OCCURRENCE OF AFLATOXIN M₁ IN PARMESAN CHEESE CONSUMED IN MINAS GERAIS, BRAZIL

Ocorrência de aflatoxina M₁ em queijo Parmesão consumido em Minas Gerais, Brasil

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RESUMO

Aflatoxina M₁ (AFM₁) pode ocorrer em leite e produtos de leite, resultante da ingestão de aflatoxina B₁ presente em rações de gado leiteiro. Um total de 88 amostras de queijo Parmesão, comercializadas no estado de Minas Gerais, Brasil, no período de março de 2004 a dezembro de 2004, foram analisadas para aflatoxina M₁ por cromatografia líquida de alta eficiência (CLAE) com detector de fluorescência, precedida de purificação da amostra por coluna de imunoafinidade. AFM₁ foi detectada em 40 das 88 amostras (46,4%). Entretanto, somente duas amostras entre estas estavam contaminadas em um nível acima do limite máximo permitido (250 ng kg⁻¹), aceito pela Comunidade Européia para AFM₁. O Brasil não apresenta tolerância máxima permitida para AFM₁ em queijo.

Termos para indexação: Aflatoxina M₁, queijo, ocorrência.

ABSTRACT

Aflatoxin M₁ (AFM₁) may occur in milk and milk products, resulting from the ingestion of aflatoxin B₁ in feedstuffs by dairy cow. A total of 88 samples of Parmesan cheese marketed in Minas Gerais state, Brazil, from March 2004 to December 2004, were analyzed for AFM₁ by high-performance liquid chromatography (HPLC) with a fluorescence detector following sample clean-up using immunoaffinity columns. AFM₁ was detected in 40 of the 88 samples (46.4%). However, only two samples among these were contaminated at a level above the maximum permissible limit (250 ng kg⁻¹) accepted by European Union for AFM₁. No AFM₁ maximum tolerance limit in cheese has been established in Brazil.

Index terms: Aflatoxin M₁, cheese, occurrence.

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INTRODUCTION

Aflatoxins are a group of mycotoxins mainly produced by common fungi Aspergillus flavus, A.parasiticus and A.nomius. These fungi are ubiquitous and can occur in a wide range of agricultural commodities, such as cereals, nuts, dried fruit and in feedstuffs. A. flavus only produces B aflatoxins, while the other two species produce both B and G aflatoxins. Aflatoxins M₁ (AFM₁) and M₂ (AFM₂), are the hydroxylated metabolites of aflatoxin B₁ (AFB₁) and B₂ (AFB₂), respectively, and may be found in milk and milk products from livestock that have ingested contaminated feed (CALONI et al., 2006; CREPPY, 2002).

Many researchers reported that there was a linear relationship between the amount of AFM₁ in milk and AFB₁ in feed consumed by the animals. It is estimated that approximately 1-6% of the AFB₁ initially present in the animal feedstuff appears as AFM₁ in milk. AFM₁ could be detected in milk 12-24 h after the first aflatoxin B₁ ingestion, reaching a high level after a few days, probably associated with the protein fraction (BAKIRCI, 2001; BATTACONE et al., 2003; DRAGACCI et al., 1995; PITTET, 1998).

AFM₁ is less mutagenic and carcinogenic than AFB₁ but it exhibits high genotoxic activity (BARNES, 1970; CANTON et al., 1975; JECFA, 2001; LAFONT et al., 1989; SINNHUBER et al., 1974). AFB₁ and AFM₁ are classified as a potential carcinogenic agent in the group 1 and 2B, respectively to humans (IARC, 1993).

Milk and milk products are a good source of many nutrients such as proteins and calcium and are mainly consumed by children. Therefore, humans are potentially exposed to these toxic metabolites and it is generally assumed that neither storage nor processing determines a reduction of AFM₁ content (DIAZ & ESPITIA, 2006; GALVANO et al., 1996; GARRIDO et al., 2003). In order to reduce this risk, many countries have regulated the levels

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A number of survey and monitoring programs have been carried out in several countries to obtain general pattern of extent of milk, cheese and yoghurt contamination (AYCICEK et al., 2005; BARRIOS et al., 1996; CARVAJAL et al., 2003; DRAGACCI & FREMY, 1996; ELGERBI et al., 2004; GALVANO et al., 2001; GÜRŞAY et al., 2006; KAMBAR, 2005, 2006; KIM et al., 2000; LÓPEZ et al., 2001; MARTINS & MARTINS, 2004; NAKAJIMA et al., 2004; ORUC et al., 2006; ROUSSI et al., 2002; SIVASTAVA et al., 2001; TEKINSSEN & TEKINSSEN, 2005).

