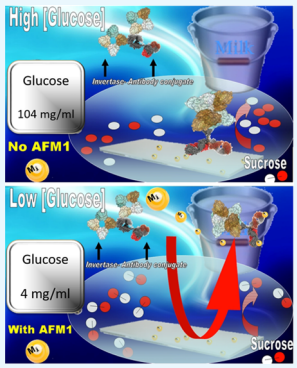
Sweet Sensor for the Detection of Aflatoxin M1 in Whole Milk

Stefano Di Giovanni,[†] Vittorio Zambrini,[‡] Antonio Varriale,[†] and Sabato D'Auria*[,†]

[†]Institute of Food Science, CNR, Via Roma, 64, 83100 Avellino, Italy

[‡]Granarolo Group, Via Cadriano 27/2, 40127 Bologna, Italy

ABSTRACT: Recently, there is an increase in interest to develop user-friendly monitoring devices in healthcare, environmental, and agrofood fields for a fast detection of contaminants. Aflatoxins (AFs) are a group of toxic substances produced by the fungi of species *Aspergillus* that contaminate cereals and dried fruits. When dairy cows ingest feed contaminated with aflatoxin B1 (AFB1), it is metabolized and transformed in the liver into a carcinogenic form aflatoxin M1 (AFM1), which is eliminated through the milk. In this work, we developed a sensor assay to detect low amounts of AFM1 directly in whole milk. For this purpose, we produced monospecific polyclonal antibody (IgGMS-M1) that was able to bind with high avidity to AFM1. Then, we conjugated the antibody to the invertase enzyme from *Saccharomyces cerevisiae*. This enzyme is able to convert sucrose into fructose and glucose. After incubation of invertase-conjugated anti-AFM1 antibody with milk containing AFM1, we measured the produced glucose by a glucometer. The produced glucose was then correlated to the amount of AFM1 present in the milk. The obtained results show that the assay is easily customizable as a portable instrument for on-site AFM1 measurements. In addition, the results point out that the assay is very sensitive since it can detect the presence of 27 parts per trillion (ppt) of AFM1 in whole milk, a value lower than the AFM1 quantities in milk and dairy products set by the European Commission (50 ppt).



1. INTRODUCTION

Recently, researchers have shown an increased interest in developing user-friendly devices to monitor the presence of contaminants in food, feed, and environment. In 2011, Lu and group published a study[1] in which they reported a method for expanding the principle of the glucose meter to detect different analytes. Sia and Curtis[2] showed the design and realization of a small device able to analyze whole blood samples in a few seconds and provided quantitative measurements.

In the agrofood sector, user-friendly devices represent a valid tool for on-site determination of contaminants. Food contaminants such as aflatoxins (AFs), a group of toxic substances produced by fungi *Aspergillus flavus* and *Aspergillus parasiticus*, represent a major public health problem due to their carcinogenic activity.[3] The ability of these species of fungi to grow on different cereals (corn, wheat, rice, and peanuts) and dried fruits extend their presence in the food chain.[4,5]

AFM1 detection in whole milk is a crucial issue for farmers for the production of good and safe milk. The current methods used to monitor the presence of AFM1 in milk are time consuming and require a highly trained personnel.[6−8] In this study, we developed a new method for the rapid detection of aflatoxin M1 (AFM1) in milk-collected daily by farmers in less than 2 h. In particular, we developed an immune-detection strip containing invertase-conjugated antibody anti-AFM1. We showed that it is possible to detect the presence of 27 parts per trillion (ppt) of AFM1 in whole milk by measuring the glucose produced by the invertase-conjugated antibody anti-AFM1 strip after 1 h of incubation. The novelty of this method is that it only requires to produce glucose by an invertase-linked immunosorbent assay (InLISA) and monitors it by a simple glucose detection. The method, therefore, provides an important opportunity to advance the rapid detection of the presence of AFM1 in whole milk.

