Actividades divulgación Proyecto AGROALNEXT_2022

Lugar	Universitat Politènica de València
Localidad	Gandia
Provincia	València
Fecha	6-8 marzo 2024
Proyecto:	Sistemas biológicos de control efectivos contra hongos micotoxigénicos y estrategias inmunoquímicas para el análisis de las micotoxinas patulina y ocratoxina A (CONPOTA)
Código proyecto	AGROALNEXT_2022/028
Grupo de investigación	

INFORME DE LA ACTIVIAD:

Dentro del congreso organizado por el proyecto Agroalnext, celebrado en las instalaciones de la Universidad Politécnica de Valencia en el campus de Gandia, se expuso un póster para la divulgación de los resultados del proyecto "Sistemas biológicos de control efectivos contra hongos micotoxigénicos y estrategias inmunoquímicas para el análisis de las micotoxinas patulina y ocratoxina A (CONPOTA)". El póster describía los avances alcanzados por nuestro grupo para el desarrollo de nuevos métodos rápidos de análisis de la micotoxina ocratoxina A en cereales. El resumen ha sido recogido en el libro de Abstract del Congreso (https://congresoagroalnext.umh.es/files/2024/04/Libro_resumenes_Agroalnext24.pdf).

UN NUEVO INMUNOENSAYO PARA EL ANÁLISIS DE OCRATOXINA A EN MUESTRAS DE ALIMENTOS

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Esta actividad ha sido comunicada a través de las cuentas de X-Twitter de @PostPath_IATA (https://twitter.com/PostPath_IATA/status/1765296445500826014)

FOTOS DE LA ACTIVIDAD:

Agroalnext/2022/028

A novel immunoassay for ochratoxin A analysis in food samples

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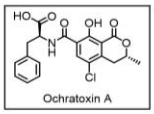
Introduction



Ochratoxin A

Ochratoxin A (OTA) is a toxic, secondary metabolite produced by several species of *Aspergillus* and *Penicillium*, which can be found in a wide variety of foodstuffs, including cereals and grape-derived products.[1] In order to protect consumers from mycotoxins, the European Commission established regulatory limits for OTA levels in several foodstuffs, being the tolerable limit in cereals and raisins of 5 and 10 µg/L, respectively.[2]

Nowadays, the enzyme-linked immunosorbent assay[3] (ELISA) is one of the most popular methods for OTA detection because of its simplicity, rapidity, and high throughput. In this study, a collection of mouse monoclonal antibodies with different binding properties and four hapten conjugates with different linker tethering sites[4] were evaluated, and a new monoclonal antibody-based ELISA was developed and validated in the antibody-coated direct competitive format for OTA analysis in relevant foodstuffs.

Immunoassay characterization

Fourteen monoclonal antibodies (mAb) were evaluated by direct competitive ELISA using homologous and heterologous tracer conjugates (**Table 1**). Antibody OTA*f*#223 combined with the heterologous enzyme tracer of hapten OTA*b* was selected for immunoassay development due to the high sensitivity achieved. The influence of pH, ionic strength, and ethanol concentration over the main analytical parameters of this immunoassay was studied (**Fig. 1**).

Table 1. IC_{50} values (µg/L) of monoclonal antibodies characterized using homologous and heterologous conjugates (n=3).

	Tracer conjugate			
mAb	**OTA*b***	**OTA*d***	**OTA*e***	**OTA*f***
OTAb#39	0.129	0.106	-[a]	-
OTAb#41	0.505	0.633	-	-
OTAb#310	0.039	-	-	-
OTAb#311	0.047	-	-	-
OTAd#16	-	0.116	-	-
OTAd#21	-	0.150	-	-
OTAd#27	-	0.227	-	-
OTAd#111	-	0.219	-	-
OTAd#114	-	0.096	-	-
OTAd#115	-	0.068	-	-
OTAd#118	-	0.104	-	-
OTAe#13	0.257	-	0.531	-
OTAe#115	-	-	0.108	-
OTAf#223	0.034	-	0.056	-

[a]The HRP-hapten tracer was not recognized.

