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Occurrence of aflatoxin M_1 in dairy products in Brazil

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ABSTRACT

The objective of this study was to investigate the incidence and occurrence of aflatoxin M_1 (AFM_1) in dairy products produced in Brazil. A total of 123 samples of three different groups of dairy products (cheese, yoghurt, and dairy drinks) consumed by Brazilians were collected during 2010. All samples including 58 cheese samples, 53 samples of yoghurt and 12 dairy drinks were purchased from grocery stores in the Ribeirão Preto-SP area. Cheese samples were classified into three categories depending on their moisture and fat contents: Minas Frescal cheese, Minas Frescal light cheese and Minas Padrão cheese. Samples were analyzed for AFM_1 by a published method. The method comprised aqueous methanol extraction, immunoaffinity column purification and isolation, reversed phase liquid chromatography separation and fluorescence detection. AFM_1 was detected in 84% of the analyzed cheese samples (>3 ng/kg) with levels ranging from 10 to 304 ng/kg in 67% of the samples. AFM_1 was detected in 95% of the yoghurt and dairy drink samples with levels ranging from 10 to 529 ng/kg in 72% of the samples. Despite the lack of a Brazilian regulatory limit for AFM_1 in yoghurt and dairy drinks the survey data of this study may offer information useful in the determination of whether the occurrence of AFM_1 in Brazilian dairy products may be considered as a possible risk for consumer health and whether Brazilian regulatory guidelines for AFM_1 in dairy products are needed.

1. Introduction

AFM_1 is a hydroxylated metabolite of aflatoxin B_1 (AFB_1) that can be found in the milk of animals that are fed with AFB_1-contaminated feed. The sources of aflatoxin contamination in animal feed varies. Sources are location dependent and the incidence and occurrence of AFM_1 contamination in animal feed from different countries varies (Prandini et al., 2009). There are many reports on AFM_1 contamination in cheese and dairy products including reports from Slovenia (Torkar & Vengust, 2008), North Africa (Elgerbi, Aidoo, Candlish, & Tester, 2004), Turkey (Akkaya, Birdane, Oguz, & Cemek, 2006; Gürbay, Engin, Çaglayan, & Sahin, 2006; Tekinsen & Uçar, 2008), Brazil (Franco, Rosim, Fernandes, & Oliveira, 2008; Prado et al., 2000; Sylos, Rodriguez-Amaya, & Carvalho, 1996) and Portugal (Martins & Martins, 2004).

Aflatoxins are toxic, carcinogenic, and/or teratogenic to humans and animals. AFM_1 is relatively stable in raw and processed milk products and cannot be destroyed by heat treatments or pasteurization. The International Agency for Research on Cancer (IARC, 1993) classified AFB_1 as a class 1 human carcinogen and AFM_1 as a class 2B possible human carcinogen (Cathey, Huang, Sarr, Clement, & Phillips, 1994; Creppy, 2002; Galvano, Galofaro, & Galvano, 1996; Moss, 2002). Because of health concerns, regulatory limits for AFM_1 exist in more than 60 countries and 34 of these countries define a maximum acceptable level of AFM_1 in milk at 0.05 μg/kg.

A recent study reported that the concentration of AFM_1 in cheese varied according to the type of cheese, water content and production technologies (Bakirci, 2001; López, Ramos, Ramadán, Bulacio & Perez, 2001). Minas Frescal cheese and Minas Padrão cheese are typical Brazilian cheeses that have about 62% and 41% moisture and 19 and 16% fat, respectively. These types of cheeses are widely consumed in Brazil. Because following good manufacturing practice helps to prevent human exposure to aflatoxins, dairy manufacturers should avoid feeding lactating cattle with aflatoxin contaminated feed and use aflatoxin free milk for cheese, yogurt and dairy drink production. The objective of this study was to investigate the incidence and occurrence of AFM_1 in Brazilian dairy products.

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2. Materials and methods

2.1. Reagents and materials

2.1.1. Materials

A total of 123 dairy food samples were purchased from supermarkets in Ribeirão Preto-SP, Brazil during 2010. The product groups and numbers selected were cheese samples (58), yoghurts (53) and dairy drinks (12). The cheese samples were classified into three categories depending on their moisture and fat contents (Table 1). Samples were stored at 5 °C until analyzed.

