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Fumonisins and their masked forms in maize products

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ABSTRACT

In recent years many papers on masked mycotoxins in maize-based products appeared, including reports on fumonisins capable to form non-covalent bonds with food macro constituents. Such so-called "hidden fumonisins" are frequently present in food at quantities higher than the free forms. The aim of this work was to assess levels of free and total (free + hidden) fumonisins (B_1, B_2 and B_3) in 88 maize products available on the Polish retail market. Isotope dilution ion trap mass spectrometery coupled to a high performance liquid chromatography was used. 57% of all tested samples contained free fumonisins at concentrations above our limit of quantification LOQ (mean 390 ± 676 μg/kg). More than 77% of the samples contained free + hidden fumonisins at concentrations above LOQ (mean 574 ± 1177 μg/kg). The highest mean fumonisins concentrations 1006 ± 1131 μg/kg, 1651 ± 2317 μg/kg, respectively for free and free + hidden forms were observed in the group of maize snacks. The lowest fumonisin concentrations were found in maize-based starch concentrate products. None of the tested products within that group had free fumonisins concentrations above LOQ, while mean concentration of free + hidden fumonisins was as low as 82 ± 42 μg/kg. In thermally processed products like corn flakes and various snacks the hidden-to-free fumonisin concentration ratio was higher than in unprocessed products like flour, groats or raw popcorn grains.

1. Introduction

Fumonisins are fungal secondary metabolites produced mainly by *Fusarium verticilloides* and *Fusarium proliferatum*, species commonly found on maize (Pascale, Visconti, Prończuk, Wiśniewska, & Chełkowski, 1997; Warfield & Gilchrist, 1999; Weidenbörner, 2001). They belong to mycotoxins that might cause adverse health effects in mammals, including hepatotoxicity, nephrotoxicity, or cytotoxicity. It was proven that chronic exposure to high doses of fumonisin B1 may results in an increased risk of esophagus cancer development (Munkvold & Desjardins, 1997; Clements, Kleinschmidt, Maragos, Pataky, & White, 2003). In this respect IARC has classified FB1 to toxicity class 2B (IARC, 2002).

Contamination of maize with fumonisins is commonly reported in southern Europe. This is expected to be related with the seasonal climate fluctuations (Pietri, Bertuzzi, Pallaroni, & Piva, 2004). In this respect European Union has set the maximum allowable levels of fumonisins (sum of FB1 and FB2) regarding unprocessed maize grain and some maize products (EC No 1126/2007). Maize grain intended for food purposes in Poland is mainly imported. However, in recent years also maize grain produced in the country was more and more frequently processed (CSO 2013). It was used to produce flour, groats, corn flakes, popcorn, snacks, gluten free bread, and infants formulas.

Maize becomes infected by *Fusarium* during the growing season. Suitable temperature and humidity increase the infestation rate and result in larger production of fumonisins (Lazzaro et al., 2012). Grain processing (including cleaning) might decrease fumonisin concentration (Pascale et al., 1997; Humpf & Voss, 2004; FAO/WHO 2012; Bryła, Roszko et al., 2013). However, maize processing at elevated temperatures might lead to formation of masked fumonisins. Alkylated derivatives of fumonisins like *N*-carboxymethyl fumonisin B1 or *N*-deoxy-D-fructose-1-yl fumonisin B1 were among the first discovered compounds. Those are formed in reactions with reductive carbohydrates at elevated temperatures (e.g. during extrusion of maize grits at 160–180 °C) (Seefelder, Hartl, & Humpf, 2001). In recent years some reports on formation of

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fumonisins esterified with fatty acids were also published. Those were identified in *Fusarium vericilloides in vitro* cultures grown on rice and maize (Bartók, Szécsi, Juhász, Bartók, & Mesterházy, 2013; Bartók, Tölgyesi, Mesterházy, Bartók, & Szécsi, 2010; Falavigna, Cirlini, Galaverna, & Dall'Asta, 2012; Falavigna, Cirlini, Galaverna, Sforza, et al., 2012). *N*-acylated derivatives of FB1 were also identified in *Fusarium* cultures grown on rice. Concentrations of the derivatives were however significantly lower than concentration of native forms (Bartók et al., 2013, 2010).

Fumonisins non-covalently bound to food macro-constituents like proteins or carbohydrates are also regarded masked fumonisins. Such compounds were identified in maize grain and maize products. Contrary to fumonisins bound to other substances with covalent bonds these are called "hidden fumonisins" (Dall'Asta et al., 2009a; Dall'Asta, Falavigna, Galaverna, Dossen, & Marchelli, 2010; Falavigna, Cirlini, Galaverna, & Dall'Asta, 2012). Concentration of these compounds might well exceed concentration of the free forms, and significantly contribute to profile of masked fumonisins (Dall'Asta, Mangia et al., 2009, Dall'Asta, Galaverna et al., 2009, Dall'Asta et al., 2010; Di Mavungu & De Saeger, 2011; Falavigna, Cirlini, Galaverna, & Dall'Asta, 2012). In addition it is believed that hidden fumonisins might be released inside the human gastrointestinal tract, therefore it seems important to assess their actual levels in food and feed for toxicological reasons (Falavigna, Cirlini, Galaverna, & Dall'Asta, 2012).

