“Hot spots” in Cocoa? Mycotoxin Distribution in cocoa beans

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Introduction

Aflatoxins and ochratoxin A (OTA) are secondary metabolites of toxigenic moulds of the genera Aspergillus and Penicillium [1, 2].

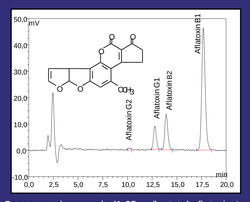
OTA primarily occurs in native starchy cereals. It has also been detected in nuts, figs, coffee beans (raw and roasted), in spices, in olive oil, wine, beer, and also in cocoa [2, 4]. Aflatoxins frequently develop in high-protein products that are grown in humid warm regions, such as nuts (peanuts, pistachios), maize, dried figs, and various spices such as pepper and paprika. Aflatoxins have also been detected in cocoa [4].

Mycotoxins are often extremely inhomogeneously distributed in raw materials that come in naturally small units, such as pistachios; this phenomenon is described using the term “hot spots”. Tests conducted on pistachios, for example, showed that a mouldy kernel can be so strongly contaminated with mycotoxins that it has a significant impact on the contamination profile of several thousand kernels [3]. Whether cocoa beans also have a tendency to form mycotoxin “hot spots” is hitherto unknown. The focus of the work conducted was on studying the statistical distribution of the total aflatoxins and OTA. within large cocoa batches and within individual cocoa beans.

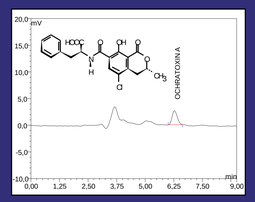
Miniaturisation of Analysis Methods

The most frequent method currently used for analysing mycotoxins is high performance liquid chromatography (HPLC) with fluorescence detection. The mycotoxins are isolated beforehand using special immunoaffinity columns.

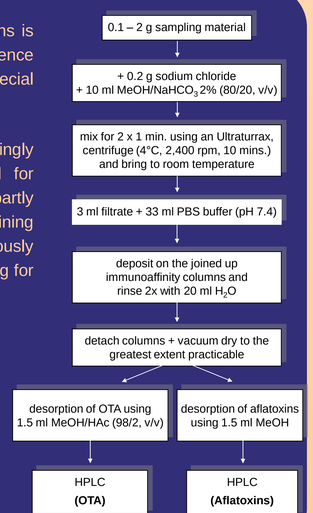
To examine individual beans it was necessary to correspondingly miniaturise the existing analysis methods that are optimised for samples weighing around 50 g to accommodate samples partly weighing <100 mg [4]. This was the only possible way of examining individual beans, split into shell and kernel, while simultaneously maintaining an acceptable detection limit in the region of 0.04 µg/kg for aflatoxins and 0.1 µg/kg for OTA..



Cocoa powder sample (1.65 µg/kg total aflatoxins)

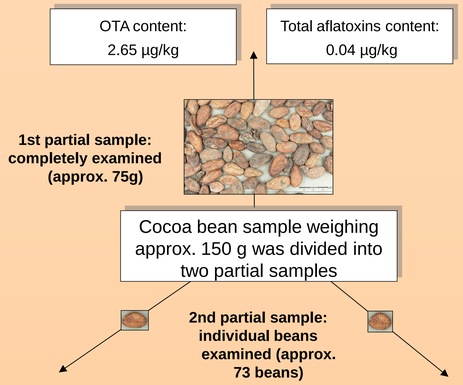


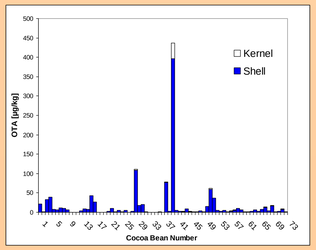
Cocoa powder sample (1.50 µg/kg OTA)

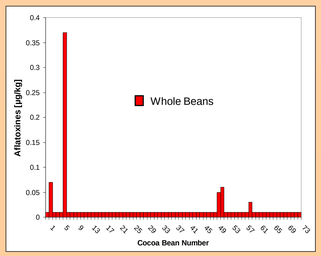


Distribution within cocoa bean batches

To conduct the envisaged examination of individual cocoa beans, a select raw cocoa bean sample from the Ivory Coast region with a total weight of around 1,200 g was first divided into two statistically equivalent portions using a sample splitter until a sample weighing 150 g was gained. Subsequently the latter was split into two further partial samples (see diagram below). The first partial sample was completely pulverised and tested for total aflatoxins and OTA. To test the second partial sample for OTA, all 73 beans were separated into shell and kernel and individually examined. By contrast, the aflatoxin tests were conducted on whole individual cocoa beans. As can be seen from the presented charts, the mycotoxin levels determined in the partial sample tested as a whole exactly match the mycotoxin levels determined by testing individual beans.







