Title Aflatoxins and ochratoxin A in stored barley grain in Spain and impact of PCR-based strategies to assess the occurrence of aflatoxigenic and ochratoxigenic *Aspergillus* spp.

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Abstract

Contamination of barley by moulds and mycotoxins results in quality and nutritional losses and represents a significant hazard to the food chain. The presence of aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2) and ochratoxin A (OTA) in stored barley in Spain has been studied. Species-specific PCR assays were used for detection of *Aspergillus flavus, A. parasiticus, A. ochraceus, A. steynii, A. westerdijkiae, A. carbonarius* and *A. niger* aggregate in mycotoxin-positive barley samples at different incubation times (0, 1 and 2 days). Classical enumeration techniques (CFU/g) in different culture media for evaluation of *Aspergillus* in sections *Flavi*, *Circumdati* and *Nigri* were also used. One hundred and five barley kernel samples were collected in Spanish grain stores from 2008 to 2010, and analyzed using a previously optimized method involving accelerated solvent extraction, cleanup by immunoaffinity column, liquid chromatographic separation, post-column derivatization with iodine and fluorescence detection. Twenty-nine samples were contaminated with at least one of the studied mycotoxins. AFB1, AFB2, AFG1, AFG2, and OTA were detected in 12.4%, 2.9%, 4.8%, 2.9%, and 20% of the samples, respectively. Aflatoxins and OTA co-occurred in 4.8% of the samples. Maximum mycotoxin levels (ng/g) were 0.61 (AFB1), 0.06 (AFB2), 0.26 (AFG1), 0.05 (AFG2), and 2.0 (OTA). The results of PCR assays indicated the presence of all the studied species, except *A. westerdijkiae.* The PCR assays showed high levels of natural contamination of barley with the studied species of *Aspergillus* which do not correspond to the expected number of CFU/g in the cultures. These results suggest that a high number of non-viable spores or hyphae may exist in the samples. This is the first study carried out on the levels of aflatoxins and OTA in barley grain in Spain. Likewise, this is the first report on the presence of aflatoxigenic and ochratoxigenic *Aspergillus* spp. in barley grain naturally contaminated with those mycotoxins using a species-specific PCR approach.