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MYCOTOXIN DETECTION OF HARVESTED MAIZE KERNELS

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KEYWORDS
Maize, mycotoxin detection, *Aspergillus*.

ABSTRACT

Mycotoxins are known to cause serious health problems in animals. Some mycotoxins such as fumonisins, aflatoxins and ochratoxins, in particular, have also been associated with human health problems. 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.) (Kumar et al. 2008). There are many factors, especially environmental conditions and agricultural practices, involved in the production of mycotoxins (Chulze, 2010).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.

Mycotoxins were detected in 67% the samples. Sixty four percent of the samples were positive for FB1 and FB2. Only 8% of the samples were positive for aflatoxins (LOD of 0.1µg/Kg). Neither OTA (LOD of 0.04µg/Kg) nor CPA (LOD of 10µg/Kg) was detected in any sample.

MATHERIALS AND METHODS

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 fumonisin B2 (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.In addition, a matrix blank was also analyzed to determine any residual mycotoxin levels.

RESULTS AND DISCUSSION

Mycotoxins were detected in 67% the samples. Sixty four percent of the samples were positive for 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. It was not detected OTA or CPA in any sample (Tables 1and 2).

Results show that the presence of a given fungus does not mean that the mycotoxin(s) associated with that fungus are also present (Pitt and Hocking 2009). *Aspergillus flavus* is a know AF and CPA producer and its presence was detected during this study. But only a small percentage of AF were detected and none of CPA was detected.The same could be observed for OTA. During this study a considerable amount of fungi belonging to the *Aspergillus* section *Nigri* were detected. This fungi are known to be potential OTA producers but this mycotoxin was not detected. The mycotoxin that was consistently detected was FB2. However, even though recent studies show that *Aspergillus niger* strains are producers of this mycotoxin, *Fusarium* species are well FB2 producers along with other fumonisins. With this results is unclear if all the FB2 detected came exclusively from the *Fusarium* species or could also be as result of *Aspergillus niger* strains production. Overall, the presence of mycotoxins in the maize batches obtained in the three regions show small values wich shows that the processing and storage of maize by the producers has been done correctly.

Region	AFG2		AFG1		AFB2		AFB1	
	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos
B. Litoral	32	0	29	3	32	0	29	3
Ribatejo	39	1	38	2	40	0	39	1
A. Alentejo	23	0	23	0	23	0	22	1
Total	94	1	90	5	95	0	90	5

Table 1 – Aflatoxin (AF) detection of maize obtained in three Portuguese Regions

Region	OTA		FB2		CPA	
	Neg	Pos	Neg	Pos	Neg	Pos
B. Litoral	32	0	15	17	32	0
Ribatejo	40	0	13	27	40	0
A. Alentejo	23	0	8	15	23	0
Total	95	0	36	59	95	0

Table 2 – Mycotoxin detection of Portuguese maize

FOOTNOTES AND ENDNOTES

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REFERENCES

Chulze S.N. 2010. Strategies to reduce mycotoxin levels in maize during storage: a review. *Food Additives and Contam*ination 27, 651−657.

Kumar, V.; Basu, M.S.; and Rajendran, T.P. 2008. Mycotxin research and mycoflora in some commercially important agricultural commodities. *Crop Protection* 27, 891-905.

Pitt, Pitt, J.I. and Hocking, A.D. 2009. Fungi and Food Spoilage, third edition. *Springer*, 328-330.