Mycotoxins in Post-harvest Maize in Three Portuguese Regions

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Abstract

The reduction of yield, quality, and nutritional value of grain cereals by filamentous fungi and subsequent contamination with mycotoxins is of great concern around the world. Mycotoxins are known to cause serious health problems in animals causing weight gain reduction, capillary fragility, reduced fertility, suppressed disease resistance, and even death. Some mycotoxins such as fumonisins, aflatoxins and ochratoxins, in particular, have also been associated with human health problems. The key mycotoxigenic moulds in partially dried grain are *Aspergillus flavus*(aflatoxins), *A. ochraceus* (ochratoxins) and some *Fusarium* species (fumonisins, trichothecenes) on temperate and tropical cereals. Aflatoxins, produced by *Aspergillus* section *Flavi* species, and fumonisins, produced by *Fusarium verticillioides*, are prominent among the mycotoxins associated to maize economic losses (*Zea mays* L.). The presence of aflatoxins and fumonisins in maize is of particular concern for maize products, as both mycotoxins are heat stable and survive the temperatures used in drying and maize processing. The presence of a given fungus does not mean that the mycotoxin(s) associated with that fungus are also present. There are many factors, especially environmental conditions and agricultural practices, involved in the production of mycotoxins.The aim of this work was to detect whether mycotoxins were present in post-harvested maize.

Ninety five maize samples collected from different agroclimatic regions of Portugal (Beira Litoral, Ribatejo, Alto Alentejo) were analyzed by HPLC for mycotoxin contamination. These samples were taken in three different steps of the storage chain and moisture content was measured immediately after sampling. Strains of *Aspergillus* section *Flavi* and *Aspergilllus* section *Nigri* were isolated after plating in MEA10. Several assays with different solvent mixes and shaking times were tested allowing the development of a simultaneous extraction based in protocols provided by Vicam of aflatoxins (AF), ochratoxin A (OTA) and fumonisins B1 and B2 (FB1 and FB2) with immunoaffinity columns. This method was validated by analysis of replicate spiked samples with 40µg/Kg of AF; 7 µg/Kg of OTA and 200µg/Kg of FB2. Cyclopiazonic acid (CPA) extraction was the one used by Mictotox LTDA. This method was validated by analysis of replicate spiked samples with 4000µg/Kg of CPA. Spiked samples were allowed to equilibrate for 24 hours prior to extraction. In addition, a matrix blank was also analyzed to determine any residual mycotoxin levels. Overall, three batches of duplicate spiked samples and one blank sample were analysed.

From the 95 samples obtained 287 strains belonging to *Aspergillus* section *Nigri* and 417 strains belonging to *Aspergillus* section *Flavi* were isolated. Mycotoxins were detected in 67% the samples. Sixty four percent of the samples were positive for FB1 and FB2 with values below 100µg/Kg (LOD of 0.1µg/Kg). Only 8% of the samples were positive for aflatoxins (LOD of 0.1µg/Kg), being AFB1, AFG1 and AFG2 detected in 5, 5 and 1 sample, respectively. AFB2 was not detected. Levels for AFB1 and AFG1 ranged from 0.1-0.5 µg/Kg. Neither OTA (LOD of 0.04µg/Kg) nor CPA (LOD of 10µg/Kg) was detected in any sample. Validation tests revealed recovery values between 61- 68 % for AF, 70% for OTA, 81 % for FB2 and 83% for CPA.

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