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Aflatoxin M_1 in milk and distribution and stability of aflatoxin M_1 during production and storage of yoghurt and cheese

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ABSTRACT

The objective of this study was to investigate the incidence and occurrence of aflatoxin M_1 (AFM_1) in Brazilian milk and infant formula. The distribution and stability of AFM_1 in cheese and yoghurt were also determined. Milk samples and infant formula samples were purchased in Ribeirão Preto-SP, Brazil and were analyzed for AFM_1 using immunoaffinity column purification, liquid chromatography separation and fluorescence detection. AFM_1 was detected in 83% of the milk samples (>3 ng/kg) with levels ranging from 8 to 437 ng/kg for fluid milk, and 20–760 ng/kg for powdered milk. No AFM_1 was found in infant formula. Processing and storage was shown to have little effect on AFM_1 content in milk and milk products. Total AFM_1 mass in milk was reduced by 3.2% in cheese and by 6% in yoghurt (pH 4.4). The mean concentration of AFM_1 in curds was 1.9-fold higher and whey was 0.6-fold lower than in unprocessed milk.

1. Introduction

Animals are exposed to mycotoxins such as the aflatoxins by consumption of feeds contaminated by mycotoxin-producing molds during growth, harvest and/or storage. When lactating cows consume aflatoxin B_1 contaminated feed, aflatoxin B_1 is metabolized to form the monohydroxy derivative, AFM_1, which is expressed in the cow's milk. The sources of aflatoxin contamination in feed vary from country to country. Consequently, the incidence and occurrence of AFM_1 contamination in milk and dairy products depend on the country of origin (Prandini et al., 2009).

There are many reports of AFM_1 contamination in milk and dairy products including reports from Iran (Nemati, Mehran, Hamed, & Masoud, 2010); South Korea (Lee, Kwak, Ahn, & Jeon, 2009); Thailand (Rwangwises & Rwangwises, 2010); Sudan (Elzupir & Elhussein, 2010); Croatia (Bilandzic, Varenina, & Solomun, 2010); Brazil (Garrido, Iha, Ortolani, & Fávaro, 2003; Iha, Barbosa, Okada, & Trucksess, 2011; Shundo, Navas, Lamardo, Ruvieri, & Sabino, 2009); Italy (Meucci, Razzuoli, Soldani, & Massart, 2010); and Taiwan (Peng & Chen, 2009).

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 (FAO, 2004).

Studies have reported that the concentration of AFM_1 in cheese varied depending to the type of cheese, water content and production technologies (Bakirci, 2001; López, Ramos, Ramadán, Bulacio, & Perez, 2001). The distribution of AFM_1 in curd and whey from production processes for typical and widely-consumed Brazilian cheese, Minas frescal cheese was investigated. The fate of AFM_1 in preparing Brazilian home-made yoghurt was also studied. Furthermore, a survey of the toxin in fluid milk as well as in infant formula was included.

2. Experimental section

2.1. Materials, chemicals and apparatus

2.1.1. Materials

A total of 83 samples (76 milk, 7 powdered infant formulas) were purchased from supermarkets in Ribeirão Preto-SP, Brazil during 2010. The products include: 17 UHT milk samples, 30 pasteurized milk samples, 6 powdered whole milk samples, 6

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powdered skim milk samples, 6 whole milk samples with additives, 5 partially skim milk samples with additives, 6 skim milk samples with additives and 7 powdered infant formula samples.

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, Tulln, Austria).

A stock solution of AFM_1 was prepared in acetonitrile at a concentration of 510 μg/mL, and its concentration was 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 concentrations of 51 and 4.1 ng/mL.

2.1.3. 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).

2.2. Analytical procedure

2.2.1. Sample preparation and extraction

2.2.1.1. Milk samples. The liquid samples were shaken manually for 5 min to ensure sample homogeneity before being opened. Aqueous powdered milk solution was prepared by diluting 26 g of product with 200 mL water followed by mixing to ensure homogeneity. Duplicate analyses were performed for each test sample. Test samples were centrifuged for 20 min, following which, 50 mL was diluted with 20 mL hot water (80 °C) and saved for IAC purification and isolation.

