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Occurrence of mycotoxins in commercial infant formulas locally produced in Ouagadougou (Burkina Faso)

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ABSTRACT

Cereals products enriched with leguminous or oleaginous are used in Burkina Faso as food complement or to avoid infant malnutrition. Infant formulas from cereals are consumed as food supplements after the weaning age. They are produced in Burkina Faso with a mixture of several cereals (maize, millet, rice, or sorghum), leguminous (bean), oleaginous (peanut, soya or sesame), sugar or salt and sometimes milk powder. The production is artisanal or semi-industrial. These infant foods from cereals should be free from mycotoxins and pathogenic bacteria. The objective of this work was to determine the occurrence of mycotoxins like aflatoxins, ochratoxin A and fumonisins in infant formulas and grains in Ouagadougou (Burkina Faso). The mycotoxins (aflatoxins, ochratoxin A and fumonisins) were determined by HPLC-FLD in 248 samples of infant formulas produced by the Recovery and Nutrition Education Centers (CRENs) and sold on the market places in Burkina Faso.

Results showed that the majority of samples of infant formulas presented high levels of mycotoxins. The frequency of contaminated samples by aflatoxin B1, ochratoxin A and fumonisins in analyzed samples were 83.9% (167/199), 7.5% (15/199) and 1.5% (3/199), respectively. The highest values registered in infant formulas were 900, 6 and 3 times higher for aflatoxin B1 (EU limit: 0.1 μg/kg), ochratoxin A (EU limit: 0.5 μg/kg) and fumonisins (EU limit: 200 μg/kg), respectively, than the EU regulation limits (1881/2006). This study presents the first results concerning the safety assessment of infant formulas regarding mycotoxins in Burkina Faso.

1. Introduction

Mycotoxins are fungal secondary metabolites which are dangerous toxins for both humans and animals (AFSSA, 2009; Ferre, 2016). These metabolites are produced and found in many foodstuffs and especially in plants during their pre-and post-harvest or during storage. Aflatoxins, fumonisins, ochratoxin A, zearalenone, and deoxynivalenol are mycotoxins that are detected in cereal crops (Ezekiel et al., 2014; Warth et al., 2012; Juan, Raiola, Mañes, & Ritieni, 2014; Zinedine & Idrissi, 2007) and in peanuts (Afolabi, Ezekiel, Kehinde, Olaolu1, & Ogunsanya, 2015). Among dangerous mycotoxins, aflatoxins (AFs), ochratoxin A (OTA) and fumonisins (FB1 & FB2) represent the greatest health risk in tropical Africa (Manjula, Hell, Fandohan, Abass, & Bandyopadhyay, 2009), in Asia (Li et al., 2014) and the rest of the world (Alborch, Bragulat, Castellá, Abarca, & Cabañes, 2012) due to their high toxicity. They are known to be carcinogenic, genotoxic, teratogenic, nephrotoxic, hepatotoxic and immunotoxic for humans (Creppy, 2002; Mahmoudi, Aryaee, Ghanbari, Ansari, & Nourafcan, 2012). Certain mycotoxins contaminating foodstuffs can cause acute poisoning with rapid onset of symptoms (diarrhea, convulsions, etc.). Other mycotoxins exhibit chronic toxicity, with cumulative effects over the long term, and can lead to cancer or immune deficiencies (Bourais & Amine, 2006). AFs and ochratoxin A were classified in carcinogenic group 1 and 2B, respectively, by the International Agency for Research on Cancer in 1993 (IARC, 1993). Among aflatoxins, aflatoxin B1 (AFB1) is the most toxic form for mammals and causes damages such as toxic hepatitis, hemorrhage,

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edema, immunosuppression and hepatic carcinoma (Speijers & Speijers, 2004). In fact, various epidemiological studies have implicated the AFs and OTA in the increased incidence of gastrointestinal and liver cancer in Africa, Philippines and China (Zinedine & Idrissi, 2007). Recently, cases of acute poisoning affecting a large geographic area in Kenya causing many deaths were reported by Centers for Disease Control and Prevention (CDC, 2004). The high incidence of aflatoxin contamination of groundnuts and cereal grains in Guinea, Gambia, Nigeria, and Senegal was correlated with an increased incidence of liver cancer (Shephard, 2004). AFs have been detected in various commodities such as maize, wheat, barley, nuts, cocoa, dried fruits, wines and spices and other foodstuffs (Juan et al., 2014; Se & Nadir, 2003). OTA contaminates cereals such as barley, wheat, maize, oat, as well as green coffee, fruit juices (grape fruit), wines and spices (Juan et al., 2014; Zinedine & Idrissi, 2007). Fumonisins have been linked with esophageal cancer in the former Transkei (South Africa) and China (Shephard, Thiel, Stockenstrom, & Sydenham, 1996). High incidence of fumonisins at low levels was reported in surveys in Eastern and Southern Africa (Manjula et al., 2009). These mycotoxins occur worldwide on maize, wheat and other cereal grains and their presence and consumption was linked to human and animal diseases through of contaminated cereals.

