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Aflatoxin M_1 survey on randomly collected milk powder commercialized in Argentina and Brazil

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ABSTRACT

Aflatoxin M_1 (AFM_1), the main monohydroxylated of aflatoxin B_1 (AFB_1) formed in liver and excreted in the milk, has toxicological proprieties comparable to those of AFB_1, albeit a lower carcinogenic potency. The presence of AFM_1 was investigated in 30 samples of powdered milk purchased in Argentina and Brazil. The samples were analyzed using an immunoaffinity column for cleanup and HPLC-FLD for determining AFM_1. The quantification limit was 0.1 μg/kg. AFM_1 was found in all the samples at levels ranging from 0.1 to 0.92 μg kg^{-1} with average concentration of 0.39 μg kg^{-1}.

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1. Introduction

Aflatoxins are toxic secondary metabolites produced mainly by fungi *Aspergillus flavus, A. parasiticus* and *A. nomius,* species that can be found in feeds. The carcinogenic effect and hepatotoxicity of these mycotoxins have been established and classified as a Group 1 (human carcinogen) by the International Agency on Research on Cancer (IARC, 2002). AFM_1 is the 4-hydroxy derivate of AFB_1 and is secreted in the milk of mammals that consume AFB_1 (JECFA, 2001, pp. 1–102). The quantity of AFM_1 in the milk depends on the concentration of AFB_1 in the contaminated feed, with a conversion rate around 0.3–6.2% (Creppy, 2002).

The international trading of milk ensures that AFM_1 is not only a problem for producing countries but it is also of concern for importing countries (Prandini et al., 2009) Many countries have declared limits for the presence of AFM_1 in milk and milk products. The milk used in the manufacture of milk powder, shall comply with the AFM_1 maximum tolerable levels of 0.5 μg kg^{-1}, referred by the General Standard for Contaminants and Toxins in Food and Feed (CAC, 2010). European Union regulations establishes that the AFM_1 maximum level in raw milk, heat-treated milk and milk-based products should not exceed 0.05 μg kg^{-1}; for infant milk and follow-on milk no more than 0.025 μg kg^{-1} is allowed (EC, 2006). The maximum level refers to the products ready to use (marketed as such or after reconstitution as instructed by the manufacturer). The Mercosur (Southern Common Market) established a limit of 0.5 μg L^{-1} for fluid milk and 5 μg kg^{-1} for milk powder (MERCOSUR/GMC, 2002).

JECFA (2001, pp. 1–102) has update AFM_1 evidence until 2001; on the other hand, several countries have carried out studies on the AFM_1 incidence in milk. Regarding milk powder, some studies were conducted from different regions. Assem, Mohamad, and Oula (2011) evaluated 14 cows and goat powder milk samples, from Lebanon and found 35.7% of the samples positive for AFM_1 with an average of 4.9 ng L^{-1}. The goat milk level was below the detection

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limit. Eight cow milk powder samples from Syrian market, showed only one sample contaminated with 0.012 μg L^{-1} (Ghanen & Orfi, 2009). In China (Pei, Zhang, Eremin, & Lee, 2009) found that 100% of 15 powder samples were contaminated; among these, 10 of them ranged between 0.16 and 0.32 μg L^{-1}, and the other five, were from levels higher than 0.32–0.5 μg L^{-1}; while Wang et al. (2012) comparing clean up column performance in milk powder, detected AFM_1 ranging from 0.077 to 0.197 μg kg^{-1}, with immunoaffinity column. In Turkey (Kabak, 2012) AFM_1 was detected in 8% of 62 baby formula samples with a concentration ranged from 0.016 to 0.022 μg kg^{-1}. In Iran, Oveisi, Jannat, Sadeghi, Hajimahmoodi, and Nikzad (2007) reported a 96.6% of infant formula contaminated with AFM_1 with an average of 0.0073 μg kg^{-1}. Rastogi, Dwivedi, Khanna, and Das (2004) studied AFM_1 contamination in infant milk products from Indian market, showing that 94–100% were positive, ranging from 0.267 to 0.350 μg kg^{-1}. The occurrence of AFM_1 in different infant formulas from Spain was detected in 37.7% of the 69 samples, with an average of 0.0031 μg kg^{-1} (Gomez-Arranz & Navarro-Blasco, 2010). Egypt detected an AFM_1 average contamination of 0.0098 μg L^{-1} corresponding to 43.2% (125) positive infant formula milk powders samples (El-Tras, El-Kady, & Tayel, 2011).

The occurrence of AFM_1 in powder milk has been also studied in few samples from Argentina and Brazil (Becker, Negrelo, Racoulte, & Drunkler, 2010; López, Ramos, Ramadán, & Bulacio, 2003; Oliveira, 2010; Oliveira & Ferranz, 2010; Shundo, Navas, Lamardo, Ruvieri, & Sabino 2009).