2.2.1.2. Liquid milk for recovery study. An appropriate amount of AFM_1 was added to 50 mL test samples (control material containing $AFM_1 < 3$ ng/kg) to obtain AFM_1 levels 24, 102 and 204 ng/L, in 3 replicates. Test portions were diluted and saved for analysis.

2.2.1.3. Cheese and yoghurt samples. The sample preparation procedure was similar to a published method (Iha, Barbosa, Fávaro, & Trucksess, 2011). An 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 were shaken manually for 2 min before being opened to ensure that the mixtures were homogeneous. The moisture contents of cheese and yoghurt samples were determined. Test samples (8 g) were mixed with extract solvent methanol:water (55:45 v/v). The amount of water in the extract solvent has been adjusted to include the water content of cheese or yoghurt sample. For cheese, 22 mL methanol and 13 mL water were added. For yogurt 22 mL methanol and 12 mL water were added. After shaking for 10 min, the mixture was centrifuged. The upper oil layer was aspirated and discarded. A 30 mL portion of the supernatant (30 mL) was placed into a 125 mL Erlenmeyer flask and 60 mL water was added. The mixture containing 18% methanol was passed through glass microfiber paper. Approximately 60 mL filtrate (approximately 4.6 g test portion) was collected and was proceed immediately with IAC chromatography.

2.2.1.4. Whey. A portion of whey (50 mL) was added to IAC for purification and AFM_1 isolation.

2.2.2. Immunoaffinity column purification and isolation

Test solutions from Section 2.2.1 were passed through an IAC secured on a column manifold. The column was then washed twice with 10 mL water. AFM_1 was eluted with methanol. The eluate was evaporated to dryness and the residue was reconstituted in LC mobile phase.

2.2.3. Liquid chromatography

LC condition: mobile phase was a mixture of water:acetonitrile (6:4, v/v) with a flow rate of 0.8 mL/min. The fluorescence detector was set at an excitation wavelength of 360 nm and an emission wavelength of 460 nm. The injection volume was 50 μL.

2.3. Stability study of AFM_1 in cheese and yoghurt

2.3.1. The effect of storage on AFM_1 in yoghurt and cheese

Three yoghurt and three cheese samples naturally contaminated with AFM_1 were stored and each was analyzed in triplicate weekly for a duration of 28 days. The initial contamination levels were 112, 204 and 297 ng/kg for cheese and 59, 61 and 90 ng/kg for yoghurt. Samples were kept at 7.7–9.5 °C in the refrigerator.

2.3.2. The effect of yoghurt fermentation on AFM_1

Three naturally-contaminated milk samples with AFM_1 were used. Two hundred mL of each sample was warmed to 45 °C, where after, 50 mL plain yoghurt was added and mixed with the sample. A 50 mL aliquot of the mixture was analyzed, the concentrations were 75.1, 94.0 and 112.9 ng/L. The remaining mixture was incubated at 45 °C for 12 h. After incubation, yoghurt samples were analyzed, in duplicate.

2.3.3. The effect of cheese production on AFM_1

Naturally-contaminated milk samples with AFM_1 concentrations of 99 ng/L were used. Eight hundred mL of each sample was added to a saucepan and heated slowly to 35 °C. A small portion of rennet (3.2 mL) was added. After stirring, the saucepan was covered and maintained at 35 °C for 1 h. The resulting curd was cut into 1 cm cubes; then cut again diagonally in both directions. The curd was let to stand for 30 min. Approximately 300 mL of whey was collected. Boiling water (300 mL) was added to the curd. After 3 min the mixture was slowly poured through cheesecloth and the whey was collected. The curd was squeezed to expel the residual whey. The curd was transferred to a small container with air vents and kept undisturbed for 5 h. Both the produced curds and whey were analyzed in duplicate for AFM_1. The cheese production procedure was repeated two more times on two separate days using other naturally-contaminated milk samples having AFM_1 concentrations of 74 and 141 ng/L.

