Study on Distribution of Mycotoxins in Cocoa Beans

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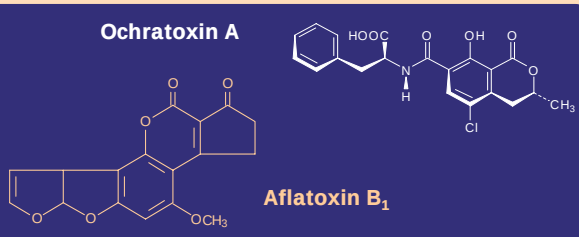
Introduction

Aflatoxins and ochratoxin A (OTA) are secondary metabolites of toxigenic moulds of the genera Aspergillus and Penicillium.
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]. The presence of these mycotoxins in foodstuffs is undesired due to their toxicological and carcinogenic potential [1, 2].

Mycotoxins are 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 so-called mycotoxin hot spots is hitherto unknown. The focus of the work conducted was on studying the statistical distribution of the mycotoxins total aflatoxins and ochratoxin A.

Ochratoxin A

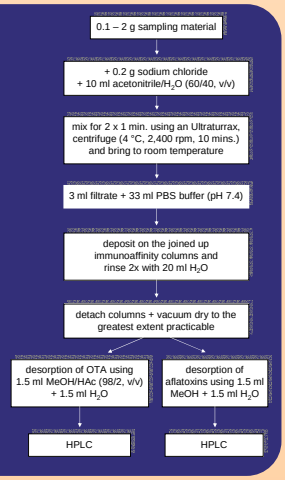


Aflatoxin B_1

Miniaturisation of Analysis Methods

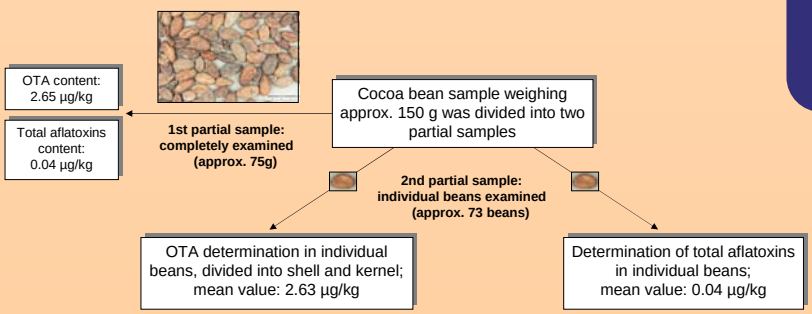
The most frequent method currently used for analysing mycotoxins is high performance liquid chromatography (HPLC) with fluorescence detection. The mycotoxins are isolated beforehand using special immunoaffinity columns.

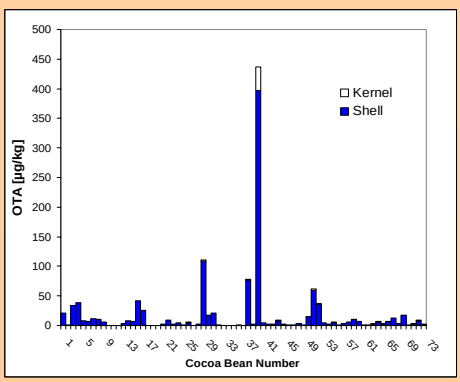
To examine individual beans it was necessary to correspondingly miniaturise the existing analysis methods that are optimised for samples weighing around 50 g to accommodate samples partly weighing < 100 mg [4]. This was the only possible way of examining individual beans, split into shell and kernel,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

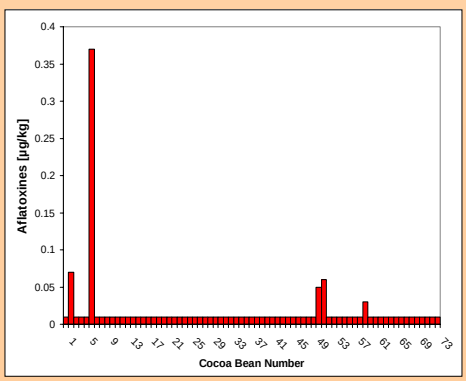


Test Setup / Results

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). The first partial sample was completely pulverised and tested for total aflatoxins and ochratoxin A. To test the second partial sample for OTA, all 73 beans were divided 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.







HPLC Conditions

	OTA	Aflatoxins
Separation column	Sperisorb ODS 2; 5 µm, 4.6 x 250 mm	
Eluent	MeOH / H_2O / HAc (70.5 / 25.5 / 4, v/v/v)	H_2O / MeOH / ACN (60 / 20 / 20, v/v/v) + 100 µl HNO_3 + 119 mg PBr
Flow	1.0 ml isocratic	1.2 ml isocratic
Injection volume	20 µl	100 µl
Column temperature	20 °C	20 °C
Derivatisation	-	KOBRA cell
Fluorescence	λ_{Ex}=330 and λ_{Em}=460	λ_{Ex}=362 and λ_{Em}=440

Conclusion

The analysis method usually used to determine aflatoxins and ochratoxin A 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].

Bibliography

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