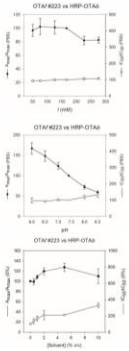


Fig 1. Influence of ionic strength, pH and methanol over the analytical parameters of the selected immunoassay.

Conclusions

A highly sensitive monoclonal antibody-based immunoassay for OTA analysis has been characterized. This direct competitive ELISA is very robust to changes in the ionic strength of the immunoreaction mixture, though pH may change the signal of the assay. Methanol has little influence over the assay performance. The matrix effects of a variety of relevant food matrices were evaluated. Water extracts exerted lower changes than methanol extracts over the assay Amax and IC_{50} values. Raisin samples contaminated with OTA were identified. Further studies will be carried out to establish the best sample treatment procedure and to quantify OTA in the positive samples.

Acknowledgements

This work has been supported by Generalitat Valenciana (Agroalnext/2022/028), MCIN/AEI/https://doi.org/10.13039/ 501100011033, and the "European Union NextGenerationEU/PRTR". The Accreditation as Center of Excellence Severo Ochoa CEX2021-001189-S funded by MCIU/AEI/10.13039/501100011033 is also fully acknowledged.

Sample analysis

Samples were acquired from local supermarkets. Muscatel and sultana raisins were homogenized with a blender. For cereal analysis, commercial flours of whole cereals from organic agriculture were employed. Food extracts (6 g) were extracted with 30 mL deionized water or 70% (v/v) methanol, and the extracts were diluted in 75 mM phosphate buffer, pH 7.4. As depicted in **Fig. 2**, low or no matrix effects over the A_{max} and IC_{50} values were observed with the water extracts of most of the food samples. A 1/5 dilution of the extract was mainly sufficient to eliminate the matrix effects, particularly if water extracts were employed. The only exception was the sultana raisins. The presence of this food sample inhibited the immunochemical reaction, thus reducing the signal, even when the sample was 500-fold diluted. This result probably indicates the presence of OTA at high levels in the sample.

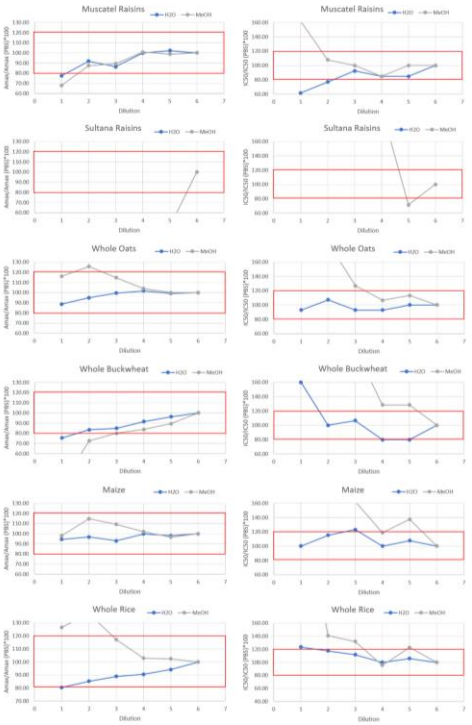


Fig 2. Matrix effects of raisins, whole rice, whole oats and maize samples over the A_{max} and IC_{50} values of the optimized immunoassay. Samples were extracted with water or 70% methanol and diluted in phosphate buffer. Dilutions 1-5 correspond to 1/2, 1/5, 1/10, 1/50 and 1/100 extract dilutions, respectively. Dilution 6 is the control without sample matrix. The red frames indicate the ±20% variation of the reference value.

References

1) T. Koszegi and M. Poór. *Toxins* **2016**, *8*, 111; 2) EC. Commission Regulation No. 1881/2006. *Off. J. Eur. Union*, **2006**, L 364/5; 3) S. D. Gan and K. R. Patel. *J. Investig. Dermatol.* **2013**, *133*, 1-3; 4) D. López-Puertollano et al., *Sci. Reports* **2018**, *8*, 9761.

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