3. Results and discussion

Table 1 summarizes results of the recovery study for AFM_1 added to milk samples. The mean recovery at added levels ranging from 24 to 204 ng/L was 93%. The mean RSD was about 6.7%. The limit of detection (LOD) was 3 ng/kg, and was determined by using the average value of blank samples plus 2 standard deviations. The limit of quantification was 8 ng/kg (almost 3 times the LOD). The results indicate that the method is adequate for the determination of AFM_1 in fluid and powdered milk at low levels. Fig. 1 shows LC

Table 1
Recoveries of AFM_1 added to milk.

Sample milk	AFM_1 added, ng/L	Recovery (%)		
		Means	SD	RSD
1	24	99.6	13.4	13.5
2	102	91.1	3.3	3.6
3	204	88.2	2.7	3.1

$n = 3$, SD, standard deviation; RSD, relative standard deviation.

chromatograms for samples of cheese, yoghurt and milk naturally contaminated with AFM_1.

A small survey was conducted for the occurrence of AFM_1 in fluid and powdered milk, milk with additives and infant formula purchased in Ribeirão Preto (SP), Brazil area. Survey results are given in Table 2. AFM_1 was detected in 13 (76%) of ultra high temperature milk samples in the range of from 8 to 215 ng/L; 26 (87%) of pasteurized milk samples, 9–437 ng/L, 12 (100%) of powdered milk samples, 20–760 ng/kg, 13 (76%) of fluid milk containing additives, 9–61 ng/L. All infant formula samples were free of AFM_1 contamination (<3 ng/L).

In all samples, the levels of AFM_1 were below the maximum limit permitted by Brazilian legislation; i.e. 500 ng/L for fluid milk and 5000 ng/kg for powdered milk (Brasil, 2011). The contamination level of this toxin is not considered a serious public health problem under Brazilian legislation. However, levels in 6 (35.3%) of the ultra high temperature milk samples, 18 (60%) of pasteurized milk samples and 1 (6%) milk sample containing additives exceeded the concentration of 50 ng/L permitted by the European Union.

Our AFM_1 levels in milk appeared to be higher than those of some other studies. In a study conducted in Iran, Nemati et al. (2010) analyzed 90 milk samples and all of them were contaminated with AFM_1 in concentration levels ranging from 2.9 to 85 ng/kg with 33% exceeding the EC limit. Lee et al. (2009), in South Korea,

