Aflatoxin M₁ occurrence in ultra high temperature (UHT) treated fluid milk from Minas Gerais/Brazil

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ABSTRACT

Aflatoxin M₁ (AFM₁) was determined in 75 samples of ultra-high-temperature (UHT)-treated fluid commercial milk from Minas Gerais, Brazil, from July to November 2009. AFM₁ determinations were carried out by HPLC. Results showed that 23 (30.7%) positive samples for AFM₁ at levels of 1000–4100 ng L⁻¹, which were above the tolerance limit for AFM₁ in milk as adopted by Brazilian regulations. It was concluded that the incidence of AFM₁ in milk marketed in Minas Gerais state is higher, warrants concern about AFM₁ in milk and milk products.

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1. Introduction

Aflatoxins are toxic fungal metabolites secondary found in foods and feeds. When cows are fed with feedstuffs containing AFB₁, this metabolite is biotransformed by hepatic microsomal cytochrome P₄₅₀ into AFM₁, in the liver, which is excreted into the milk of lactating animals. The presence of AFM₁ in milk is considered to be undesirable because this toxin has been categorized by the International Agency for Research on Cancer (IARC) as a Group 1 carcinogen (IARC, 2002, pp. 171–300). The carcinogenic characteristics of this toxin have led several countries to establish limits for the maximum quantity of AFM₁ allowed in milk. The maximum acceptable limits of AFM₁ established by the Brazilian Ministry of Health (Brasil, 2002) are 500 ng L⁻¹ for fluid and 5000 ng kg⁻¹ for powder milk following the MERCOSUL Technical Regulations (MERCOSUL/GMC, 2002). The European Commission prescribes that the maximum level of AFM₁ in raw milk, heat treated milk and milk for the manufacture of milk-based-products should not exceed 50 ng kg⁻¹ (EC, 2006). In US’ and China’s regulations on levels of AFM₁ in milk is limited at 500 ng kg⁻¹; in Austria and Switzerland the maximum level is even lower at 10 ng kg⁻¹ for infant food commodities (FAO, 1997); in Syria, the Ministry of Health established the maximum acceptable limit of AFM₁ at 200 ng kg⁻¹ for fluid milk and 50 ng kg⁻¹ for powder milk (FAO, 2004). The Iranian national standard and FDA limit is 500 ng L⁻¹ (Rahimi, Bonyadian, Rafei, & Kazemeini, 2010), the same level for the Korean legal regulation (Lee, Kwak, Ahn, & Jeon, 2009). The Morocco limits are 50 ng kg⁻¹ to milk and products; 30 ng kg⁻¹ for milk and products for infant under 3 years, 500 ng kg⁻¹ for milk powder and 30 ng kg⁻¹ for milk powder for infant under 3 years (Zinedine & Mañes, 2009). However, many countries have no legal maximum limit of AFM₁ in milk and dairy products.

Brazilians authors reported the presence of AFM₁ in milk in Brazil states (Oliveira, Oliveira, Soares & Silva, 2009; Oliveira, Rosmaninho, & Rosim, 2006; Pereira et al., 2005; Sassahara, Netto, & Yanaka, 2005; Shundo, Navas, Lamardo, Ruvieri, & Sabino, 2009) and several papers reported the incidence of AFM₁ in others countries: China (Pei, Zhang, Eremin, & Lee, 2009), Croatia (Bilandzic, Varenina, & Solomun, 2010), Indonesia (Nuryono et al., 2009), Iran (Fallah, 2010; Ghazani, 2009; Rahimi et al., 2010), Kuwait (Dashhi et al., 2009), Pakistan (Hussain, Anwar, Asi, Munawar & Kashif, 2010), South Korea (Lee et al., 2009), Sudan (Elzupir & Elhussein, 2010), Syria (Ghanem & Orfi, 2009). The occurrence of AFM₁ in milk makes it a particular risk for humans.
because of its importance as a foodstuff for adults and especially children, the major consumers, who are more susceptible to the adverse effects of these mycotoxins.

The aim of this study was to evaluate the occurrence of AFM1 in fluid milk UHT in the Minas Gerais state, Brazil.