2. EXPERIMENTAL SECTION

2.1. Materials. All reagents were of the highest commercially available quality and were used as received. 1-[3-(Dimethylamino)-propyl]-3-ethylcarbodiimide (EDC), carboxymethoxylamine hemihydrochloride, EAH Sepharose 4B resin, bovine serum albumin (BSA)-aflatoxin M1 (AFM1), invertase, and all buffers were purchased from Sigma-Aldrich. AFM1 was purchased from Apollo Scientific. Nitrocellulose transfer membrane Protran and enhanced chemiluminescence (ECL) detection reagents, used in western blot experiments, were purchased from Schleicher & Schuell and Amersham Biosciences, respectively. Goat polyclonal antibody to rabbit IgG-horseradish peroxidase (HRP) conjugate (secondary antibody) was purchased from Abcam. BSA was from Pierce. Glutamine-binding protein (GlnBP) was purified in our laboratory according to Staiano.[9] Ovalbumin (OVA) was purchased from Amersham Biosciences. Aflatoxin-HRP was purchased from Beacon Analytical Systems (ORSell). Protein A Sepharose 4 Fast Flow and EAH Sepharose 4B resins were purchased from GE Healthcare. Sera against AFM1 were from Covalab.

2.2. Antibodies Antiaflatoxin M1 Production and Purification. Rabbits were immunized through a standard

Received: May 6, 2019
Accepted: July 11, 2019
Published: July 29, 2019

DOI: 10.1021/acsomega.9b01300
ACS Omega 2019, 4, 12803−12807

protocol, by intradermal injection of commercial BSA-aflatoxin M1. At the end of the immunization period, blood from rabbits was recuperated and centrifuged to isolate serum from blood cells. A 2.0 mL of sample (1 mL for each rabbits) was diluted 1/1 in 50 mM Tris/HCl, pH 7.0 (binding buffer) and applied to 0.5 mL of resin Protein A Sepharose 4 Fast Flow (GE Healthcare). The IgG samples were purified as reported by Varriale.[10] The immunoglobulin G (IgG) samples were eluted with glycine (0.1 M), pH 2.8, and promptly buffered with 1.0 M Tris/HCl, pH 8.8. The IgG elutions were checked following the absorbance at λ = 278 nm, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out to assess the purity of the samples. The obtained total IgG was dialyzed against 10 mM potassium phosphate buffer, pH 7.4.

2.3. Synthesis of Aflatoxin M1 Derivative and Production of Aflatoxin M1-GlnBP Conjugate. The AFM1 derivative compound was obtained as described by Chu[11] using 1.0 mg of AFM1 (99%, 3.05 μmol, Sigma-Aldrich). To avoid interference, the AFM1 derivative was conjugated to the glutamine-binding protein (GlnBP). The following procedure was used: 3 μmol (1.0 mg) AFM1 derivative was suspended in ethanol and mixed with an aqueous solution of 4.84 μmol EDC. The solution was diluted up to 840 μL with 10 mM potassium phosphate buffer, pH 6.0, and incubated for 20 min at room temperature. Then, the mixture was incubated with 4.84 nM of GlnBP, dissolved in 160 μL of 10 mM potassium phosphate buffer, pH 6.0, and again incubated for 24 h at room temperature under continuous stirring.

2.4. Aflatoxin M1-EAH Sepharose Resin Preparation and Purified Antibodies against Aflatoxin M1. The affinity resin was obtained by conjugating the AFM1 derivative to EAH Sepharose 4B. For this purpose, the protocol reported by Varriale[10] was used. In brief, 1.2 mL of Sepharose resin suspension was washed sequentially with deionized water at pH 4.5 (160 mL), with 0.5 M NaCl (100 mL), and again with deionized water at pH 4.5 (100 mL). Then, the resin was packed into a polystyrene column, suspended in 2.0 mL of water at pH 4.5, and gently shaken.

AFM1-oxime (15.3 μmol) diluted with methanol was preincubated for 10 min with 400 μmol EDC (0.1 M at pH 4.5) and then added to a slurry resin and gently shaken overnight at 4 °C. At the end of this step, the resin was washed with a solution of 0.5 M NaCl in 50% methanol (15 mL) and treated with 15 mL of 0.1 M acetic acid, pH 4.0, 0.5 M NaCl in 50% methanol (blocking buffer), 0.1 M Tris/HCl, pH 7.0, and 0.5 M NaCl in 50 % methanol (washing buffer). The obtained EAH-AFM1 resin was used to purify the monospecific antibody against AFM1. In brief, 8.0 mg of IgG (2.0 mg/mL) was diluted to 1:1 with 50 mM Tris/HCl, pH 7.0, and incubated on the affinity column. The column was previously washed with 20 column volumes of 50 mM Tris/HCl, pH 7.0, to eliminate the presence of unspecific antibodies, and then, a solution of 0.1 M glycine/HCl, pH 3.0, was used to elute the monospecific antibody. Throughout this paper, the term IgGM1-MS will refer to purified monospecific IgG against AFM1. The samples were collected, and the concentration of the antibodies was determined by the absorbance value at λ = 278 nm, using as $\varepsilon^{1\%}$ = 14. The samples were recovered and stored at 4 °C.