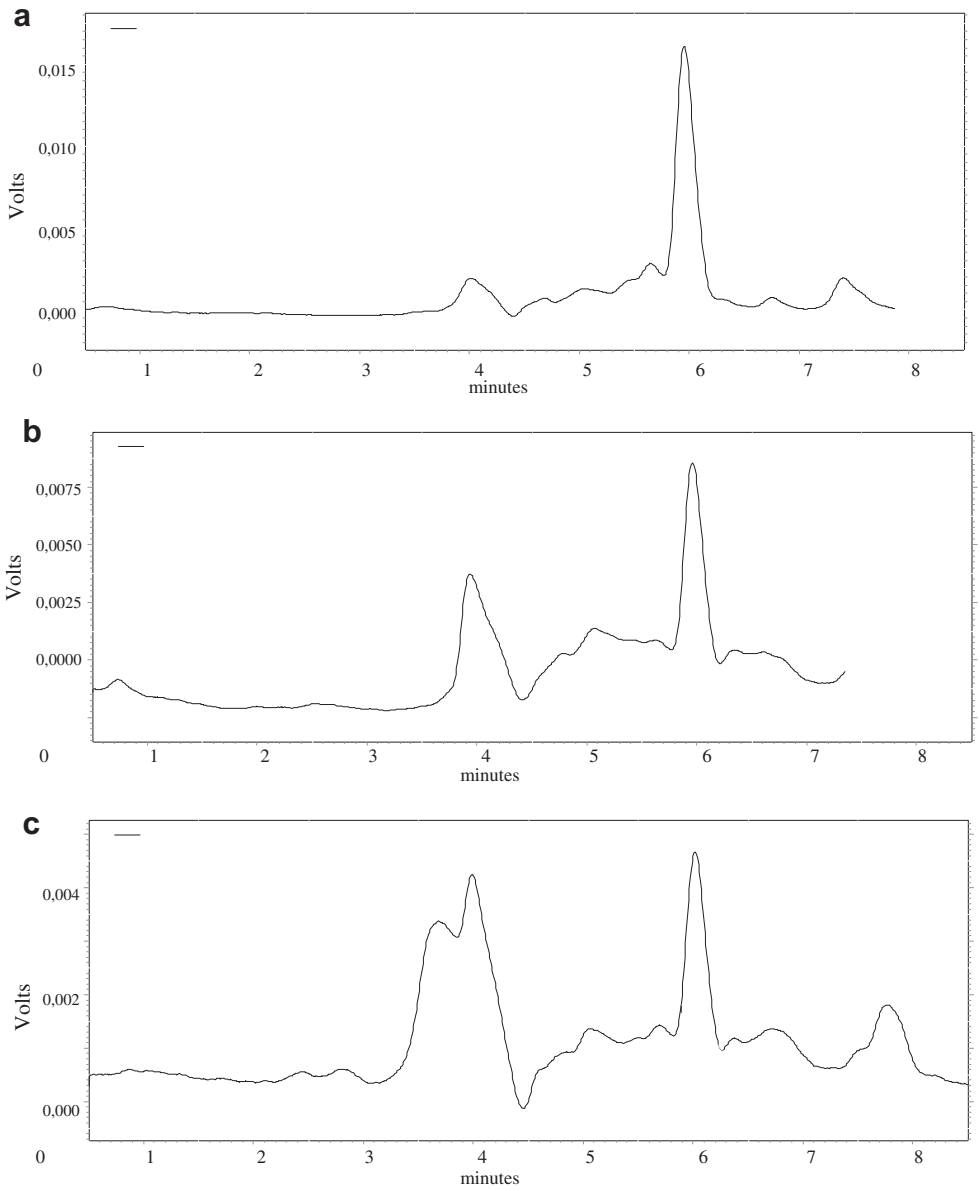

Fig. 1. Chromatograms for samples of (a) milk, 159 ng/L; (b) cheese, 198 ng/kg and (c) yoghurt, 103 ng/kg, naturally contaminated with AFM_1.

Table 2
Incidence of AFM_1 in milk commercialized in Ribeirão Preto.

Total of 83 samples	Range of AFM_1 concentration (ng/L)[a]						
	<3	3–8	9–100	101–250	251–500	>500	>3
Ultra high temperature milk ($n = 17$)	3	1	9	4	0	0	14
Pasteurized milk ($n = 30$)	4	0	18	4	4	0	26
Powdered whole milk ($n = 6$)	0	0	2	1	3	0	6
Powdered skim milk ($n = 6$)	0	0	2	2	0	2	6
Whole milk with additives (vitamin D and iron, $n = 6$)	0	1	5	0	0	0	6
Partially skim milk with additives (calcium and reduction of lactose, $n = 5$)	0	1	4	0	0	0	5
Skim milk with additives (vitamin A and D, calcium and fiber, $n = 6$)	0	2	4	0	0	0	6
Powdered infant formula ($n = 7$)	7	0	0	0	0	0	0

[a] Powdered milk, ng/kg.

analyzed 100 raw milk samples, in which 48 were contaminated with AFM_1 with detected levels ranging from 2 to 80 ng/L. In Thailand, Rwangwises and Rwangwises (2010), found AFM_1 in all 240 raw milk samples at levels ranging from 50 to 101 ng/L. In Croatia (Bilandzic et al., 2010), 61 raw milk samples were found to be contaminated with AFM_1 at levels of from 0.6 to 58.7 ng/L Elzupir and Elhussein (2010) analyzed 44 bulk dairy cattle milk samples, in Sudan, of which 42 (95.45%) were contaminated with AFM_1 at levels from 220 to 6900 ng/L. In Brazil, Shundo et al. (2009) found AFM_1 with contamination levels from 10 to 200 ng/L in 119 (95.2%) milk samples. And Garrido et al. (2003) analyzed 139 milk samples and detected AFM_1 in 29 (20.9%) at 50–240 ng/L. In Italy, a total of 185 cow's milk-based infant formula samples were analyzed and AFM_1 was detected in 2 samples at contamination levels of 11.8 and 15.3 ng/L (Meucci et al., 2010). In Taiwan, Peng and Chen (2009) studied 16 infant formula samples, detecting no AFM_1 in any sample at an LOD of 11.9 ng/kg.