2. Materials and methods

2.1. Samples

A total of 75 samples of fluid milk Ultra High Treated (UHT) from different city of Minas Gerais state, Brazil, were collected randomly during 6 months (July to November 2009) and analysed for AFM1. All information on the samples was taken from the labels.

2.2. Reagents and standard

AFM1 standard was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile and methanol were HPLC grade and all other chemicals were analytical grade. Water was purified in a Milli-Q system on 18.2 MΩ/cm.

2.3. AFM1 determination

Analysis of the samples for AFM1 determination were performed as recommended by the manufacturer of the immunoaffinity columns (AFLAPREP® M – R-BIOPHARM RHÔNE LTD).

2.3.1. Extraction and clean up

Milk samples were analysed for the presence of AFM1 using an immunoaffinity column for clean up and HPLC with fluorescence detection. The milk samples were centrifuged at 2000 × g for 15 min and the upper fat layer was discarded. The skimmed milk (50 ml) was passed through an immunoaffinity column (AFLAPREP® M – R-BIOPHARM RHÔNE LTD). The column was washed with 2 × 10 ml of PBS buffer (pH 7.4) to remove extraneous non-specific material. The AFM1 bound to the antibody was released by the elution with 1.25 ml of methanol:acetonitrile (20:30) and 1.25 ml of water to give a 2.5 ml total volume.

2.3.2. Chromatographic quantification

Identification and quantification of the AFM1 residues was achieved by high performance liquid chromatography (HPLC), using a Class-VP system (Shimadzu, Kyoto, Japan) equipped with a LC 10AT-VP HPLC pump, a fluorescence detector (model RF 10Ax1) at wavelengths of 362 and 418 nm for excitation and emission, respectively. The HPLC column was a VP-ODS (150 × 4.6 mm, Shimadzu, Kyoto, Japan) and guard column VP-ODS, 4 × 4.6 mm. AFM1 was derivatized post-column using a electrochemical cell KOBRA® Cell. The isocratic mobile phase consisted of solution water:acetonitrile:methanol (68:24:4; v/v) and the flow rate was 1.0 ml/min, 50 μl of sample extracts were injected into the HPLC equipment for determination of AFM1. Calibration curve of AFM1 (Y = 1037.3X – 184.16 R² = 0.9992) was prepared using standard solutions of Aflatoxin M1-Solution OEKANAL®. Sigma–Aldrich at concentrations of 150, 250, 500, 1000, 10,000, 20,000 and 50,000 ng L⁻¹. Under these conditions, the retention time for AFM1 was 4.2 min.

Recovery test were performed by spiking aflatoxin-free milk samples with known amounts of AFM1 and revealed mean recovery rates of 89.3% and relative standard deviations equal to 3.7%.

The detection limit for AFM1 was 150 ng L⁻¹, as considered by the minimum amount of toxin that could generate a chromatographic peak three times over the baseline standard deviation.

3. Results and discussion

The occurrence and levels of AFM1 obtained are presented in Table 1.

Analysis of 75 samples showed that 23 (30.7%) samples contained AFM1 and in 52 (69.3%) samples AFM1 was not detected. All of the contaminated samples were found to be higher than the maximum acceptable limits for fluid milk (500 ng L⁻¹) of the Brazil. Previous results revealed the AFM1 levels under 290–2,100 ng L⁻¹ in 12 samples of UHT milk marketed in Viçosa city of Minas Gerais, Brazil (Oliveira et al., 2009), but as compared to studies in Brazil and studies in other countries the range of contamination was relatively much higher.