The aim of this study was to evaluate the AFM_1 concentration in milk powder commercialized in Argentina and Brazil countries.

2. Materials and methods

2.1. Samples

During 2012, a total 30 different trade mark samples of powdered milk were randomly purchased in Argentina (21) and Brazil (9) from supermarkets and grocery stores. Of these samples, 20 were whole (or fat) milk, 9 skimmed milks and one infant milk formula; one of the whole milk came from goat.

The package size ranged between 0.150 kg and 1 kg. The samples were picked from shelves, where the number of products was less than 10, to fit the European Norm EC (2006) N° 401/2006, when the package sizes were smaller than 1 kg, more packages were bought to have a representative sample of lot. Samples were kept in their original packages, properly identified, and stored under refrigeration (4 ± 1 °C) until the day of the analyzes at the Laboratory of Mycotoxicology and Food Contaminants and Mycotoxins of the Federal University of Santa Catarina, Southern Brazil.

2.2. Standard, reagents and solution

The AFM_1 standard was purchased from Sigma (No. A6428-5UG, Saint Louis, USA). The standard reference solution utilized for accuracy was Aflatoxin M_1 solution of Supelco (No. 46319-U, Bellefonte, USA) with AFM_1 concentration of 9.699 ± 0.078 μg mL^{-1}.

Acetonitrile and methanol Panreac (No. 361881 and 361091, Barcelona, Spain, respectively) and hexane Veteco (Rio de Janeiro, Brazil) were HPLC grade. Sodium hydroxide, monobasic sodium phosphate and sodium chloride, all from Vetec (Rio de Janeiro, RJ, Brazil), dibasic sodium phosphate and potassium chloride Biotec (Sao Paulo, Brazil), trifluoroacetic acid (TFA) Merck-Schuchardt (Darmstadt, Germany) were analytical grade. Water was purified in a Milli-Q system on 18.2 MΩ/cm.

Five hundred mL of PBS buffer was prepared using 0.13 g monobasic sodium phosphate, 0.57 g sodium phosphate dibasic, 3.51 g sodium chloride and 0.1005 g of potassium chloride (pH: 7.4).

2.3. Apparatus

The HPLC equipment was from Gilson (No 712, Villiers-le-Bel, France) consisting of an isocratic pump (No 305), a manifold module (No 805), manual injector (20 μl loop), fluorescence detector (FLD, model 121) with 095442 emission (range between 430 and 470 nm) and 095312 excitation (range of 305–395 nm) filters. The chromatographic column used was a C_{18} 150 × 4, 5 mm, 5 μm particle size, ACE (Edinburgh, Scotland) and a centrifuge at 6000 rpm, Hermle, model 200AZ (Wehingen, Germany) was selected for centrifugation.

2.4. AFM_1 quantification

All powdered milk samples were analyzed for the presence of AFM_1 using an immunoaffinity columns for clean-up and HPLC/FLD for determination based on the method of Aflatest WB M1TM, according to Vicam protocol, No G1024 USA (VICAM, 2013), with modifications. Briefly, 10 g of each sample were weighed on an

Table 1
Levels of aflatoxin M_1 in milk powder samples from Argentina and Brazil collected randomly from supermarkets in 2012 and their comparison to literature data.

Country of precedence		Milk powder type	Sample number	Aflatoxin M_1 (μg kg^{-1})
Commercialized	Produced			
Argentina	Argentina	Whole	1	0.50
			2	0.25
			3	0.15
			4	0.17
			5	0.32
			6	0.35
			7	0.27
			8	0.14
			9	0.88
			10	0.34
			11	0.10[a]
Argentina	Argentina	Skimmed	12	0.41
			13	0.44
			14	0.25
			15	0.44
			16	0.22
			17	0.61
			18	0.81
			19	0.92
			20	0.37
Argentina	Argentina	Infant formula	21	0.32
Brazil	Brazil	Whole	22	0.74
			23	0.53
			24	<LOQ[b]
			25	<LOQ[b]
			26	0.25
			27	0.14
			28	0.37
Brazil	Argentina	Whole	29	0.27
			30	0.81

Country	Positive/total samples[c]	LOD	Aflatoxin M_1 (μg kg^{-1})	References
			Average (with ND = 0)	
Argentina & Brazil	28/28	0.03	0.39	This work
Brazil	1/4	0.1	0.002	Becker et al., 2010
Argentina	4/5	0.1	0.10	López et al., 2003
Brazil	50/50	0.03	0.54	Oliveira, 2010
Brazil	62/65	0.03	0.61	Shundo et al., 2009

LOD: limit of detection; **LOQ**: limit of quantification.
[a] Goat milk.
[b] <LOQ (LOQ: 0.10 μg kg^{-1}).
[c] Cow powder milk.

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