2.1.2. Chemicals and supplies

The chemicals and supplies used in the study were: AFM_1 standard (A6428, Sigma Chemical Company, St Louis, MO); methanol and acetonitrile, LC grade (EM Science, Gibbstown, NJ, USA); immunoaffinity column (IAC), AflaStar Fit 3 (Romer Labs, Tulin, Austria).

A stock solution of AFM_1 was prepared in acetonitrile at a concentration of 510 μg/mL, and its concentration determined according to AOAC International Official Methods 986.16, 971.22 and 970.44 (AOAC 2008). Working solutions were prepared by appropriate dilution in acetonitrile. Appropriate portions of the stock solution of AFM_1 were evaporated and diluted with mobile phase to give the following concentrations: 0.5; 1.0; 2.0; 4.0 ng/mL. For AFM_1 spiking solutions, appropriate portions of the stock solution of AFM_1 were evaporated and diluted with methanol to give a concentration of 50 μg/mL.

2.2. Apparatus

Equipment used in this study included an LC system (Shimadzu Instruments, Kyoto, Japan) with a fluorescence detector, a Rheodyne L.P. injector with a 50 μL loop (Rheodyne, Cotati, CA, USA) and a Shim-pack CLC-ODS (M), 4.6 × 250 mm, 5 μm column (Shimadzu, Kyoto, Japan); spectrophotometer (Hitachi, Tokyo, Japan); vortex mixer (Fanem, São Paulo, Brazil); centrifuge (Fanem, São Paulo, Brazil); and column manifold (Supelco, Bellefonte, PA).

2.3. IAC/LC procedures

The procedures used were an IAC cleanup, LC separation, and fluorescence determination method (Iha, Barbosa, Fávaro, & Trucksess, 2011). The entire package of cheese was cut into small pieces, placed in a food blender, and blended for about 5 min to a homogeneous paste. Bottles of yoghurt and milk drinks were shaken manually for 5 min before being opened to ensure that the mixtures were homogeneous. Duplicate analyses were performed for each test sample. Test sample (8 g) was mixed with methanol:water (55:45, including water in the test sample, v/v) and centrifuged, then diluted with water. The resulting slurry was filtered with a glass microfiber filter paper (934AH, GF/B circles, 90 mm, Whatman, England). A portion of the filtrate was passed through an IAC secured on a column manifold. The column was then washed with water. AFM_1 was eluted with methanol. The eluate was evaporated to dryness and the residue was reconstituted in LC mobile phase comprising water:acetonitrile (6:4, v/v) with a flow rate of 0.7 mL/min. The fluorescence detector was set at excitation wavelength 330 nm and emission wavelength 460 nm. The injection volume was 50 μL.

Table 1
Moisture and fat content of Brazilian cheese.

Cheese samples	Moisture (g/100 g)	Fat (g/100 g)
Minas frescal light	>55	10–24.9
Minas frescal	>55	25–44.9
Minas padrão	37–47	26–53
Prato	36–45.9	45–59.9

3. Results and discussion

Prior to this survey, the authors developed and validated the IAC cleanup and LC separation and quantitation method (Iha et al., 2011). Mean recoveries for AFM_1 added to cheese, yoghurt, and dairy drinks at levels ranging from approximately 100–500 ng/kg were >70%. The mean RSDr was <10%. The limit of detection (LOD) was 3 ng/kg and the limit of quantification was 10 ng/kg. The performance of the method exhibited good accuracy and reproducibility for dairy products.

Results of the survey are given in Table 2. Fig. 1 shows LC chromatograms of samples of cheese, yoghurt and a dairy drink naturally contaminated with AFM_1. A total of 123 samples of the three different groups of dairy products (cheese, yoghurt, and dairy drinks) consumed by Brazilians were collected during 2010. AFM_1 was detected (>3 ng/kg) in 49 cheese samples (84%). Thirty nine (39) of the cheese samples (67%) were contaminated with AFM_1 in the range from 10 to 304 ng/kg. AFM_1 was detected (>3 ng/kg) in 62 yoghurt and dairy drink samples (95%), and 47 of the yoghurt samples (72%) and 10 of the dairy drinks (83%) were contaminated with AFM_1 at levels ranging from 10 to 529 ng/kg and 10–50 ng/kg, respectively.