Based on the milk samples taken from the city of Ribeirão Preto, Brazil, the occurrence of AFM_1 does not appear to be a serious public health hazard under Brazilian legislation. Although it is not necessary to continue monitoring the incidence and levels of aflatoxin M_1 in milk samples, surveillance could be appropriate.

A published method was used to analyze AFM_1 in cheese and yoghurt (Iha, Barbosa, Fávaro, et al., 2011; Iha, Barbosa, Okada, et al., 2011). The mean recoveries were 71% for cheese at spiked levels ranging from 100 to 517 ng/kg, and 76% for yoghurt spiked at levels ranging from 66 to 260 ng/kg. The mean RSDs were 5.9% for cheese and 10% for yoghurt. The detection limit was 3 ng/kg and the quantification limit was 10 ng/kg for the two products. Because the shelf-life of commercial yogurt and Minas frescal cheese is

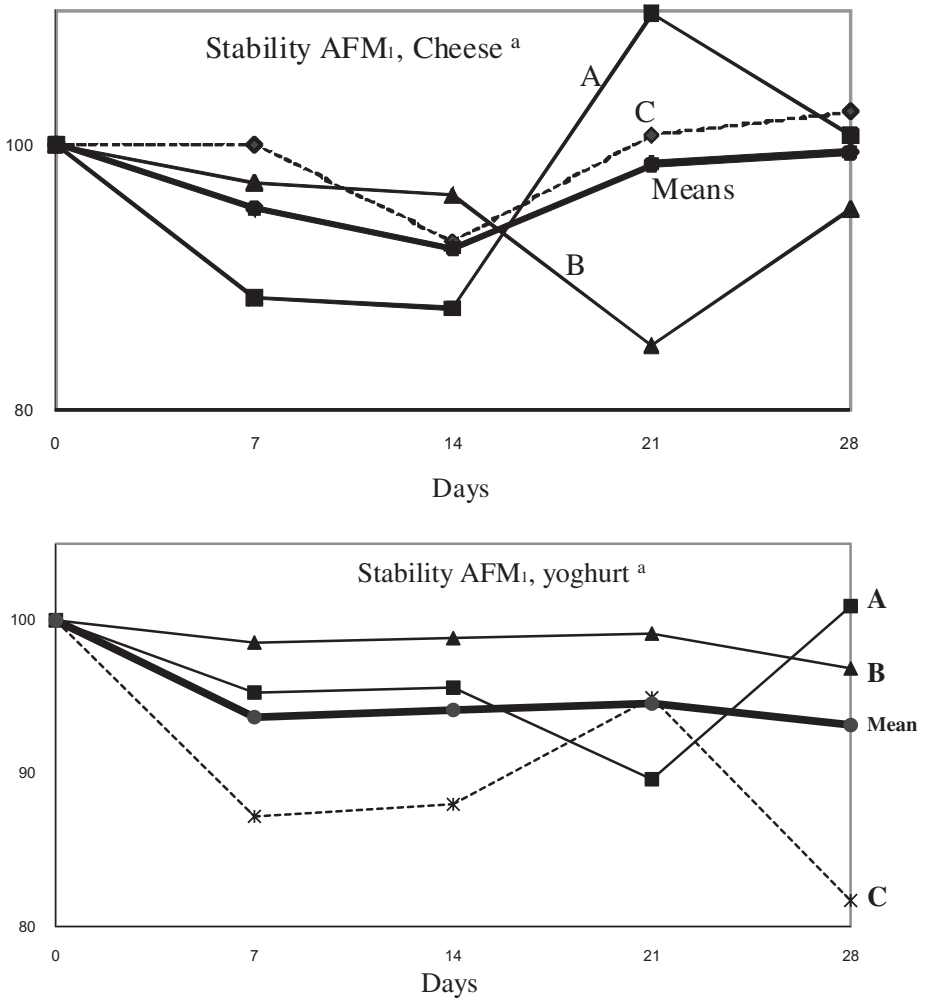

Fig. 2. Stability of AFM_1 in cheese (a) and in yoghurt (b). [a]A, B, and C were 3 naturally-contaminated samples and each data point was average of triplicate analyses.

