Fumonisins B₁ and B₂ in Black Tea and Medicinal Plants

M. LÍGIA MARTINS,¹ H. MARINA MARTINS,¹ AND FERNANDO BERNARDO²*

¹Laboratorio Nacional Investigação Veterinária, Serviço de Micologia, Estrada de Benfica, 701-1549-011, Lisboa, Portugal; and ²Faculdade de Medicina Veterinaria, Polo Universitário da Ajuda, Rua Professor Cid dos Santos, 1300-417, Lisboa, Portugal

ABSTRACT

Fumonisins are mycotoxins produced by Fusarium moniliforme that are prevalent in cereals and other agricultural products. These mycotoxins have been pointed to as a natural cause of equine leukoencephalomalacia, porcine pulmonary edema, and human esophageal cancer. A total of 87 samples, 18 black tea samples and 69 samples of four different medicinal plants (chamomile, leaves of the orange tree, leaves and flowers of the linden tree, and corn silk), for infusions preparations were acquired from supermarkets in Lisbon, Portugal. The samples were analyzed for the incidence and levels of fumonisin B₁ (FB₁) and fumonisin B₂ (FB₂) by high-performance liquid chromatography. The detection limit was 20 µg/kg for both FB₁ and FB₂. FB₁ was detected in 55 (65.5%) of the 87 samples. The highest number of positive samples was found in black tea (88.8%), with levels ranging from 80 to 280 µg/kg. Relative to the medicinal plants, the leaves of the orange tree had higher concentrations of FB₁ (range, 350 to 700 µg/kg) followed by leaves and flowers of the linden tree (range, 20 to 200 µg/kg). The samples of corn silk and chamomile had less contamination of FB₁, with concentrations ranging from 50 to 150 µg/kg and 20 to 70 µg/kg, respectively. None of the samples tested had contamination of FB₂. This is the first report of the natural occurrence of fumonisins in black tea and medicinal plants in Portugal. We reinforce the necessity to implement risk management measures for safety control of this kind of product.

MATERIALS AND METHODS

Sampling. A total of 87 samples (18 samples of black tea and 69 samples of four different kinds of medicinal plants) were collected from different supermarkets in Lisbon, Portugal. The commercial and scientific names of these samples are as follows: black tea (Camellia sinensis L.) (18 samples), leaves of the orange tree (Citrus sinensis O.) (18 samples), leaves of the linden tree (Tilia grandifolia L.) (18 samples), corn silk (Zea mays L.; Stigmata maydis) (15 samples), and chamomile (Matricaria chamomilla L.) (18 samples).

Detection and quantification of FB₁ and FB₂ by high-performance liquid chromatography. Extraction, purification, and high-performance liquid chromatography (HPLC) quantification
TABLE 1. Incidence and levels of fumonisin (FB₁) in black tea and medicinal plants

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. (%) of samples</th>
<th>No. (%) with levels &lt;20a</th>
<th>Range (µg/kg)</th>
<th>Meanb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black tea</td>
<td>16/18 (88.8)</td>
<td>2 (11.1)</td>
<td>80–280</td>
<td>149</td>
</tr>
<tr>
<td>Leaves of orange tree</td>
<td>12/18 (66.6)</td>
<td>6 (33.3)</td>
<td>350–700</td>
<td>537</td>
</tr>
<tr>
<td>Leaves or flowers of linden tree</td>
<td>12/18 (66.6)</td>
<td>6 (33.3)</td>
<td>20–200</td>
<td>98</td>
</tr>
<tr>
<td>Corn silk</td>
<td>9/15 (60.0)</td>
<td>6 (40.0)</td>
<td>50–150</td>
<td>91</td>
</tr>
<tr>
<td>Chamomile</td>
<td>8/18 (44.4)</td>
<td>10 (55.5)</td>
<td>20–70</td>
<td>64</td>
</tr>
<tr>
<td>Total</td>
<td>57/87 (65.5)</td>
<td>30 (34.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Negative for FB₁.
b Arithmetic mean. Concentrations below detection limit have been regarded as zero.