AFM_1 is a common contaminant worldwide. Consequently, many countries have regulatory limits for AFM_1 in dairy products, with limits varying from 50 to 500 ng/kg (Van Egmond, 2004). In Turkey, the regulatory limit is 250 ng/kg for AFM_1 in cheese. According to Ardic, Karakaya, Atasever, and Adiguzel (2009), Aycicek, Aksoy, and Saygi (2005), and Gürbay et al. (2006), AFM_1 in cheese appears to be a serious public health problem in Turkey. In Kuwait, Dashti et al. (2009), analyzed 40 cheese samples and found 32 contaminated with AFM_1 (23.8–452 ng/kg). One sample was above the regulatory limit of 250 ng/kg. In Slovenia, Torkar and Vengust (2008) studied the presence of yeasts, molds and AFM_1 in cheese and concluded that the presence of AFM_1 in Slovenian cheese did not present a serious risk for human health. In Brazil, the regulatory limit is 2500 ng/kg for AFM_1 in cheese (Brasil, 2011), there were several studies of AFM_1 incidences in dairy products. Sylos et al. (1996) detected no mycotoxin in 12 Minas cheese samples. Prado et al. (2000) analyzed 57 Minas cheese samples and found AFM_1 from 20 to 6920 ng/kg in 45 samples. Franco et al. (2008) detected AFM_1 in 15 out of 24 samples at 30–1500 ng/kg and 3 cheeses contained above 5000 ng/kg.

In Portugal (Martins & Martins, 2004) and Italy (Galvano et al., 2001), yoghurt samples were contaminated with low levels of AFM_1. The highest concentrations were 50 and 65 ng/kg, respectively. Two other studies in Turkey (Akkaya et al., 2006; Gürbay et al., 2006) found a higher incidence of AFM_1 in yoghurt and slightly higher contamination levels. Sylos et al. (1996) reported no contamination of AFM_1 in yoghurt, possibly because the LOD of the

Table 2
AFM_1 in Brazilian dairy products.

Samples ($n = 123$)	Number of samples within range of aflatoxin M_1 concentration (ng/kg)				
	<10	11–50	51–100	101–250	>251
Minas frescal light ($n = 20$)	9	8	1	1	1
Minas Frescal ($n = 30$)	5	12	5	5	3
Minas Padrão ($n = 8$)	5	2	1	0	0
Whole yoghurt ($n = 9$)	2	5	1	0	1
Semi-skimmed yoghurt ($n = 21$)	6	12	2	0	1
Skimmed yoghurt ($n = 23$)	8	13	2	0	0
Semi-skimmed dairy drink ($n = 3$)	0	3	0	0	0
Whole dairy drink ($n = 9$)	2	7	0	0	0