Distribution within individual cocoa beans

In preparation for the envisaged examinations, a certain cocoa bean sample originating from the Ivory Coast and having a total weight of around 1,000 g was first divided into equal portions several times over using the sample divider. One of the resultant divided samples was subsequently halved using a MAGRA cutting unit (made by Tserba/CH) as shown in Figure 1 and a selection of the ensuing cocoa bean halves was examined for OTA content levels using the described miniaturised sample processing procedure.

A total of 57 halved cocoa beans (including their shells), hence 114 bean halves, were analysed within the scope of this assay. Of these, 24 cocoa bean halves tested positive for OTA (at a limit of detection of LOD ≤0.1 µg/kg). Figure 2 shows the distribution of OTA content levels found in those 17 cocoa beans of which one or both corresponding bean halves tested positive for OTA. Values below the LOD are included in the figures for half the LOD (0.05 µg/kg).

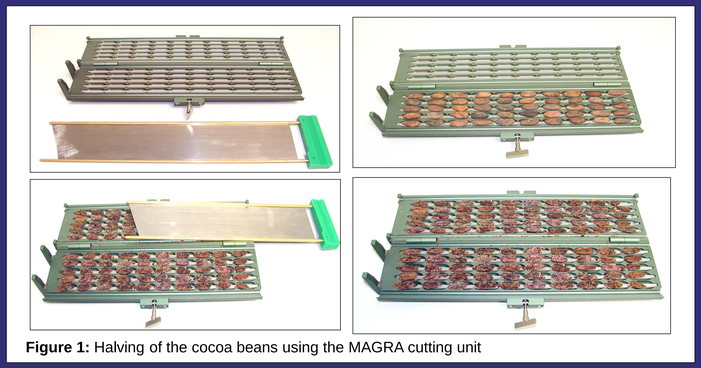


Figure 1: Halving of the cocoa beans using the MAGRA cutting unit

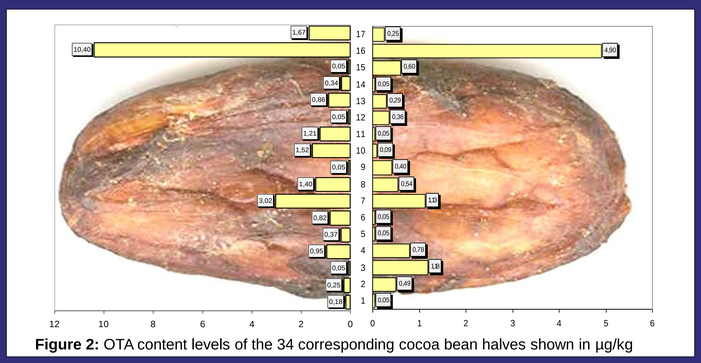


Figure 2: OTA content levels of the 34 corresponding cocoa bean halves shown in µg/kg

Conclusions

The analysis method usually used to determine aflatoxins and OTA was successfully miniaturised to meet the requirements of the conceptual formulation. This also created considerable time and materials savings due to the low extraction volume involved.

The examinations conducted to determine distribution characteristics showed a more asymmetrical distribution of the individual levels both for aflatoxins and for ochratoxin A. In view of the exact consistency between the mycotoxin levels determined in the partial sample as a whole and those detected in the individual bean sample tests, it is to be assumed that, in comparison to other mycotoxin-containing goods (peanuts or pistachios), the individual cocoa beans have a relatively low value distribution level. Thus real “hot spots” were not detected in the examined batch. Furthermore, it was again shown that the content of OTA is predominantly to be found in the cocoa shell and not in the cocoa kernel [5].

In examining cocoa beans halved in the cutting test, 17 of the 57 examine cocoa beans tested positive for OTA (LOD <0.1 µg/kg). Furthermore, it was found that there were sometimes considerable variations in OTA content levels even in the corresponding cocoa bean halves of the same cocoa bean. OTA content levels ranged up to 5.49 µg/kg in one and the same individual cocoa bean.

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[2] Deutsche Forschungsgemeinschaft (1990) Ochratoxin A - Vorkommen und toxikologische Bewertung. VCH Verlagsgesellschaft, Weinheim
[3] Steiner E, Brunschweiler K, Leimbacher E, Schneider R (1992) Aflatoxines and fluorescence in brasil nuts and pistachio nuts. J Agric Food Chem 40: 2453-2457
[4] Raters M, Matissek R (2003) Neue Studien zur Analytik und zum Vorkommen von Ochratoxin A in Kakao und kakaohaltigen Erzeugnissen (not published)
[5] Raters M, Matissek R (2005): Study on distribution of mycotoxins in cocoa beans. Mycotoxin Research Vol 21, No 3: 182-186

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