In this study concentrations of free and masked fumonisins have been evaluated in various group of low- and medium-processed maize products available on the Polish retail market. Free fumonisin forms, hidden esterified fumonisins, as well as fumonisins covalently bound via carboxyl group to other substances present in food easily hydrolyze in alkaline media, releasing its side chain off the native fumonisin molecule. Such reaction is depicted in Fig. 1.

2. Materials and methods

2.1. Chemicals and reagents

Only solvents of HPLC grade supplied by Rathburn (Walkerburn, UK) were used in this study. Formic acid, acetic acid, potassium hydroxide of prior to analysis grade were supplied by POCH (Gliwice, Poland). Molecularly imprinted polymeric SPE cartridges (FumoZON AFFINIMIP) were provided by Polyintell (Val de Reuil, France).

2.2. Samples

Fumonisins were determined in 88 samples of commercially available maize-based food products from a variety of manufacturers bought in local supermarkets in 2013, including groats (n = 15), starch concentrates (n = 6), noodles (n = 14), flour (n = 20), corn flakes (n = 19) and maize snacks (n = 14). Sampling was performed on a random basis. Prior to analysis the samples were stored at room temperature except for bread samples which were stored frozen below minus 18 °C.

2.3. Standards and reference materials

Native standards of FB1, FB2 & FB3 (50 μg/mL), labeled $^{13}C_{34}$-FB_1 (25 μg/mL), $^{13}C_{34}$-FB_2, $^{13}C_{34}$-FB_3 (10 μg/mL), and hydrolyzed HFB1 (25 μg/mL) were supplied by Biopure (Tulln, Austria). HFB2 and HFB3 standards were synthetized in a laboratory scale by alkaline hydrolysis of native FB2 and FB3's. Briefly, FBs standard solutions were transferred into a test tube with a ground glass joint. Solvent was evaporated under a gentle stream of nitrogen, then 2 M KOH aqueous solution was added. Digestion was performed for 24 h at room temperature. The solution was quantitatively transferred with acetonitrile into a separator funnel. Aqueous layer was extracted two more times with acetonitrile, organic extracts were combined evaporated to dryness using a rotary evaporator operated at 40 °C, then re-dissolved in acetonitrile. For further calculations a complete hydrolysis of fumonisins was assumed.

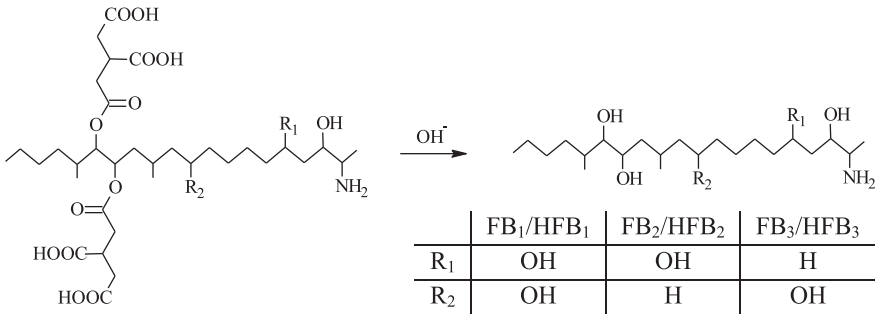
Biopure (BRM 003017) certified reference material of maize was used in validation experiments.

2.4. Analytical procedures

Free and hidden fumonisins were determined using our previously published method without any major modifications (Bryła, Jędrzejczak et al., 2013; Bryła et al., 2014; Bryła, Szymczyk, Jędrzejczak, & Obiedziński, 2015).

2.4.1. Free FBs

Briefly, samples were ground using a laboratory grinder. 2.5 g of sample was transferred into a glass beaker, spiked with 10 μl of the solution with $^{13}C_{34}$-FB1, $^{13}C_{34}$-FB2 and $^{13}C_{34}$-FB3 labeled internal standards and homogenized with 10 mL of methanol: acetonitrile: water solution 25:25:50 (v/v/v) for 3 min. The solution was centrifuged at 10,730 × g, 5 mL of the supernatant was mixed with 5 mL of water and used for analysis. Extracts were purified using polymeric SPE's. Cartridges were conditioned with 2 mL of acetonitrile and 2 mL of water at 3–4 droplets per second flow rate. Two 4 mL portions of the diluted extract were loaded on the cartridge. Cartridge was washed with 6 mL of acetonitrile: water mixture 40:60 (V/V) and subsequently eluted with 4 mL of formic acid: methanol solution 2:98 (V/V) into a 25 mL round bottom flask. Solution was evaporated to dryness using a rotary evaporator and re-dissolved in 1 mL of methanol: water: acetic acid mixture (10:89.9:0.1) and sonicated in an ultrasound bath to improve transfer of the analyte and sample dissolution. Samples were



	FB_1/HFB_1	FB_2/HFB_2	FB_3/HFB_3
R_1	OH	OH	H
R_2	OH	H	OH

Fig. 1. Degradation of fumonisin molecules in alkaline media.