approximately 4 weeks, the stability study was conducted for a duration of 28 days, at refrigeration temperatures (7.7–9.5 °C). Fig. 2(a) and (b) shows levels of AFM_1 for 3 naturally-contaminated cheese or yoghurt samples analyzed weekly in triplicate for a duration of 4 weeks. Each data point in curves A, B, and C was the average of 3 analyses. The average of each data point was used to plot the curve of means. The decrease of AFM_1 concentration during the experimental period was approximately 3.2% for cheese and 6% for yoghurt (pH 4.4). The decrease could be due to analytical variation.

Our findings were similar to those found in a previously performed study (Oruc, Cibik, Yikmaz, & Kalkanli, 2006) wherein the stability of AFM_1 in two kind of cheese was determined; i.e. for kashar cheese over 60 days and for traditional white pickled cheese over 90 days. Results showed that the toxin was stable during cheese storage and ripening.

Govaris, Roussi, Koidis, and Botsoglou (2002) studied the stability of AFM_1 in yoghurt artificially contaminated with concentrations of 0.050 and 0.100 μg/L, during storage for 4 weeks, at 4 °C, at two pH levels, *viz.* 4.0 and 4.6. The results show that at a pH of 4.6, AFM_1 levels did not significantly ($p > 0.01$) change; however, yoghurt having a pH of 4.0, AFM_1 showed a significant decrease ($p < 0.01$) after the third and fourth weeks of storage at both concentrations levels. The authors concluded that the decrease of AFM_1 could be a function of the low pH (4.0).

The results of our stability study of AFM_1 during fermentation are shown in Table 3, with the mean of the decrease of AFM_1 in the 3 concentrations being 6.4%. Our results show discrepancies with two published studies. Govaris et al. (2002) reported that during fermentation of yoghurt, the levels of AFM_1 decreased significantly ($p < 0.01$) from the initial levels present in milk. They concluded that this decrease in AFM_1 levels might be attributed to factors such as low pH, formation of organic acids or other fermentation by-products, and even to the presence of *Lactobacillus* sp. In another study, Bakirci (2001) found that the AFM_1 increases 13% higher than that of bulk-tank milk samples, but it was not significant statistically.

Table 4 shows the distribution of AFM_1 in curd and whey during Minas frescal cheese production. The total AFM_1 content decreased by 28.9, 13.5 and 34.2% for the three trials. This loss could be due to the use of cheesecloth and a home production environment or there may have been interaction between the toxin and the protein in the products, another explanation could be that the analytical recoveries were different. The recovery of added AFM_1 from milk was 93% and from cheese was 71%. The results given in Table 4 were not corrected for analytical recovery. The concentrations of AFM_1 in the curds over three trials were 1.86, 2.30 and 1.44-fold higher than in milk used to make the cheeses. Results were lower than those from other studies. Two and 4-fold increases previously have been reported (Bakirci, 2001; Manetta et al., 2009; Oruc, Cibik, Yikmaz, & Gunes, 2007; Oruc et al., 2006). The relatively higher water content (residual whey) in the samples of Minas frescal cheese, about (60%), might have contributed to the difference. Our mean AFM_1 concentration in whey was about 39% of the milk used for cheese production while in the other studies the range was from 40 to 60% (Bakirci, 2001; Manetta et al., 2009; Oruc et al., 2006, 2007).

Table 3
Aflatoxin M_1 in milk and yoghurt before and after processing.

