doi:10.2533/chimia.2023.879 Chimia 77 (2023) 879–880

FH HES
Universities of Applied Sciences
Fachhochschulen – Hautes Ecoles Spécialisées

Mycotoxin Recovery and Matrix Effect in Fava Bean Starch and Protein Isolate Measured by LC-MS/MS

Anika Hoffmann[a], Souha Essassi[b], Wolfram Brück[a], and Saša M Miladinović*[a]

*Correspondence: Dr. S. Miladinović, E-mail: sasa.miladinovic@hevs.ch
[a]HES-SO Valais-Wallis, University of Applied Sciences, Institute of Life Technologies, CH-1950 Sion; [b]University of Carthage, INSAT, 1080, Tunis, Tunisia

Abstract: Mycotoxins are dangerous fungal food contaminants. Customized sample preparation techniques are essential for LC-MS/MS analysis of mycotoxins in unique protein-rich food matrices to enhance recovery and reduce matrix effects.

Keywords: Matrix effect · Mycotoxins · Plant-based food · Protein-rich flours · Recovery

Mycotoxins are secondary metabolites produced by fungi such as *Aspergillus* and *Penicillium*. These fungi are found in a variety of food products including cereals, spices, apples, coffee beans, and dried fruits.[1] Mycotoxins are chemically stable compounds that can survive food processing. The consumption of contaminated food products can lead to serious health issues such as food poisoning or cause hepatoxic, carcinogenic, or immunosuppressive effects in mammals. The class of mycotoxins includes over a hundred different substances, but a few are considered critical and need to be monitored in food. These critical mycotoxins include aflatoxins (AFB1, AFB2, AFG1, AFG2), fumonisins (FB1, FB2), ochratoxin A (OTA), deoxynivalenol (DON), patulin, zearalenone (ZEN), HT-2 and T-2 toxin.

The plant-based food market is a fast-growing area with an estimated size of 11.3 billion USD in 2023.[2] Therefore, products manufactured from flours made from *e.g.* chickpeas, lentils, or fava beans gain in popularity. With the increase of plant-based products, the topic of food safety concerning mycotoxin contaminations gets increasingly important.

Liquid chromatography tandem mass spectrometry (LC-MS/MS) is a powerful technique used to identify and quantify mycotoxin contents at trace levels.[3] The LC-MS/MS allows the simultaneous detection of many mycotoxins present in the sample. However, the results may be influenced by the matrix effect, which can cause fluctuations in ionization efficiency, potentially resulting in either ion suppression or enhancement, ultimately leading to inaccuracies in concentration determination. In quantitative LC-MS/MS, the matrix effect can affect precision and accuracy and it is usually corrected by using internal [^{13}C]-labelled mycotoxin standards.

Here, we have evaluated the matrix effect and recovery of 11 mycotoxins in differently composed fava bean food matrices: a fava bean starch and a fava bean protein isolate. Mycotoxins were spiked to 2.5 grams of unprocessed fava bean powder across six different concentration levels. Afterwards, the spiked mycotoxins were extracted using 10 mL of the organic solvent (acetonitrile/water/formic acid, 80:19.9:0.1, *v/v/v*). As a reference for recovery measurements, extracts of the blank samples were post-spiked with mycotoxins to match 100 % of recovery. The matrix factor was calculated by comparing analyte signals from spiked powder extract versus the signal in neat solution.[3]

Mycotoxins were separated using the Agilent 1290 Infinity II UPLC system equipped with ZORBAX RRHD Eclipse (100 × 2.1 mm, 1.8 nm) C18 column. The LC system was coupled to the Agilent 6490 triple quadrupole mass spectrometer operating in MRM mode. MRM transitions were described elsewhere.[3] A typical LC-MS/MS chromatogram is given in Fig. 1.

The results for the mycotoxin recovery in the two different types of fava bean matrices are presented in Fig. 2.

It is seen that the recovery of mycotoxins in the starch matrix falls between 80 to 140%. On the otherhand, the protein isolate had consistent recovery values of around 50%. The exception is the low recovery of FB1 and FB2 which remain to be below 10% within the protein isolate powder. Fumonisins are polar molecules with carboxyl and hydroxyl groups attached to their aliphatic backbone (Fig. 1, inset). It is possible that these functional groups interact with proteins and stick to their surface which could explain low recovery here since the protein isolates consist of 90% of proteins.

