Zearalenone in cocoa-containing milk products, desserts and chocolate

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The *Fusarium* mycotoxin zearalenone (ZEN) is frequently found as a contaminant in all major cereal grains and other plant material, including feedingstuff for dairy cows. A number of studies have shown that carry-over of ZEN from feed into cow's milk is minimal. However, because ZEA is highly lipophilic, the initial aim of this study was to examine whether ZEN may occur selectively enriched in the fat fraction of milk, which comprises only 4-5% of the total milk volume. For sample preparation, a simple extraction method for ZEN from whipped cream (30-35% fat) was developed, which involves intermediate alkaline cleavage of ZEN (Majerus et al., 2009). An enzyme immunoassay (EIA) was used for detection. In pure whipped cream samples (n = 38), no ZEN was detectable above the detection limit (2 µg/kg). However, within this survey two samples of ready-to-use whipped cream spray containing cocoa were found positive for ZEN at 5-6 ng/g. Therefore the focus of the study was directed on cocoa-containing milk products. Seven out of 28 samples of milk mix drinks with cocoa were positive for ZEN at levels of 1.5-6.5 µg/kg (mean 3.6 µg/kg). Chocolate desserts (n = 63) were frequently posititive (52%) for ZEN, at levels of 1.1-9.8 µg/kg (mean 3.4 µg/kg). The contamination level corresponded with the percentage of cocoa in a product and with the type of cocoa. The highest amounts of ZEN were found in products with high contents of dark chocolate. Further analysis of chocolate confirmed this trend. All samples of dark cocoa chocolate (n = 11) and white cocoa chocolate (n = 2) were positive for ZEN at 3-29 µg/kg, while 75% of brown milk chocolates (n = 4) were positive at 2.3-5.2 µg/kg. Selective confirmatory analyses by HPLC-FLC and LC-MS/MS qualitatively confirmed the presence of ZEN, although quantitative agreement was poor. To check the hypothesis that cacao was the source of ZEN, cacao beans were purchased via internet from three different sources. Each one batch was from Venezuela and from Ecuador, one batch was from a western African country (not specified). Shells but not nibs from Venezuelan and western African beans were positive for ZEN (approx. 10 ng/g). Mycological culture of beans on malt extract agar yielded three morphologically different fungal isolates, two were tentatively identified as *Aspergillus* spp and were negative for ZEN. One fungal isolate was identified as *Eurotium rubrum* by morphological means and by ITS sequencing, which after culture on malt extract agar for 13 days produced ZEN at levels of approx. 100 ng/g agar plug. *E. rubrum* is not known to produce ZEN and further analysis to confirm these results are needed. This is the first report about natural occurrence of ZEN in cocoa-containing food products. Although the levels of ZEN in all cocoa-containing products were quite low, the widespread occurrence of this toxin requires further consideration, because the average consumption of cocoa products is relatively high.

Reference: Majerus P., Graf N., Krämer M. (2009) Rapid determination of zearalenone in edible oils by HPLC with fluorescence detection. Mycotoxin Res 25: 117-121