FOOD SAFETY AND TOXICOLOGY

ENZYMATIC SYNTHESIS OF B-GLUCOSIDES OF THE TRICHOTHECENE TOXINS DEOXYNIVALENOL, NIVALENOL AND HT-2 TOXIN

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ABSTRACT

Glycosylation is an important plant defense mechanism and glucoconjugates of *Fusarium* toxins often co-occur with their parent compounds in cereal based food and feed. Such derivatives have been termed "masked" mycotoxins, implying that they are not routinely detected and reconstitution of the parent toxins during food processing or digestion is possible. Of particular importance is deoxynivalenol-3-*O*-ß-D-glucopyranoside, but glucosides of other relevant trichothecenes such as nivalenol (NIV), T-2 (T2) and HT-2 (HT2) toxin have been identified as well. The toxicological relevance of trichothecene glucosides is not fully understood and it is crucial to synthesize such compounds in sufficient amounts to make toxicological studies possible. Standards of glucosides of NIV, T2 and HT2 toxins are so far not commercially available, which also limits screening of their occurrence in cereal samples. Better understanding the role of glycosylation in plant-pathogen interaction with strains producing different toxins may also be of interest for breeders aiming to increase *Fusarium* resistance of cereal crops. Previously, our group has identified and studied several DON-conjugating plant UDP-glucosyltransferases (UGTs). A rice UGT (OsUGT79) was expressed in *E. coli* and biochemically characterized. *In vitro* biochemical assays showed that the recombinant enzyme is able to equally conjugate DON, NIV and HT2, but is inactive with T2 toxin. Interestingly, preliminary assays with a related UGT from barley (HvUGT13248) indicate that this enzyme prefers NIV over DON as substrate. OsUGT79 was used to synthesize mg quantities of DON-3-glucoside, NIV-3-glucoside and HT2-3-glucoside. NMR spectroscopy confirmed the formation of a trichothecene-3-*O*-ß-D-conjugate in each case. We are now able to enzymatically synthesize DON-3-glucoside as well as the novel standards NIV-3-glucoside and HT2-3-glucoside, which is a crucial step forward in evaluating the natural occurrence and toxicity of these masked mycotoxins in animal feeding trials.

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TRICHOTHECENE MYCOTOXIN LEVELS DETECTED IN WINTER WHEAT IN ONTARIO, CANADA FROM 2009-2015

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ABSTRACT

Fusarium head blight caused by *Fusarium graminearum* is a serious disease of wheat (*Triticum aestivum* L.). Deoxynivalenol (DON) is the mycotoxin most commonly detected in contaminated wheat grain in Ontario. The objective of this study was to evaluate the level of trichothecene mycotoxins in winter wheat grain in Ontario from 2009, 2010, 2013, 2014 and 2015. The harvested grain was sampled to determine DON, 15-acetyl DON, 3-acetyl DON, nivalenol (NIV), T-2 and HT-2 toxins using a GC-MS system with a detection limit of 0.06, 0.05, 0.05, 0.12, 0.06 and 0.04 μg/g, respectively. In 2015, DON level was detected using ELISA method with a detection limit of 0.25 μg/g. The average DON level was 0.7 μg/g, 0.3 μg/g, 3.3 μg/g, 0.2 μg/g and 2.5 μg/g in 2009, 2010, 2013, 2014 and 2015, respectively. 15-acetyl DON and 3-acetyl DON were not detected in 2009, 2010 and 2014 in Ontario. However, they were detected in 2013 in soft white winter wheat at one or two locations. NIV was not detected in any sample in 2009, 2010 and 2014 while it was detected just in one sample in 2013 at level 0.14 μg/g. T-2 and HT-2 toxins were detected in one sample in 2009 at level 0.07 μg/g and 0.06 μg/g, respectively, while they were not detected in 2010 and 2014. In 2013, T-2 and HT-2 ranged from 0.08 μg/g to 0.14 μg/g and from 0.04 μg/g to 0.80 μg/g, respectively. In 2013, DON level was high in general, but lower mean levels of DON were detected in hard red wheat than in soft white wheat. DON level was low in general in 2014, and the highest in cv 'Wentworth' at Ridgetown location (1.6 μg/g). In conclusion, several times higher average levels of DON were detected in 2013 and 2015 compared to previous years, with some winter wheat lines showing a level of tolerance to mycotoxin accumulation. Future monitoring of trichothecene mycotoxins in winter wheat in Ontario is recommended.

STATE OF THE ART IN MULTI-MYCOTOXIN DETERMINATION BY LC-(HR)-MS/MS

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ABSTRACT

Methods based on liquid chromatography – tandem mass spectrometry (LC-MS/MS) for the simultaneous determination of mycotoxins in different commodities are steadily increasing in popularity. In the presentation we will show applications using highly sensitive low resolution triple and quadruple mass spectrometers (QqQ), as well as the possibilities offered by high resolution quadruple time-of-flight (QTOF) instruments. All of the applications are based on a simple sample preparation procedure using acidified aqueous acetonitrile extraction, without further clean-up, and electrospray ionisation interfaces. Challenges in the development of such multi-analyte methods and the advantages and disadvantages of the different approaches will be discussed.

One of the obstacles in accurate quantification are matrix effects caused by suppression or enhancement of the analyte signal due to co-eluting matrix components. The use of stable isotopically labelled standards is an effective approach to compensate for these matrix effects. An accurate, reliable and fast method for the quantification of mycotoxins, currently regulated in the European Union in solid foodstuff, was developed, validated and will be presented.

A second method for the determination of several hundreds of mycotoxins and other fungal and bacterial metabolites in food and feed samples will also be discussed. In this approach, two specific transitions are optimized for each analyte and are acquired in a predefined retention time window. This method has been in-house validated for 295 analytes in four model food matrices, but currently comprises over 500 compounds.

Finally, the application of a database and spectral library using LC-QTOF-MS/MS will be presented. The acquired accurate mass spectra were corrected to their theoretical mass-to-charge ratio by a probabilistic approach before incorporation into the library. Once the MS/MS library has been created, standards are not required for the identification of compounds, moreover, this approach also allows post-acquisition data analysis.