2.5. Western Blotting Experiments. SDS-PAGE at 12% of proteins, AFM1-GlnBP, and GlnBP (10 μg each) was performed and then was transferred overnight at 4 °C onto a nitrocellulose membrane. The membrane was blocked for 1 h at room temperature in 50 mL of the blocking buffer (PBS containing 5% skim milk, 0.2% Tween-20, and 0.05% Triton (PBS-TT)).

At the end of the blocking step, two washing steps with PBS-TT and PBS sequentially (10 min for each washing) were performed before incubating the membrane with anti-AFM1 (1:500 diluted in the blocking buffer) for 1 h at room temperature.

After these steps, the membrane was washed (with PBS-TT and PBS) incubated with the secondary antibody (goat antirabbit HRP conjugate, 1:3000 in the blocking buffer) for 1 h at room temperature and then it was washed and incubated with the detection reagent ECL.

2.6. Indirect Enzyme-Linked Immunosorbent Assay (ELISA). The titer of anti-AFM1 produced was determined by an indirect ELISA test. In brief, the antigen GlnBP-AFM1 was diluted at different concentrations in the coating buffer (0.1 M PBS, pH 7.4) and used to coat the wells of microplates (100 μL per well). As control, BSA was used in this experiment. At the end of the overnight incubation and the washing steps (three times with 0.1 PBS and 0.05% Tween, at pH 7.4), a blocking step was performed through incubation with PBS containing Tween (PBS-T) detergent and 5% milk (100 μL each well) for 2 h at room temperature.

Then, the wells were washed and incubated with serially diluted antibody (from 1/500 to 1/12 000) IgGM1-MS at room temperature for 1 h. Finally, the wells were rinsed three times with PBS-T and incubated with horseradish peroxidase-conjugated antirabbit IgG antibodies, diluted 1:4000 in PBS-T, 1% milk, and incubated for 1 h at room temperature. The wells were washed with PBS-T, the enzyme substrate 3,3′,5,5′-tetramethylbenzidine was added (100 μL per well), and the color reaction was quenched after 5 min by addition of 1.0 M H_2SO_4 (100 μL per well). The absorbance was measured at 450 nm. The antibody titer was graphically determined by plotting the reciprocal of the antibody dilution vs absorbance for each dilution of antibodies (data not shown). The antibody titer was determined as reported by Varriale,[10] and the value for purified IgGM1-MS was found to be 1/25 000.

2.7. Competitive ELISA. The resulting purified monospecific IgG anti-AFM1 (IgGMS-M1) were used for an indirect competitive ELISA. In this assay, 25 ng of GlnBP-AFM1 was used for each well. Each experiment was performed in triplicate. For the competitive assay, the selected antibody dilution (1:6000 that is 1.0 μL in 6.0 mL of buffer) was preincubated with an increasing amount of AFM1 diluted in a methanol solution before performing the standard ELISA. We performed the preincubation step for 30 min of IgGMS-M1 with increasing concentrations of AFM1, from 0.125 to 8 ppt. After this step, we performed the standard protocol of ELISA as reported above. Data management and analysis were performed using the OriginPro 7.5 program.

2.8. Production of IgGMS-M1-Invertase Conjugate. To obtain a suitable conjugate with IgGs, the invertase was activated by glutaraldehyde as a homo-bifunctional linker.[6] For this purpose, 10 mg of yeast invertase was dialyzed four times for 2 h at room temperature against 250 mL of 0.01 M sodium phosphate buffer at pH 6.8. Afterward, glutaraldehyde was added to a final concentration of 1.25% to 1.0 mL of invertase solution (6.5 mg/mL). The solution was placed under mild stirring conditions overnight at room temperature. Afterward,