Milk + plain yoghurt		Yoghurt after 12 h incubation	
Number	ng/L	ng/L	%
1	75.1	71.6	95.3
2	94.0	88.3	94.0
3	112.9	103.3	91.5
Means	94.0	87.7	93.6

$n = 2$.

Table 4
Aflatoxin M_1 distribution during cheese production.

Trial	Samples	Amount (mL or g)	AFM_1 (ng/L, kg)	Total mass (ng)	% Total AFM_1 mass	% of AFM_1[a] mass distribution
1	Milk	800	99.4	79.5	100	
	Whey	820	36.6	30.0	37.7	53.6
	Cheese	143.3	185.4	26.6	33.4	46.7
	Decrease			23.0	28.9	
2	Milk	800	73.9	59.1	100	
	Whey	730	34.6	25.2	42.7	49.3
	Cheese	151.4	171.2	26.0	43.8	50.7
	Decrease			8	13.5	
3	Milk	800	141.2	98.8	100	
	Whey	845	41.5	35.1	35.5	54.0
	Cheese	147.5	203.0	29.9	30.3	46.0
	Decrease			33.8	34.2	
Means	Milk	800	104.8	79.2	100.0	
	Whey	798.3	37.6	30.10	38.6	52
	Cheese	147.4	186.5	27.5	35.8	48
	Decrease			21.6	25.5	

$n = 2$.
[a] Calculation on base of whey + cheese = 100%.

4. Conclusion

Based on the milk samples taken from the city of Ribeirão Preto, Brazil, the occurrence of AFM_1 does not appear to be a serious public health hazard under Brazilian legislation. The effects on AFM_1 of cheese and yoghurt storage are minimal. The fermentation process of yoghurt manufacture has no effect on AFM_1. The total AFM_1 content in milk and in cheese and whey prepared from the milk decreased approximately 25%. The concentrations of AFM_1 in cheese and whey were 1.9- and 0.4-fold of that for the milk used for cheese production.

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References

AOAC official methods of analysis. (2008) (18th ed.). Gaithersburg. MD: AOAC International.
Bakirci, I. (2001). A study on the occurrence of aflatoxin M1 in milk and milk products produced in Van province of Turkey. *Food Control, 12*, 47–51.
Bilandzic, N., Varenina, I., & Solomun, B. (2010). Aflatoxin M1 in raw milk in Croatia. *Food Control, 21*, 1279–1281.
Brasil. (2011). *Ministério da Saúde. Agência Nacional de Vigilância Santária. Resolução RDC n° 7, de 18 de fevereiro de 2011. Aprova o Regulamento Técnico sobre limites máximos tolerados (LMT) para micotoxinas em alimentos* (p. 66). Brasilia, DF: Diário Oficial [da] Republica Federativa do Brasil. n° 46, Seção 1.
Cathey, C. G., Huang, A. G., Sarr, A. B., Clement, B. A., & Phillips, T. D. (1994). Development and evaluation of a minicolumn assay for the detection of aflatoxin M_1 in milk. *Journal of Dairy Science, 77*, 1223–1231.
Creppy, E. E. (2002). Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicology Letters, 127*, 19–28.
Elzupir, A. O., & Elhussein, A. M. (2010). Determination of aflatoxin M1 in dairy cattle milk in Khartoum State, Sudan. *Food Control, 21*, 945–946.
FAO. (2004). http://www.google.com/url?sa=t&rct=j&q=FAO+Worldwide+Regulations+for+Mycotoxins+++A+Compendium+&source=web&cd=1&ved=0CFEQFjAA&url=http%3A%2F%2Fwww.fao.org%2Fdocrep%2F007%2Fy5499e%2Fy5499e0o.htm&;ei=HzKoT9DIAoav6AHJ8fTCBA&usg=AFQjCNFdDOvQd0_hkjAnqRT8u2o6hP6uSA.

Worldwide regulations for mycotoxins in food and feed in 2003. Rome, Italy: FAO. Food and nutrition paper 81. www.fao.org/docrep/007/y5499e/y5499e0o.htm.