In Brazil there are limited surveys for AFM in milk (GARRIDO et al., 2003; OLIVEIRA et al., 1997, 2006; PARREIRAS & GOMES, 1987; PEREIRA et al., 2005; PRADO et al., 1999; SABINO et al., 1989; SOUZA et al., 1999; SYLOS et al., 1996; TAVEIRA & MÍDIO, 2001) and milk products as yoghurt and cheese (PRADO et al., 2000, 2001; SYLOS et al., 1996).

AFM, is relatively stable during pasteurization, sterilization, preparation, and storage of various dairy products (GALVANO et al., 1996; GÜRŞAY et al., 2006; HASSANIN, 1994). Contrasting data have been reported on the influence of cheese preparation on AFM recovery. The first studies, performed in the years of 1971-1974, showed variable losses of AFM during cheese manufacturing. In contrast, later investigations reported increases in AFM concentration in cheese as a function of cheese type, technologies, and the amount of water eliminated during processing (BASKAYA et al., 2006; KAMBAR, 2006).

The consumption of cheese is widespread in Brazil. For this purpose, this study was designed to determine the presence and levels of AFM in Brazilian Parmesan cheese consumed in Minas Gerais state, Brazil.

MATERIALS AND METHOD

Samples

A total de 88 samples of Parmesan cheese were obtained randomly from different groceries and supermarkets located in several cities of Minas Gerais state, Brazil, from March 2004 to December 2004. The samples were taken in 200-250 g quantities and analyzed within 1 month from collection.

Aflatoxin M₄ determination

Analysis was carried out in duplicate as described by Dragacci et al. (1995) and Tuinstra et al. (1993) briefly outlined as below.

Ten g of samples of cheeses were put in a bowl with dichloromethane (80 mL) and 10 g of Hyflo-supercel and the whole was blended for 2 min at high speed. After washing with further dichloromethane (40 mL), the mixtures were filtered and the filtrate was then evaporated at 60 °C under nitrogen. The residue was dissolved in methanol (1 mL), water (30 mL) and n-hexane (50 mL), transferred in a separatory funnel and the aqueous phase collected. The hexane phase was then washed twice with water (10 mL) and the water phases also collected.

The aqueous collections were passed through an immunoaffinity column obtained from VICAM (Aflatest, Vicam, Watertown, MA, USA), according to the instructions of the manufacturer. Subsequently, the column was washed with water and then the toxin was eluted by acetonitrile, evaporated to dryness under a gentle stream of nitrogen and reconstituted to 500 µl with acetonitrile-water (30:70, v/v) before the injection of 50 µl into the liquid chromatographic system. Each extract was injected in triplicate.

AFM was quantified by reversed-phase HPLC (LC-10 AD, Shimadzu, Japan, linked to a Auto Sampler SIL 10AF and Shimadzu RF551 fluorescence detector: excitation 366 nm, emission 428 nm), using a Shim-Pack CLC-ODS column, 5 µm, 250 mm x 4.6 mm i. d., preceded by a guard column Shim-Pack G-ODS, 5 µm, 10 mm x 4 mm i. d. The mobile phase was isocratic, acetonitrile-isopropyl alcohol-water (8:12:80, v/v/v), filtered through a 0.45-µm filter membrane,degassed and used at a flow rate of 1 ml/min (DRAGACCI et al., 2001). Under these conditions, the retention time of AFM, was approximately 8.6-9.5 min. HPLC solvents were of HPLC grade (EM Science or J. T. Baker) and other chemicals were of analytical reagent grade. A calibration curve of AFM was obtained using standard from Sigma (Code A-6428; St. Louis, MO, USA), at concentrations of 0.5-8.0 ng ml⁻¹. The concentrations were determined according to AOAC (1995). The correlation coefficient was higher than 0.999. The limit of detection and quantification, was 10 ng kg⁻¹ and 20 ng kg⁻¹, respectively.
RESULTS AND DISCUSSION

The incidence of AFM₁ contamination in Parmesan cheese was not very high, since 46.4% (40/88) of all samples were positive. Only two samples (2.3%) were contaminated with AFM₁, with 270 ng kg⁻¹ and 660 ng kg⁻¹, over the permissible level of 250 ng kg⁻¹ as accepted in most European countries. Fourteen samples (15.9%) were contaminated with low levels ranging from 10 to 19 ng kg⁻¹. Twenty four samples (27.3%) were contaminated with levels ranging from 20 to 250 ng kg⁻¹ and mean concentration of aflatoxin M₁ of 54.8 ng kg⁻¹. The results are shown in Table 1.

Few surveys have been undertaken to assess the level of AFM₁ in some types of cheese consumed in Brazil (Table 2).

Sylos et al. (1996) detected no AFM₁ in cheese commercialized, in 1990, in Campinas/Brazil. However, the analytical method employed was a thin-layer chromatography which is less sensitive than high-performance liquid chromatography (HPLC).

Prado et al. (2000) detected in 56 (74.7%) of 75 white cheese samples AFM₁ contamination and 20 (26.7%) of the positive samples were found higher than 250 ng kg⁻¹.