DOI: 10.1021/acsomega.9b01300
ACS Omega 2019, 4, 12803−12807

the excess of glutaraldehyde was removed by dialyzing three times for 2 h at 4 °C against 250 mL of 100 mM sodium phosphate buffer, pH 6.8, and 150 mM NaCl. The molar ratio between the invertase and IgGs in this reaction was 5:1. To achieve this, 200 μL of a new preparation of IgGs at a concentration of 8 mg/mL (1.6 mg total), obtained from the rabbit sera, was dissolved in 800 μL of solution comprising 500 mM sodium carbonate buffer, pH 9.6, and placed in a 2.0 mL Eppendorf tube. Later, 1.0 mL of invertase containing 6.5 mg of invertase activated with glutaraldehyde was added. It was placed under mild stirring for overnight at 4 °C. Subsequently, 100 μL of a solution of 200 mM ethanolamine, pH 7.0, was added and the solution was placed under stirring for 2 h at 4 °C. The conjugate was centrifuged at 16 000*g* for 20 min at 4 °C and dialyzed five times against 200 mL of 50 mM Tris/HCl buffer, pH 7.4. The sample was recovered and stored at 4 °C. Different dilutions 1:100 (2 μL of conjugate and 198 μL of sample for each assay) were used for each experiment, and the experiments were performed in triplicate.

2.9. Production of the Aflatoxin Strip. The purified conjugate IgGMS-M1-INV was tested on nitrocellulose strips previously incubated with different protein solutions.

The strips (1.0 x 3.0 cm^2) were placed in an Eppendorf tube and incubated with 1.0 mL of different protein solutions. For this purpose, 150 μg of OVA and 150 μg of GlnBP were used as negative controls. This experiment was performed to verify the ability of the conjugates to detect a commercial protein conjugated to various aflatoxins used in the competitive ELISA kit. A solution of peroxidase-conjugated aflatoxin (50 μL, aflatoxin-HRP, 6 mg/mL) was diluted in 1.0 mL of TBS (20 mM Tris/HCl, 130 mM NaCl) before incubating with the nitrocellulose strip for 1 h at room temperature under mild stirring. Subsequently, the nitrocellulose strips were incubated with 1 mL of 0.5% OVA TBS-T (0.05% Tween-20) for 1 h at room temperature under mild stirring. Three washing steps were performed in 1.0 mL of TBS-T, and the strips were incubated overnight at 4 °C with a dilution of IgGMS-M1-INV. The IgGMS-M1-INV conjugates were used at a dilution of (1:100 of 0.5 % OVA in TBS-T). Three washing steps were performed (10 min with TBS-T). The strips were incubated at room temperature with 500 μL of a buffer solution containing an excess of substrate (2:3 of 100 mM acetate buffer, pH 4.6, and 1:3 of a solution at 10% w/v sucrose) to verify the invertase activity. After performing various incubations at varying durations, withdrawals of 33 μL and final 100 μL were tested with the D-glucose kit (Megazyme) for the detection of glucose produced by the immunoconjugate IgGMS-M1-INV. To perform a test in real matrices, the conjugate (IgGMS-M1-INV) was added to a solution of milk and methanol for extraction/dilution of AFM1. The assay was performed by diluting the IgGMS-M1-INV conjugate in a solution of whole milk at 33% in 0.25% OVA TBS-T; in all samples, 10% of methanol with or without AFM1 dilution was added. All experiments were performed three times.

3. RESULTS AND DISCUSSION

3.1. Synthesis of the Aflatoxin M1 Derivative and Aflatoxin M1-GlnBP Conjugate. AFM1 (Figure 1A) lacks anchoring sites for conjugation with biomolecules. Consequently, a short linker arm was introduced by condensation on the carbonyl to form an oxime.[11] The obtained AFM1-oxime (Figure 1B) derivative was conjugated through the well-known EDC conjugation procedure at two different protein

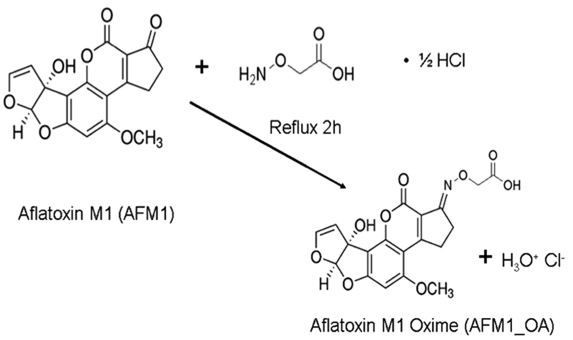


Figure 1. Aflatoxin M1 structure and synthesis of the AFM1 derivate used in the conjugation reaction with GlnBP.

carriers. BSA-AFM1 conjugate was used to produce polyclonal antibodies against AFM1.[12,13] The total IgGs obtained were purified (Figure 2A) and tested for their ability to bind with AFM1. For this purpose, to avoid carrier interference, the AFM1 derivative was conjugated to GlnBP isolated from *Escherichia coli*.[9]

In Figure 2B, the results of the ELISA tests are reported. The purified monospecific IgG anti-AFM1 (IgGMS-M1) are able to recognize GlnBP-AFM1 and BSA (immunization carrier), but they are not able to bind to GlnBP (negative control). The same results were obtained from the western blotting experiment (Figure 2C).

