New Examinations of Mycotoxin Carryover to Cocoa Beans

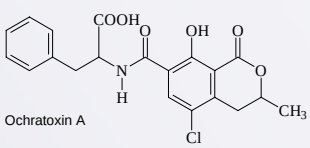
LCI Cologne

Marion Raters, Susanne Beucker, Reinhard Matissek

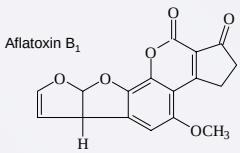
LCI *
Food Chemistry Institute of the Association of the German Confectionery Industry
Cologne/Germany, www.lci-koeln.de

Introduction

The mycotoxins ochratoxin A (OTA) and the aflatoxins B_1, B_2, G_1, and G_2 are secondary metabolites of the mould species Aspergillus and Penicillium. OTA primarily occurs in native starchy cereals. Aflatoxins frequently grow in high-protein products of moist, warm regions. Both were also detected at low concentration levels in cocoa and cocoa products [1-5].



Ochratoxin A



Aflatoxin B_1

Compared to other raw materials, the mycotoxin content levels found in cocoa are not only lower, but their distribution within a batch is distinctly more homogeneous. So-called "hot spots" have not been determined in cocoa [4]. A key issue affecting distribution would seem to be the time point when contamination with mycotoxin-producing moulds and the formation of mycotoxins took place. It is presumed that in the case of cocoa, contamination levels are not so much affected by individual beans as by the fermentation unit as a whole.

The objective of this work was to use a model assay to test for a possible carryover of the aforementioned mycotoxins to cocoa beans during fermentation.

Fermentation

The fermentation of cocoa beans takes place, among other things, in so-called fermentation boxes (Figure 1). As soon as the sugar-containing fruit pulp is exposed to air, it is contaminated by ever present microorganisms of very diverse types and by insects. The fermentation process begins with the fermentation of the sugar substances in the fruit pulp [6].

Processes during fermentation

- Liquefication of the fruit pulp and detachment from the shell through the development of microorganisms and yeast cells
- Temperature rises up to 50 °C; pH value of the cocoa seeds falls from 6.5 to around 4 due to the formation of acetic acid
- acetic acid content in the fermentation juice falls from 2.5 to 1.6 %; pH value of the fruit pulp increases from 3.5 to 5


Figure 1: Fermentation box

Model Assay

To create conditions most closely resembling reality, the beans of a fresh cocoa fruit with fruit pulp still attached were placed into an artificial fermentation solution (Figure 2). At periodic intervals, 2 beans (to balance out possible fluctuations) were respectively removed, dried, and separated into shell and kernel.

Model conditions

- "Fermentation solution" with defined content of OTA (3.15 µg/l) and aflatoxins (aflatoxin B_1 5.22 µg/l)
- HAc aqueous solution; pH value: 3.5
- Temperature: 35 °C (drying oven); loosely sealed container


Figure 2: Cocoa beans with attached pulp in the "fermentation solution"

Literature

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[5] Tafuri A, Ferracance R, Ritieri A (2004) Ochratoxin A in Italian market cocoa products. Food Chemistry 88: 487-494

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Results

The results show that both aflatoxin B_1 (here as a tracer for the aflatoxins B_1, B_2, G_1 and G_2) and OTA carry over from the "fermentation solution", first into the cocoa shells and, with a short time lag, into the kernel (Figure 3). The carryover into the shells reached a maximum level after only 2 days (only 28 hours in the case of aflatoxin B_1). As regards the carryover of the observed mycotoxins to the cocoa kernels, it can be seen that the aflatoxin B_1 content levels diminish again after peaking at around 4 µg/kg, whereas the OTA levels rise continuously over the entire testing period.

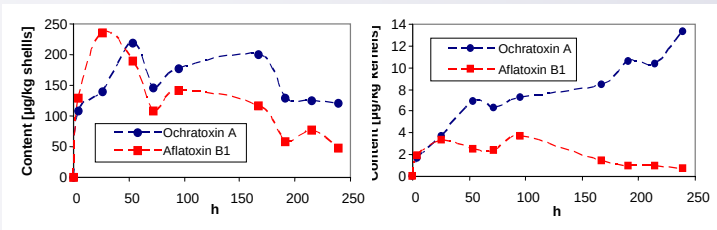

Figure 3: Aflatoxin B1 and OTA levels in the shells and kernels of the "fermented" cocoa beans

In the "fermentation solution" (Figure 4), the concentration level of aflatoxin B_1 is initially quicker to fall than that of OTA. After 26 hours, the cocoa beans have already taken up 45 % of the aflatoxin B_1 content in the solution and only 15 % of the OTA content. Seen over the entire testing period, the cocoa beans absorbed around 54 % of the original aflatoxin B_1 content and 44% of the original OTA content from the surrounding solution.

As an explanation for the different absorption behaviour of the observed mycotoxins aflatoxin B_1 and OTA, it is presumed that the mycotoxins, depending on their respective polarity and due to certain swelling processes, are absorbed at different rates by the various histological layers of the cocoa bean.

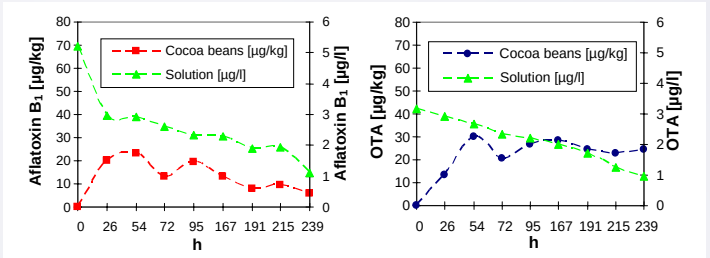

Figure 4: Aflatoxin B_1 and OTA levels in the "fermented" cocoa beans and in the "fermentation solution"

CONCLUSIONS

A model essay was used to test for a possible carryover of the aflatoxins and ochratoxin A during the fermentation process. It was shown that already in the first two days of the assay, there was a significant carryover of both mycotoxins from the surrounding solution into the cocoa shells and then into the cocoa kernels. This goes to show that fermentation plays a decisive role in determining the mycotoxin contamination picture of cocoa beans as regards aflatoxins and OTA.

The various absorption patterns of the observed mycotoxins still require further research. This is possibly caused by chemico-physical effects, such as the different polarities of the mycotoxins.

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