Other authors in Brazil found positives samples for AFM1 in raw, pasteurized, powdered and UHT milk at lower levels. Pereira et al. (2005) detected the toxin in milk from farms of the South of Minas Gerais state; the animals had been fed with AFB1 contaminated ration. The aflatoxin concentrations found are within tolerated standard according to Brazilian legislation, in 19 (52.8%) from 36 raw milk samples in trace values of about 74.1 ng L⁻¹ and in 13 (38.2%) from 34 pasteurized milk samples, in trace values of about 58.9 ng L⁻¹. Sassahara et al. (2005), analysed 42 samples of raw milk from 40 farms producing type B milk, located in 12 municipal districts in the North of Paraná State, 10 (24%) were contaminated and 3 (7%) were above the Brazilian limit. Oliveira et al. (2006), determined AFM1 in 48 samples of grade A-C and UHT fluid commercial milk from São Paulo, Brazil, from July to December 2004 and the results showed 37 (77.1%) positive samples for AFM1 at levels of 11–251 ng L⁻¹, which were below the tolerance limit as adopted by Brazilian regulations. Shundo et al. (2009), analysed the presence of AFM1 of powdered milk, pasteurized milk and UHT milk in the city of São Paulo and from a total of 125 samples, 119 (95.2%) were contaminated with AFM1 but none of the samples exceeded the Brazilian legislation. In others countries, several authors (Dashki et al., 2009; Ghanem & Orfi, 2009; Ghazani, 2009; Lee et al., 2009; Nuryono et al., 2009; Pei et al., 2009; Rahimi et al., 2010) have stated that AFM1 levels indicate that the necessary precaution will have to be taken to minimize the AFM1 contamination in milk and milk products from their countries. The above mentioned studies in general have demonstrated that AFM1 levels above the maximum acceptable limits of the European Union (50 ng kg⁻¹). It can be concluded that AFM1 levels in the samples purchased in their countries, appear to be a serious public health problem at the moment and the AFM1 level in milk is closely related to the mould contamination level in animal feed and other related factors.

It has been stated, in fact, that the contamination of milk and milk products with AFM1 displayed variations according to geography; country; season; environmental conditions; inability of certain agricultural systems; low availability of green fodder; excessive use of concentrated feed, cottonseed cake, corn, soybean, threshed wheat straw, paddy straw, wheat bran; contamination of the fed and the grain with aflatoxins during storage (Bilandzic et al., 2010; Ghazani, 2009; Pei et al., 2009; Prandini, Sigolo, Filippi, Laporta, & Piva, 2009; Rahimi et al., 2010; Tajkarimi et al., 2007). Some of these factors and especially the inappropriate feeding of animals located in urban and semi-urban areas of the Minas Gerais.

<table>
<thead>
<tr>
<th>N° positive/ tested (%)</th>
<th>Number and percent of samples with AFM1, in ng L⁻¹ ranges</th>
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<tr>
<td></td>
<td>NDações</td>
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<tr>
<td>23/75 (30.7)</td>
<td>42 (69.3)</td>
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*ND (not detected).
state with fed contaminated with AFB1, contributed to the high concentration rate of AFM1.

For Prandini et al. (2009) and Rahimi et al. (2010), the most effective way of controlling AFM1 in the food supply is to reduce contamination with AFB1 of raw materials and supplementary feedstuffs for dairy cattle. Pei et al. (2009), said, cows’ feed samples from various cowsheds must be routinely evaluated for aflatoxin and those with excessive contaminations should be discarded to keep the dairy cow’s feeds away from fungal contamination as much as possible. Ghazani (2009) concluded that dairy farmers must be educated by the government authorities on potential health consequences of aflatoxins. Nuryono et al. (2009) suggested that once the production processes do not essentially affect the concentration of AFM1, due to its heat stability, the main strategy to diminish exposure risk, both for animals and human beings, is an appropriate preventive monitoring program. Nevertheless, the most important aspect that has to be considered is the incidence and contamination levels of AFM1 in Brazil and some of the countries seem to be a serious problem. There is a need to routinely monitor AFM1 levels in dairy products as a food quality control measure for human health because the contamination with this toxin is a serious problem for public health, especially children because the consumption of milk and milk products by them is very high. Implementing a food control system, such as the HACCP system, in the food industries is suggested as an efficient means for limiting mycotoxin contamination in Kuwait’s food supply (Dashti et al., 2009).

4. Conclusions

The present study showed that there is a risk of milk being contaminated with AFM1 which consequently affects the public health in Minas Gerais state, Brazil. Accordingly, the levels of AFM1 in UHT milk should be controlled and monitored continuously. Therefore, it is important to monitor the level of AFB1 in feedstuffs of dairy animals, it is recommended that AFM1 analysis and control must be taken seriously by the dairy industry in Minas Gerais to reduce AFM1 contamination and improve the quality of milk and milk products.

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