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Exposure assessment to fumonisins B_1, B_2 and B_3 through consumption of gluten-free foodstuffs intended for people affected by celiac disease

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ABSTRACT

Fumonisins are mycotoxins produced by Fusarium species and affecting maize crops. Some analogues of fumonisins are known for their toxic and possible carcinogenic effects on humans and animals. Because of their occurrence in corn-based food, diet is the main source of exposure to these mycotoxins, especially among people affected by celiac disease. Hence, the purpose of this paper was to evaluate the amount of fumonisins B1, B2 and B3 in maize-based products and to assess the exposure of people affected by celiac disease to fumonisins. The sample consisted of 154 gluten-free products analyzed according to method UNI EN 14352:2005. Results showed a heterogeneous contamination by fumonisin B1, B2 and B3, although below limits of Commission Regulation No 1126/2007 and consistent with other European literature data. Exposure to fumonisins was evaluated for different age groups. In some cases exposure to fumonisins could not be ignored since the total intake could exceed EFSA Provisional Maximum Tolerable Intake up to 150%. Therefore, in the light of an overall contamination by fumonisins the total dietary exposure could be underrated not only in people affected by celiac disease, but also in non-celiac population.

1. Introduction

Mycotoxins are secondary metabolites of some fungi species, generally belonging to *Aspergillus*, *Penicillium*, *Alternaria* and *Fusarium* genera, that could exert toxic effects on humans and animals. Among molds, *Fusarium* species are very common contaminants of agricultural crops and they are able to produce a great variety of mycotoxins such as fumonisins, trichothecenes, deoxynivalenol and zearalenone (Cirillo et al., 2003; Cirlini et al., 2012; Schollenberger et al., 2005; Yazar and Omurtag, 2008). In recent years, there has been an increasing interest in fumonisins due to their widespread occurrence in cereals, especially in maize and maize-based foodstuff and less frequently in vegetables such as asparagus, garlic and beans (Castells et al., 2008; Creppy, 2002; Seefelder et al., 2002). As introduced above, fumonisins are a group of structurally related mycotoxins produced by many *Fusarium* species including *Fusarium verticilloides* (formerly known as *F. moniliforme*), *F. proliferatum*, and *F. nygamai* which often show endophytic behaviour, infesting host plant tissues, without causing disease symptoms (Bennett and Klich, 2003; Zhang et al., 2013). Since 1988, when fumonisin was firstly isolated, 28 analogues are known so far which may be divided into four main series: A, B, C and P, even though most common form belongs to B-series. (Gelderblom et al., 1988; Rheeder et al., 2002; Sewram et al., 2005). In strict chemical terms, fumonisin is structurally characterized by 20 carbon aminopolyhydroxy-alkyl chain esterified with two molecules of tricarballylic acid (TCA) (Fig. 1) (Dall'Asta et al., 2008; Rheeder et al., 2002). As explained earlier, B form is the most frequently occurring fumonisin, different from other homologues in the composition of side chains whereby three common recurring analogues are known as Fumonisin B_1, B_2 and B_3. As stated above, these compounds are known for their toxic and possible carcinogenic, nephrotoxic and hepatotoxic effects on humans and animals. Existing studies have documented the toxic effects of fumonisins that are likely to be due to their structural similarity to sphingosine

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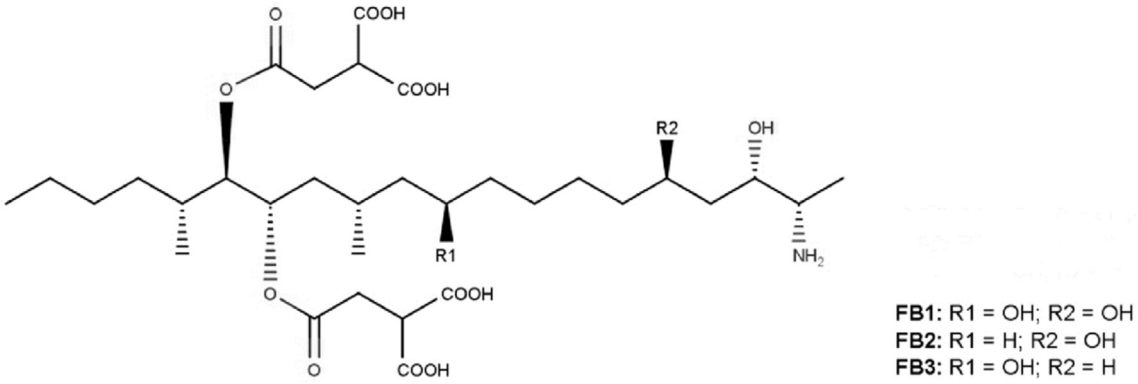

Fig. 1. Structure of fumonisin B_1 (FB1), fumonisin B_2 (FB2) and fumonisin B_3 (FB3).