3.2. Competitive Indirect ELISA with IgGMS-M1. In Figure 3, the competitive ELISA experiments are reported in which the binding efficiency of IgGMS-M1 was tested in the presence of different concentrations of AFM1. The data show that the purified IgGMS-M1 are able to recognize the AFM1 conjugate to GlnBP. This allowed the setup of a sensitive assay for the detection of AFM1 up to 125 ppq (0.125 ppt). In fact, an increasing inhibition of IgGMS-M1 binding from 40% up to 80% on coated GlnBP-AFM1 inside the wells was obtained (Figure 3).

3.3. Production of the IgGMS-M1-Invertase Conjugate. A suitable strip assay derivatized with the IgGMS-M1-invertase conjugate was tested on whole milk samples. First, we prepared an invertase-IgGMS-M1 conjugate using glutaraldehyde as homo-bifunctional cross-linkers[14] and obtained the IgGMS-M1-INV conjugate. Then, a dilution of this preparation (IgGMS-M1-INV) was used in the dot blot experiment for the detection of AFM1 directly in milk (data not shown). For the production of the AFM1 strip, several strips of nitrocellulose of 1 cm^2 were incubated with aflatoxin-HRP, GlnBP, and OVA as negative controls for about 2 h and then incubated with an excess of OVA (5 mg/mL) to saturate all of the binding sites on the nitrocellulose strips. The IgGMS-M1-INV was diluted (a ratio of 1:100) and incubated overnight at 4 °C. After three washing steps, it was possible to detect the invertase activity only on the strip on which aflatoxin-HRP was spotted and not with other immobilized proteins such as OVA and GlnBP.

3.4. Competitive Strip Assay in Real Matrix (InLISA). In Figure 4, the principle of the assay using the developed strip is shown. The absence or the presence of AFM1 in the sample modulates the glucose concentration produced. In particular, the absence of the toxin in the milk sample allows for binding of IgGMS-M1-INV on the surface of the strip. Consequently, a high concentration of glucose will be detected. On the contrary, the presence of the toxin in the milk sample will

DOI: 10.1021/acsomega.9b01300
ACS Omega 2019, 4, 12803−12807

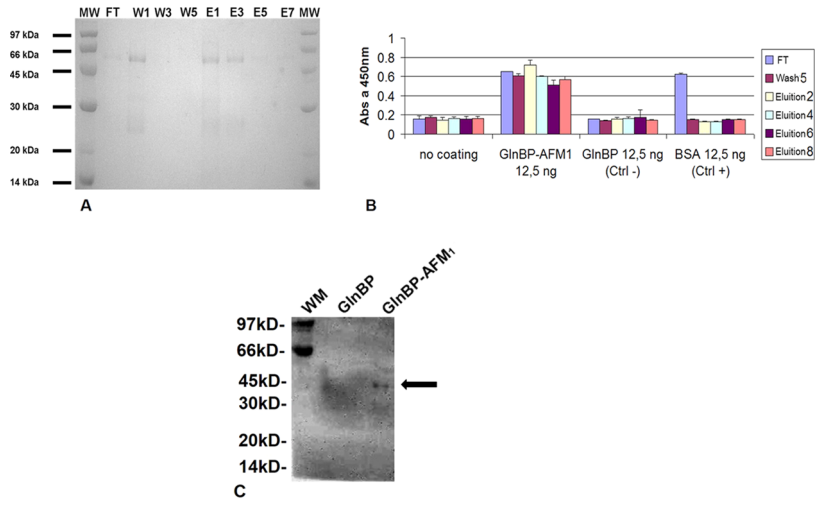


Figure 2. New SDS-PAGE of anti-AFM1 purified antibodies (IgGMS-M1) on EAH-AFM1 resin (A). ELISA test performed against different antigens (BSA/GlnBP/GlnBP-AFM1) with different collected samples during immunoaffinity purification (B) and western blotting (C).