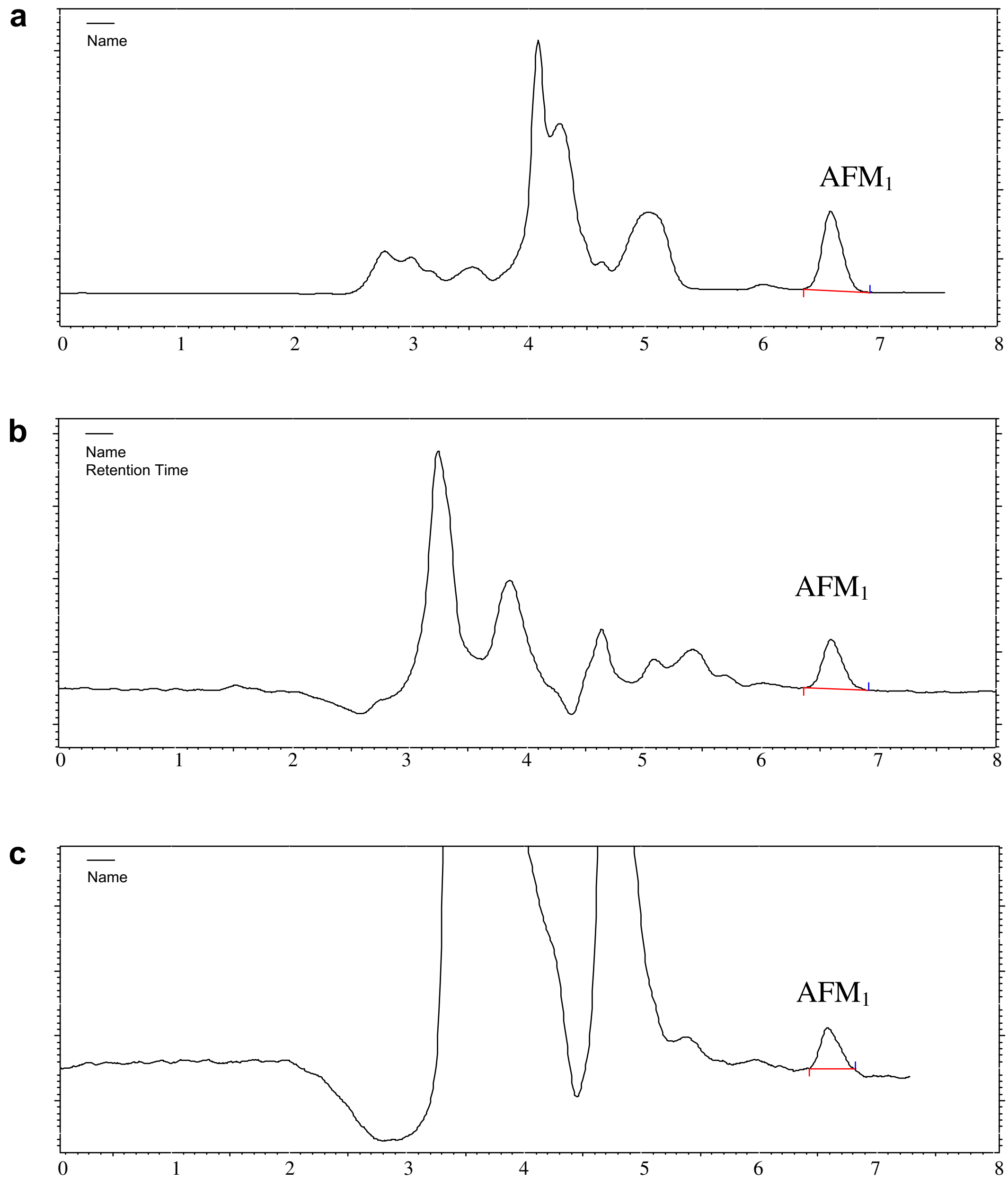

Fig. 1. LC chromatogram of sample naturally contaminated with AFM_1 (a) cheese, 365 ng/kg, (b) yoghurt, 62 ng/kg and (c) dairy drink, 38 ng/kg.

method of analysis was higher than in the other studies. There is a Brazilian regulatory limit for AFM_1 in liquid milk of 500 ng/L (Brasil, 2011) but there is no Brazilian regulatory limit for AFM_1 in yoghurt and dairy drinks.

Table 2 shows that the level of AFM_1 in cheese was higher than that in yoghurt and dairy drinks. This is not unexpected since the preparation procedures for the three types of dairy products are quite different. Milk is concentrated five times during the making of Minas cheese but is not concentrated in yoghurt milk fermentation. Therefore, the concentration of AFM_1 found in yoghurt would be similar to the milk being used for the process. However, the concentration of AFM_1 found in dairy drinks would be less than that of the milk being used for the process because many other food ingredients are added to the milk during preparation of the products.

Results of our survey indicate that milk from Brazil used for the production of yoghurt and dairy drinks was of good quality, with low concentrations of AFM_1. However AFM_1 levels in milk could vary from year to year depending on levels of aflatoxins in cattle feed. Our study offers limited data collected in 2010. It is important to continue yearly surveillance studies in order to compile data for a more comprehensive assessment of AFM_1 contamination in dairy products. The survey data could also be used for risk assessment evaluation and will contribute to an understanding of whether the levels of AFM_1 in these commodities pose a health concern. Finally, further study will be required to determine whether Brazilian regulatory limits for AFM_1 in yoghurts and dairy drinks are needed.

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