and sphinganine, interfering with ceramide synthase and altering sphingosine to sphinganine ratio (Bryła et al., 2014; Dall'Asta et al., 2012; Domijan, 2012; EFSA, 2014; Gazzotti et al., 2011; IARC, 2002; Pagliuca et al., 2005). Occurrence of oesophageal cancer in human was related to ingestion of food contaminated by fumonisins in South Africa, China and USA as well as in Northen Italy where consumption of "Polenta" (a typical cornmeal based dish) is higher than other regions (Wan et al., 2013). Besides there is some evidence that fumonisins could also exert toxic acute effects, showing transient clinical symptoms featuring abdominal pain and diarrhoea (Bennett and Klich, 2003; Myburg et al., 2002; Peraica et al., 1999; Raiola et al., 2015; Sydenham et al., 1991). Because of their recognized harmful effects, maximum amount of fumonisin in maize food is regulated by Commission Regulation No 1126/2007 amending Regulation (EC) No 1881/2006 setting maximum levels for fumonisins in maize and maize-based products (EC, 2007). Because of their occurrence in maize-based food, it is commonly assumed that diet is the main source of exposure to these mycotoxins, especially in some countries where consumption of maize is a daily habit, or due to dietary restrictions as in celiac patients (Brera et al., 2014).

Celiac disease is a chronic widespread and immuno-mediated disease induced in genetically predisposed individuals by the ingestion of food that contains gluten. It affects about 1% of population and it is one of the most common chronic disease among children, even though some evidence suggests that the prevalence of celiac disease among children could be greater than 1% (Catassi et al., 1999; Ivarsson et al., 2013; Schuppan and Zimmer, 2013).

As celiac disease is a chronic and a permanent disorder, patients throughout their lifetime cannot eat any food that contains even little amount of wheat, oat, rye, barley and products thereof. Whereas maize and rice as well as potatoes and few other cereals and pseudocereals can be safely employed as carbohydrate source in the diet of celiac patients, since these products do not contain gluten (Saturni et al., 2010). Therefore, people affected by celiac disease, must replace with maize or rice based products every source of carbohydrates according to their personal taste and preference. Nowadays, a lot of gluten-free products, containing variable amounts of maize flour (up to 100%) are available on the market. For the reasons outlined above, daily ingestion of maize-based food in people affected by celiac disease could be much greater than non-celiac individuals and consequently their intake of fumonisins could be considerable, in the light of the Provisional Maximum Tolerable Daily Intake (PMTDI) equal to 2000 ng/kg body weight/day (EFSA, 2014). In recent years, an increasing amount of literature addressed the possible safety concern due to modified forms of mycotoxins (also known as "masked mycotoxins"), not detectable through standard methods, that could contaminate foodstuff (Berthiller et al., 2013; Bertuzzi et al., 2016; Bryła et al., 2015; Dall'Asta et al., 2008). Masked fumonisins are also known as bound and hidden fumonisins depending on whether they are covalently or non-covalently bound to other molecules (Berthiller et al., 2009; Bryła et al., 2016; De Boevre et al., 2015). To date, there is a general lack of research on toxic effects exerted by modified forms of fumonisins. Some studies focused on other Fusarium masked mycotoxins addressing their probable conversion in the toxic form after digestion (Berthiller et al., 2011; Dall'Erta et al., 2013). Whereas much uncertainty still exists about the fate of masked fumonisins once ingested and about their toxicity after digestion (Dall'Asta et al., 2010; Hahn et al., 2015).

Thus, the purpose of this paper was to evaluate the amount of B_1, B_2 and B_3 analogues of fumonisins in gluten-free maize-based products, in order to ascertain the exposure of people affected by celiac disease to such compounds, making an important contribution to the current literature.

2. Materials and methods

2.1. Sampling

154 gluten-free products intended for people affected by celiac disease were bought at dedicated shops and supermarkets in Naples and nearby cities (Italy). Samples consisted of: biscuits, breakfast cereals, canned corn, bread, pasta, cookies, cornmeal, rice, sweet snacks (cereal bars, cakes and pastries) and savoury snacks (hardtacks, extruded products). As shown in Table 1, every sample was made of a variable amount of maize (Table 1). Rice was included in the sampling because previous studies indicated that it could show significant levels of fumonisins (Petrarca et al., 2014; Tanaka et al., 2007). Other ingredients of the samples included

Table 1
Mean percentage of maize flour in analyzed food categories.

Food category	Mean ± SD %	Min - max %
Biscuits	37.0 ± 22.9	0.0–70.0
Breakfast cereals	82.3 ± 33.2	0.0–100.0
Canned corn	100.0 ± 0.0	100.0–100.0
Bread	68.8 ± 34.2	0.0–40.0
Pasta	84.1 ± 15.0	59.0–100.0
Corn meal	100.0 ± 0.0	100.0–100.0
Rice	0.0 ± 0.0	0.0–0.0
Sweet snacks	11.8 ± 10.5	0.0–50.0
Savoury snacks	51.8 ± 36.1	0.0–90.0