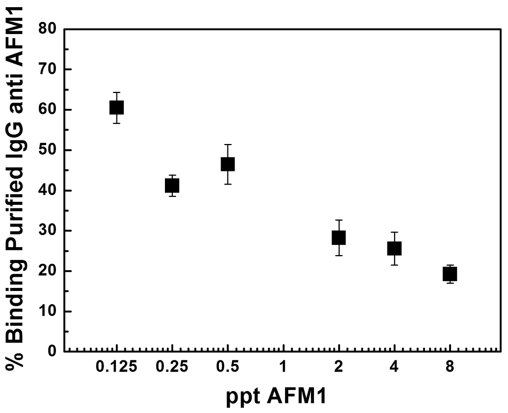


Figure 3. Indirect competitive ELISA in the presence of an increasing concentration of AFM1 from 0.125 ppt up to 8 ppt, during the preincubation time of IgGMS-M1 purified on EAH-AFM1 resin.

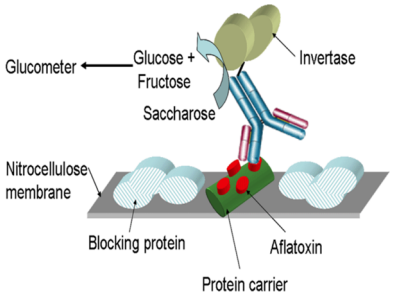


Figure 4. Cartoon representation of the immunoreaction of the IgGMS-M1-INV conjugate on the strip derivatized with aflatoxin-protein.

get out the IgGMS-M1-INV from the solution and only few molecules will be able to bind at the strip. As a consequence, a low amount of glucose will be produced.

In Figure 5, the absorption values of the absorbance at 510 nm against the AFM1 concentration are reported. The data show that the absorption is directly proportional to glucose concentrations, and after an incubation of 1 h in the absence of AFM1, 1.5 μg of glucose is produced. In the presence of AFM1, a decrease in glucose production by 80% and 50% was recorded after preincubation with the IgGMS-M1-INV

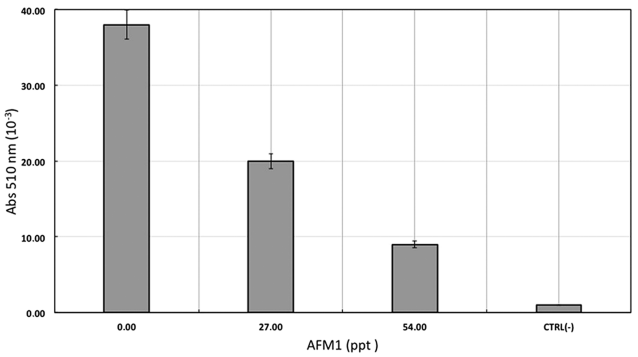


Figure 5. Direct competitive (InLISA) assay in the presence of 27 and 54 ppt of AFM1 during the preincubation time of the IgGMS-M1-INV conjugate in whole milk. Positive control: strip derivatized with aflatoxin-protein incubated with IgGMS-M1-INV without AFM1; negative control: strip derivatized with ovalbumin incubated with IgGMS-M1-INV in whole milk, also without AFM1.

conjugate in diluted milk-AFM1 at 54 and 27 ppt, respectively. A positive control was performed on the strip derivatized with aflatoxin-protein incubated with IgGMS-M1-INV without AFM1, and a a negative control was performed on a strip derivatized with OVA incubated with IgGMS-M1-INV in whole milk without AFM1.

4. CONCLUSIONS

In this work, we have developed an innovative assay for the detection of AFM1 directly in whole milk. The assay is based on the principle that the amount of toxin present in milk can be easily correlated to the amount of glucose produced by the invertase.

In the literature, the main advantages of the use of glucose detection as an analytical tool are reported.[15,16] In fact, due to its simple usability, glucometer can be used by millions of unskilled young and old potential users around the world. The possibility to engineer commercial glucometers to sense lower amounts of glucose will allow to perform the analyses directly on-site.

DOI: 10.1021/acsomega.9b01300
ACS Omega 2019, 4, 12803−12807

AUTHOR INFORMATION

Corresponding Author

*E-mail: sabato.dauria@cnr.it. Phone: +39-0825299101. Fax: +39-0825299100.

ORCID

Antonio Varriale: 0000-0003-0985-9877

Sabato D'Auria: 0000-0002-3082-8947

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was partially funded by the Granarolo Group.