of FB₁ and FB₂ were performed by the technique described by Shephard et al. (19). Briefly, the technique involves blending 25 g of sample with methanol and water and then filtering. An aliquot of the filtered sample was applied to a Bond-Elut strong anion-exchange (SAX) cartridge 3cc (model 020850, Waters, Milford, Mass.), which had been previously equilibrated and washed with methanol and water. The toxins were eluted with an acetic acid–methanol solution. The eluate was evaporated to dryness and the dried sample was redissolved in methanol. An aliquot was derived with o-phthalaldehyde (P-0657, Sigma-Aldrich Quimica S.A., Madrid, Spain) and 2-mercapto-ethanol (M-6250, Sigma Chemical Co., St. Louis, Mo.) before separation on a reverse-phase HPLC system, with fluorescence detection (emission, 440 nm; excitation, 355 nm). An isocratic mobile phase of methanol and 0.1 M sodium dihydrogen phosphate (80:20), adjusted to pH 3.3 with orthophosphoric acid, was used at a flow rate of 1 ml/min. The detection limit for the method was 20 µg/kg for both FB₁ and FB₂. Fumonisins were quantified by correlating the peak area of the extracts to that of the reference standards. Standards of FB₁ and FB₂ were purchased from Sigma-Aldrich (F-2643 and F-3771, respectively). The recoveries of FB₁, and FB₂, were determined in duplicate by spiking standards (20 to 200 µg/kg and 50 to 200 µg/kg, respectively) into blank samples of orange leaves. The average recovery was 80.0% for FB₁ and 77.0% for FB₂.

RESULTS AND DISCUSSION

A total of 87 samples (18 samples of black tea and 69 samples of four different kinds of medicinal plants) were collected from different supermarkets in Lisbon, Portugal. All samples were analyzed for FB₁ and FB₂. Fifty-seven (65.5%) of these samples contained FB₁ concentrations ranging from 20 to 700 µg/kg. FB₂ was not detected in any samples (Table 1).

Of the 18 black tea samples, 16 (88.8%) contained FB₁ concentrations ranging from 80 to 280 µg/kg (mean, 149 µg/kg).

Relative to the 69 samples of medicinal plants, the leaves of the orange tree (66.6%) contained higher concentration of FB₁ (range, 350 to 700 µg/kg; mean, 537 µg/kg), followed by leaves and flowers of the linden tree (66.6%; range, 20 to 200 µg/kg; mean, 98 µg/kg). The samples of corn silk (60.0%; range, 50 to 150 µg/kg; mean, 91 µg/kg) and chamomile (44.4%; range, 20 to 70 µg/kg; mean, 64 µg/kg) had lower contamination of FB₁.

At present, little is known about the presence of mycotoxins in these products. Other researchers reported studies in medicinal plants with mycotoxins other than fumonisins. Martins et al. (11) referred to a study carried out to evaluate the possible presence of aflatoxins and ochratoxin A by HPLC in green and black tea; in that study, none of the 70 samples analyzed contained any detectable amounts of aflatoxins. Ochratoxin A was found in 24 (34%) of the 70 samples in concentrations ranging from 20 to 124 µg/kg. Abeywickrama and Bean (2) detected aflatoxin B₁ concentrations of 0.5 µg/g by thin-layer chromatography in medicinal plant (Aerva lanata) samples. Another study, conducted by Halt (8), reported ochratoxin A in one of the seven samples of medicinal plant material and herbal tea contaminated with Aspergillus flavus. These samples were also analyzed for aflatoxins and zearalenone by thin-layer chromatography, but they were not detected in any samples. Hitokoto et al. (9) also reported the absence of mycotoxins (aflatoxins, sterigmatocystin, and ochratoxin A) in 49 powdered herbal drugs, suggesting that because many medicines are made of material that originates from plants and animals, there may be a risk of mycotoxicosis in patients after oral administration of such medicines.

According to the Food and Agriculture Organization, (6) only Switzerland has established regulations for FB₁ and FB₂ in maize products at 1 µg/kg. Nevertheless, the data available on the toxicity and carcinogenicity of FB₁ indicate that these mycotoxins should be regarded as a potential risk to human health. Therefore, it is necessary to obtain data on the distribution and contamination levels of fumonisins in other human food.

REFERENCES