Several countries have established or proposed regulatory limits for mycotoxins in foods. European Union countries edited regulations to limit their presence in the foods in Europe (EU Regulation, 1881/2006). These regulations have been revised several times. In Africa some countries such as South Africa, Nigeria, or Ghana have regulations on mycotoxins and produce significant research, especially on aflatoxins and fumonisins (Ezekiel et al., 2014). However regulatory limits in sub-Saharan are absent or rarely in place or not properly implemented and regular surveillance is often a major issue. Burkina Faso, a country of West Africa did not set yet a mycotoxin regulation (FAO, 2003; Warth et al., 2012). In Burkina Faso, cereals have a social, economic and nutritional importance for the people. According to the Ministry of Agriculture and Food Security in 2014, the cereal production in 2013–2014 was estimated at 4 869 723 tons/year. The presence of mycotoxins has an impact on health and especially affects rural sub-Saharan populations because they often consume affected crops as staple diet and because crops in tropical and subtropical regions are more susceptible to contamination due to favorable climatic conditions (Bankole & Adebanjo, 2003). However, it was proved that cereal products enriched with leguminous or oleaginous were effective as food complement or to avoid infant malnutrition in Burkina Faso (Compaoré et al., 2011; Kayalto et al., 2013). Previous studies have shown that cereals such as maize, millet, rice and wheat, as well as leguminous and peanuts were contaminated with mycotoxins in Burkina Faso (Nikiéma, Traoré, & Singh, 1995; Ouattara-Sourabié, Nikièma, & Traoré, 2011; Sanou, 2000; Warth et al., 2012). Since infant formulas are based on cereals, these data could alerted us on a potential risk for children's health (Juan, Raiola, Mañes, & Ritieni, 2014; Pereira, Fernandes, & Cunha, 2015). The aim of this study was to assess the level of aflatoxins, ochratoxin A, and fumonisins in infant formulas sold and consumed in Ouagadougou, Burkina Faso.

2. Materials and methods

2.1. Samples of infant formulas and grains

Sampling was conducted between June 2013 and December 2014 around the city of Ouagadougou, capital of Burkina Faso. A total of 199 samples of different formulations of flour consumed by low-weight children were selected by CRENs scientists. Infant formulas produced in semi-industrial units and small craft industries were taken on the production sites or in super markets or grocery stores. Moreover, from cereal sellers, 49 samples of flours and various cereals and oilseeds were also collected. A total of 248 samples of 300–500 g were collected aseptically in plastic bags and were transported in cool boxes at 4 °C to the laboratory (LaBESTA) of the research center for food and nutrition biological sciences (CRSBAN) of the University of Ouagadougou. All samples were conditioned and sent to the laboratory of food safety of the UMR Qualisud, CIRAD in Montpellier (France) for determination analysis of mycotoxins.

2.2. Mycotoxins quantification

Performance data of mycotoxin analysis methods are summarized in Table 1.

2.2.1. Aflatoxins and ochratoxin A quantification

The sample (25 g) was homogenized with 50 mL methanol-water (80:20; v/v) and 5 g of NaCl at high speed for 2 min with a blender (Waring France). The extract was centrifuged at 6000 rpm for 10 min. Two mL of the filtrate was diluted with 18 mL of PBS buffer. Ten mL of this diluted sample was passed through an immuno affinity column-IAC column (Aflaochraprep, R-Biopharm). The IAC was washed twice with 10 mL of PBS each time before being eluted with 2 mL methanol. The eluting fraction was then evaporated and 1 mL of methanol-water (50:50; v/v) was added. The obtained fraction was collected into a glass bottle, identified by High Performance Liquid Chromatography (HPLC) and quantified by spectrofluorescence (Shimadzu RF 20A, Japan) after derivatization post column with electrochemical system (Kobra Cell™ R. Biopharm Rhône Ltd, Glasgow, UK). Fluorescence detection for AFs was set at 365 nm excitation and 435 nm emissions and OTA was set at 333 nm excitation and 460 nm emissions. The mobile phase A was water-methanol (55:45; v/v), 119 mg of potassium bromide and 350 μL of nitric acid and the mobile phase B was water-methanol (20:80; v/v), 119 mg of potassium bromide and 350 μL of nitric acid. AFs and OTA standard solutions were used for the construction of a five-point calibration curve of peak areas versus concentration (ng/mL). The operating conditions were as follows: injection volume of 100 μL of sample and standard solutions; C18 reverse-phase HPLC column, Uptisphere type, ODS, 5 μm particle size, 5 ODB, 250 × 4.6 mm, with identical pre-column, thermostatically controlled at 40 °C; isocratic flow rate of 0.8 mL/min. Mobile phase gradient: mobile phase A: 0% (0–26 min); 65% (26–45 min); 0% (45–50 min); 41% (20–25). The detection and quantification limits on aflatoxins were 0.3 μg/kg and 1 μg/kg, respectively. The detection and quantification limits on ochratoxin A were 0.05 μg/kg and 0.1 μg/kg, respectively. The contents were calculated from a calibration curve established with aflatoxins (TSL-108, Biopharm Rhône Ltd, Glasgow, UK) and ochratoxin standards (TSL-504, Biopharm Rhône Ltd, Glasgow, UK).

2.2.2. Fumonisins quantification

The sample (25 g) was homogenized with 50 mL of methanol-water (80:20; v/v) and 5 g of NaCl at high speed for 2 min with a blender (Waring France). The extract was centrifuged at 6000 rpm for 10 min. Ten mL of filtrate was diluted with 40 mL of PBS buffer. Ten mL of this diluted sample was passed through an IAC column (Fumoniprep, R-Biopharm), followed by washing with 10 mL of PBS buffer. The IAC column was washed twice with 10 mL of PBS for each time before being eluted with 1.5 mL of methanol and 1.5 mL of water. The eluate was collected and derivatized with O-phthaldialdehyde (OPA) prior to analyze by HPLC and quantified by spectrofluorescence (Shimadzu RF 20A, Japan). Fluorescence detection for fumonisins was set at 335 nm excitation and 440 nm