Values of the matrix factor as a measure of matrix effect are presented in Table 1 for the different fava bean matrices. An enhancement of the mycotoxin signal is found in the starch sample (MF>1) whereas the signal suppression was seen in the protein isolate sample (MF<1). The impact of the matrix effect was sig-

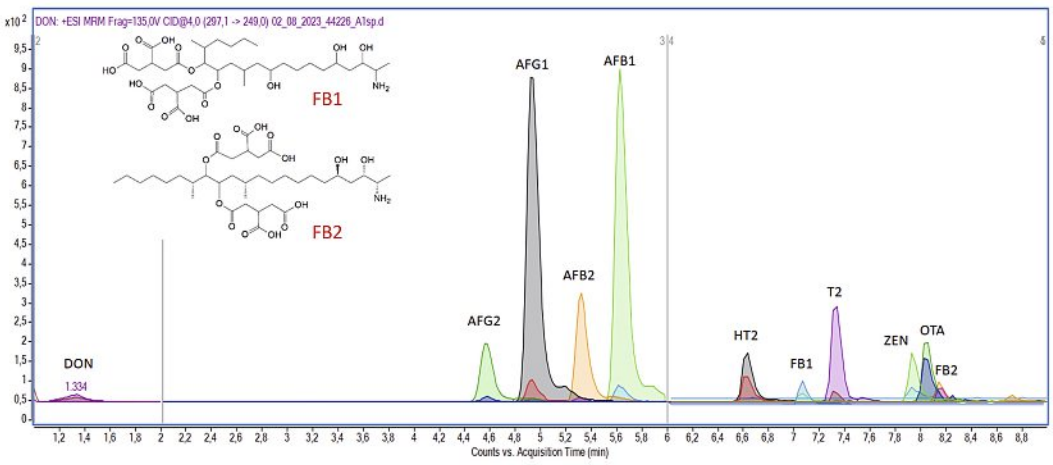

Fig. 1. LC-MS/MS total ion chromatogram of 11 mycotoxins measured in MRM mode. Inset: structures of FB1 and FB2.

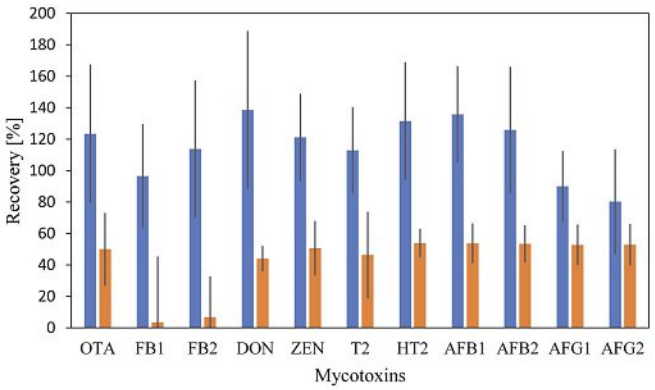

Fig. 2. Recovery of spiked mycotoxin in organic extract of spiked fava bean starch (blue) and protein isolate powder (orange).

nificant in the case of fumonisins. To increase fumonisin recovery, it is possible to include additional aqueous extraction step 3. We have noticed that with that additional sample preparation step, the matrix effect on the other 9 mycotoxins significantly increased resulting in poor sensitivity. Therefore, sample preparation included only organic extraction.

Table 1. Matrix factor for different fava bean matrices.

Mycotoxin	Fava Bean Starch	Fava Bean Protein Isolate
OTA	1.4	0.4
FB1	1.3	0.05
FB2	1.6	0.1
DON	1.4	0.4
ZEN	1.5	0.5
T2	1.5	0.5
HT2	1.3	0.5
AFB1	1.4	0.5
AFB2	1.4	0.5
AFG1	1.3	0.5
AFG2	1.4	0.5

These results demonstrate that different sample preparations are needed for samples with a high protein content to analyze different classes of mycotoxins by LC-MS/MS. The aqueous extraction step is crucial to achieving good recovery of fumonisins.[4] On the other hand, this extraction step caused a substantial matrix effect for other mycotoxins. The matrix effect is not negligible for these unique matrices and further research needs to be done to characterize the impact of matrix effect in different protein concentrate samples. Furthermore, new more universal sample preparation strategies are needed to improve the extraction efficiency and recovery in these types of protein-enriched matrices to allow simultaneous analysis of mycotoxins.

Received: October 6, 2023

[1] https://www.who.int/news-room/fact-sheets/detail/mycotoxins
[2] https://www.futuremarketinsights.com/reports/plant-based-food-market
[3] L. Gámiz-Gracia, A. M. Garcia-Campaña, N. Arroyo-Manzanares, *Toxins* **2020**, *12*, 272. https://doi.org/10.3390/toxins12040272
[4] E. Varga, T. Glauner, R. Köppen, K. Mayer, M. Sulyok, R. Schuhmacher, R. Krska, F. Berthiller, *Anal. Bioanal. Chem.* **2012**, *402* (9), 2675–2686. https://doi.org/10.1007/s00216-012-5757-5