REFERENCES

(1) Xiang, Y.; Lu, Y. Using personal glucose meters and functional DNA sensors to quantify a variety of analytical targets. *Nat. Chem.* **2011**, *3*, 697−703.

(2) Sia, S. K.; Chin, Curtis D. Analytical chemistry: Sweet solution to sensing. *Nat. Chem.* **2011**, *3*, 659−660.

(3) da Rocha, M. E. B.; da Chagas Oliveira Freire, F.; Erlan Feitosa Maia, F.; Florindo Guedes, M. I.; Rondina, R. Mycotoxins and their effects on human and animal health. *Food Control* **2014**, *36*, 159−165.

(4) Tola, M.; Kebede, B. Occurrence, importance and control of mycotoxins: A review. *Cogent Food Agric.* **2016**, *2*, No. 1191103.

(5) D'Mello, J. P. F.; Macdonald, A. M. C. Mycotoxins. *Anim. Feed Sci. Technol.* **1997**, *69*, 155−166.

(6) Curticapean, A.; Curticapean, M. Validation of New HPLC Method Determination of Aflatoxin M1 in Raw Milk. *J. Environ. Prot. Ecol.* **2013**, *14*, 503−508.

(7) Barbieri, G.; Bergamini, C.; Ori, E.; Resca, P. Aflatoxin M1 in Parmesan cheese: HPLC determination. *J. Food Sci.* **1994**, *59*, 1313−1331.

(8) Stefanovic, S.; Spiric, D.; Petronijevic, R.; Nedeljkovic Trailovic, J.; Milicevic, D.; Nikolic, D.; Jankovic, S. Comparison of two Analytical Methods (ELISA and LC-MS/MS) for Determination of Aflatoxin B_1 in Corn and Aflatoxin M_1 in Milk. *Procedia Food Sci.* **2015**, *5*, 270−273.

(9) Staiano, M.; Scognamiglio, V.; Mamone, G.; Rossi, M.; Parracino, A.; Rossi, M.; D'Auria, S. Glutamine-binding protein from *Escherichia coli* specifically binds a wheat gliadin peptide. 2. Resonance energy transfer studies suggest a new sensing approach for an easy detection of wheat gliadin. *J. Proteome Res.* **2006**, *5*, 2083−2086.

(10) Varriale, A.; Pennacchio, A.; Pinto, G.; Oliviero, G.; D'Errico, S.; Majoli, A.; Scala, A.; Capo, A.; Pennacchio, A.; Di Giovanni, S.; Staiano, M.; D'Auria, S. Fluorescence Polarization Assay To Detect Steroid Hormone Traces in Milk. *J. Agric. Food Chem.* **2015**, *63*, 9159−9164.

(11) Chu, F. S.; Ueno, I. Production of Antibody Against Aflatoxin B1. *Appl. Environ. Microbiol.* **1977**, *33*, 1125−1128.

(12) de Champdorè, M.; Bazzicalupo, P.; De Napoli, L.; Montesarchio, D.; di Fabio, G.; Cocozza, I.; Parracino, A.; Rossi, M.; D'Auria, S. A new fluorescence competitive assay for the detection of patulin toxin. *Anal. Chem.* **2007**, *79*, 751−757.

(13) Di Giovanni, S.; Varriale, A.; Marzullo, V. M.; Ruggiero, G.; Staiano, M.; Secchi, A.; Pierno, L.; Fiorello, A. M.; D'Auria, S. Determination of benzyl methyl ketone—a commonly used precursor in amphetamine manufacture. *Anal. Methods* **2012**, *4*, No. 3558.

(14) Hermanson, G. T. *Bioconjugate Techniques*, 3rd ed.; Academic Press, 2013.

(15) Centonze, D.; Quinto, M.; Carelli, D.; Di Paolo, S.; Montemurno, E.; Gesualdo, L. Interferenze nella determinazione della glicemia effettuata con i glucometri elettrochimici nel paziente ospedalizzato. *G. I Diabetol. Metab.* **2006**, *26*, 160−171.

(16) Tonyushkina, K.; Nichols, J. H. Glucose meters: a review of technical challenges to obtaining accurate results. *J. Diabetes Sci. Technol.* **2009**, *3*, 971−980.

DOI: 10.1021/acsomega.9b01300
ACS Omega 2019, 4, 12803−12807