Bioavailability of aflatoxins in cultured fish and animal livers using an in-vitro dialysability

approach

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INTRODUCTION

Aflatoxins (AFs) represent a group of toxic metabolites of mould that significantly affect human and animal

health. Animal tissues can retain AF residues, including AF metabolites, giving rise to potential health issues. They can be transferred to the human body from food (including meat). To understand how AFs can transform, sufficient knowledge of the amount of AFs in foodstuffs and the fraction of AFs absorbed by the human body is required. Human bioavailability approaches, mainly encompassing processes such as digestion, absorption, transport, utilisation and elimination, are useful strategies for understanding the fraction of nutrients and pollutants that can theoretically be released into the gastrointestinal tract (GI) and become available for intestinal absorption. Human bioavailability approaches are split into in-vivo and in-vitro assays. The current study is focused on the development of an in-vitro bioaccessibility method, which helps to indicate the maximum fraction of AFs in the muscle and liver that can theoretically be released from foodstuffs into the GI tract (bioaccessible fraction). This then becomes available for entering into the bloodstream.

METHODOLOGY

The AF content in (raw and cooked) muscle and liver samples was extracted with 5 mL of 60:40 acetonitrile/0.1 M aqueous KH_2PO_4 (pH 7.0) solution and the extract was subjected to a pre-concentration method based on a vortex-assisted liquid-liquid microextraction (VALLME) procedure which consisted of mixing 25 mg of NaCl with 5.0 mL of extract and adding 400 μL of chloroform as an extractant before vortexing at 2000 rpm for 1 min. The chloroform extract was fully dried under an N_2 flow and re-dissolved in 100 μL of methanol and injected into the UHPLC-MS-MS instrument. The in-vitro dialysability procedure was performed with gastric solution (16 % (m/v) pepsin in 0.1 M HCl) and intestinal solution (4.0 % (m/v) pancreatin and 2.5 % (m/v) bile salt solution prepared in 0.1 M sodium hydrogen

carbonate, pH 7.4) under the optimum conditions. AF content in dialysable (in PIPES solution) and non-dialysable fractions was evaluated by following the validated VALLME method. A mass balance study was used to assess the accuracy of the bioavailability study and statistically compared (ANOVA test, 95 % confidence interval) with the AF concentration in the standards/foodstuffs prior to the in-vitro bioavailability approach.

RESULTS

Raw samples showed bioavailability ratios of 41–45 % for aflatoxin B1 (AFB1), 28–38 % for aflatoxin B2 (AFB2), and 42 % for aflatoxin G2 (AFG2). Aflatoxin G1 (AFG1) was not detected. The cooking process (steaming or grilling) was found to change AF bioavailability (higher bioavailability ratios were observed in cooked samples). The cooking process was found to affect the dialysability of AFs in different ways. Steaming does not affect AF bioavailability. Dialysability ratios (42–54 % for AFB1, 29 % for AFB2, and 43 % for AFG2) were quite similar to those found in raw samples. However, grilling increases AF bioavailability, and AFB1 dialysability was found to be within the 54–59 % range, while values of 37 and 56 % were obtained for AFB2 and AFG2, respectively. Statistical dialysability ratios for fried samples were found to be significantly higher ($p < 0.05$) than those found in raw and steamed samples and in AF aqueous standards. AFB2 was found to be transformed into other compounds during the in-vitro process, and the presence of AFB2 and AFB2 transformation/degradation products was investigated and confirmed by high-resolution mass spectrometry (HRMS).

DISCUSSION

Cooked samples, predominantly fried samples, produced slightly higher dialysable AF concentrations than raw samples. These findings align with those obtained when assessing the AF content in raw and cooked samples. They are explained by taking into account the moisture content of the samples, which is reduced from 78-83 % in raw samples to 69-76 % in steam samples and 43-59 % in fried samples. A mass balance study was performed by comparing the sum of the AF levels in the dialysable and non-dialysable fractions with AF content in the samples. AFB2 indicates a significant statistical difference between AFB2 in the sample and the sum of AFB2 concentrations in the dialysable and non-dialysable fractions. These results align with those obtained when using AFB2 aqueous standards and should be attributed to AFB2 transformation/degradation into other compounds during the in-vitro assay. Therefore, AFB2 is degraded during the in-vitro dialysability process and several degradation products are present in both dialysable and non-dialysable fractions.