Galvano, F., Galofaro, V., & Galvano, G. (1996). Occurrence and stability of aflatoxin M_1 in milk and milk products: a worldwide review. *Journal of Food Protection, 59*(10), 1079–1090.

Garrido, N. S., Iha, M. H., Ortolani, M. R. S., & Fávaro, R. M. D. (2003). Occurrence of aflatoxins M1 and M2 in milk commercialized in Ribeirão Preto-SP, Brazil. *Food Additives and Contaminants, 20*(1), 70–73.

Govaris, A., Roussi, V., Koidis, P. A., & Botsoglou, N. A. (2002). Distribution and stability of aflatoxin M1 during production and storage of yoghurt. *Food Additives and Contaminants, 19*(11), 1043–1050.

Iha, M. H., Barbosa, C. B., Fávaro, R. M. D., & Trucksess, M. W. (2011). Chromatographic method for aflatoxin M1 in cheese, yoghurt and dairy drinks. *Journal of AOAC International, 94*(5), 1513–1518.

Iha, M. H., Barbosa, C. B., Okada, I. A., & Trucksess, M. W. (2011). Occurrence of aflatoxin M1 in dairy products in Brazil. *Food Control, 22*, 1971–1974.

International Agency for Research on Cancer. (1993). *Monographs on the evaluation of carcinogenic risks to humans: Heterocyclic amines and mycotoxins, Vol. 56*. Lyon, France: IARC.

López, C., Ramos, L. L., Ramadán, S. S., Bulacio, L. C., & Perez, J. (2001). Distribution of aflatoxin M1 in cheese obtained from milk artificially contaminated. *International Journal of Food Microbiology, 64*, 211–215.

Lee, J. E., Kwak, B. M., Ahn, J. H., & Jeon, T. H. (2009). Occurrence of aflatoxin M1 in raw milk in South Korea using an immunoaffinity column and liquid chromatography. *Food Control, 20*, 136–138.

Manetta, A. C., Giammarco, M., Giuseppe, L., Fusaro, I., Gramenzi, A., Formigoni, A., et al. (2009). Distribution of aflatoxin M1 during Grana Padano cheese production from naturally contaminated milk. *Food Chemistry, 113*, 595–599.

Meucci, V., Razzuoli, E., Soldani, G., & Massart, F. (2010). Mycotoxin detection in infant formula milks in Italy. *Food Additives and Contaminants, 27*(1), 64–71.

Moss, M. O. (2002). Risk assessment for aflatoxins in foodstuffs. *International Biodeterioration and Biodegradation, 50*, 137–142.

Nemati, M., Mehran, A. M., Hamed, P. K., & Masoud, A. (2010). A survey on the occurrence of aflatoxin M1 in milk samples in Ardabil, Iran. *Food Control, 21*, 1022–1024.

Oruc, H. H., Cibik, R., Yikmaz, E., & Gunes, E. (2007). Fate of aflatoxin M1 in kashar cheese. *Journal of Food Safety, 27*, 82–90.

Oruc, H. H., Cibik, R., Yikmaz, E., & Kalkanli, O. (2006). Distribution and stability of aflatoxin M1 during processing and ripening of traditional white pickled cheese. *Food Additives and Contaminants, 23*(2), 190–195.

Peng, K. Y., & Chen, C. Y. (2009). Prevalence of aflatoxin M1 in milk and its potential liver cancer risk in Taiwan. *Journal of Food Protection, 72*(5), 1025–1029.

Prandini, A., Tansini, G., Sigolo, S., Filippi, L., Laporta, M., & Piva, G. (2009). On occurrence of aflatoxin M_1 in milk and dairy products. *Food and Chemical Toxicology, 47*, 984–991.

Rwangwises, N., & Rwangwises, S. (2010). Aflatoxin M1 contamination in raw milk within the central region of Thailand. *Bulletin of Environmental Contamination and Toxicology*, . doi:10.1007/s00128-010-0056-3.

Shundo, L., Navas, S. A., Lamardo, L. C. A., Ruvieri, V., & Sabino, M. (2009). Estimate of aflatoxin M1 exposure in milk and occurrence in Brazil. *Food Control, 20*, 655–657.