Prado et al. (2001) examined AFM₁ levels in yellow cheese samples and in all brands analysed AFM₁ was detected in the range of 20-540 ng kg⁻¹ and mean level of 150 ng kg⁻¹. They found that 2 samples out of 9 exceeded 250 ng kg⁻¹.

Some previous studies in different countries have reported that AFM₁ was not found in cheese samples. On the other hand some have reported high levels or low-levels of AFM₁ in cheese samples (SARIMEHMETOGLU et al., 2004).

Pietri et al. (1997) checked 223 samples of Grana Padano cheese manufactured in 4 years (1991-1994) and it has emerged that only one sample exceeded the maximum tolerated level in cheese in some European countries (250 ng kg⁻¹). Most samples (91%) were found in the range of 5-100 ng kg⁻¹ and only 15 (67%) was found in the range of 100-250 ng kg⁻¹.

Oruc & Sonal (2001) examined AFM₁ levels in cheese from Bursa, Turkey and found in 89.5% of cheese samples with range of 0-810 ng kg⁻¹.

Sarimehmetoglu et al. (2004) detected in 327 (81.75%) of 400 cheese samples AFM₁ contamination. The number of cheese samples which exceeded the limits of 250 ng kg⁻¹ was 110 (27.5%).

Aycicek et al. (2005) examined AFM₁ levels in dairy products and the contamination was 90.58% (202 samples of 223) whereas AFM₁ was not detected in 21 samples (9.42%).

### Table 1 – Incidence and levels of AFM₁ in Parmesan cheese samples determined by HPLC from Brazil.

<table>
<thead>
<tr>
<th>No. positive/tested (%)</th>
<th>Number and percent of samples with AFM₁ in ng kg⁻¹ ranges</th>
<th>AFM₁(ng kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40/88 (46.4)</td>
<td>ND  10-19  20-250 &gt;250</td>
<td>Range⁹ Averageᵇ  90th Percentil</td>
</tr>
<tr>
<td></td>
<td>48  14  24  2</td>
<td>20-660  54.8±34.3  60</td>
</tr>
</tbody>
</table>

ND: not detected, < 10 ng kg⁻¹
Limit of detection: 10 ng kg⁻¹
Limit of quantification: 20 ng kg⁻¹

⁹Minimum - Maximum

ᵇMean of positive samples (= 20 ng kg⁻¹) ± SD (standard error)

### Table 2 – Incidence and level of AFM₁ in cheese samples from Brazil

<table>
<thead>
<tr>
<th>Type of cheese</th>
<th>No. positive/tested</th>
<th>Incidence</th>
<th>Range (ng kg⁻¹)ᵇ</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Minas” cheese</td>
<td>0/12</td>
<td>0</td>
<td>NA</td>
<td>Sylos et al. (1996)</td>
</tr>
<tr>
<td>Mozzarella</td>
<td>0/12</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Cheddar</td>
<td>0/12</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>White cheese</td>
<td>56/75</td>
<td>74.7%</td>
<td>20-6.920</td>
<td>Prado et al. (2000)</td>
</tr>
<tr>
<td>Yellow cheese</td>
<td>9/9</td>
<td>100%</td>
<td>20-540</td>
<td>Prado et al. (2001)</td>
</tr>
</tbody>
</table>

ᵇMinimum-Maximum
NA- not applicable
Kamber (2005) verified a total of 60 samples of Turkish cheese types, to determine the presence and the level of AFM$_1$. The concentration of AFM$_1$ was found to range from 51-115 ng kg$^{-1}$. The mean values of AFM$_1$ were 82.5 ng kg$^{-1}$ in Ceci cheese samples and 62.4 ng kg$^{-1}$ in Kars Kashar cheese samples. None of the AFM$_1$ levels were above the limit of 250 ng kg$^{-1}$.

More recently, AFM$_1$ levels were determined in 600 cheese samples from some provinces of Turkey. AFM$_1$ was detected in 30 (5%) cheese samples and in 6 (1%) exceeded the Turkish legal limits of 250 ng/kg (YAROGLU et al., 2005). However, Baskaya et al. (2006) checked 363 cheese samples in Istanbul from 2002 to 2004, and AFM$_1$ levels in 80 (22.04%) were higher than 250 ng kg$^{-1}$.

CONCLUSION

Although in some cases AFM$_1$ concentrations in the cheese samples were found to be low, cheese is a very strong source of AFM$_1$ among dairy products. For this reason, milk and dairy products have to be inspected continuously for AFM$_1$ contamination. Finally, since there is not enough study in Brazil about the content of milk and dairy products, more studies are required to be done. Beside this, it is important to determine AFM$_1$ levels in certain cheese samples consumed in Brazil. Monitoring the AFM$_1$ in milk products in Brazil is the first step to support the establishment of legislation that could diminish risk to consumers and protect commercial activities.

REFERENCES


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