Deoxynivalenol (DON), also known as vomitoxin, is one of a group of closely related secondary fungal metabolites—the trichothecenes—and is produced predominantly by several species of the genus *Fusarium*, especially *Fusarium graminearum*. The present study was carried out to evaluate the natural occurrence of DON in different kinds of wheat-based breakfast cereals widely consumed by the population. A total of 88 commercially available samples of wheat-based breakfast cereals were randomly collected from different supermarkets in Lisbon, Portugal. The samples were analyzed using immunoaffinity column, and DON was quantified by liquid chromatography. Detection limit was 100 µg/kg. Average recovery of DON was 80%. Of 88 analyzed samples, 72.8% contained levels of DON between 103 and 6,040 µg/kg, with mean level of 754 µg/kg, and 24 samples (27.2%) were not contaminated (<100 µg/kg). These results indicate an incidence of this mycotoxin in these products, and the authors suggest a monitoring for the prevention of molds and mycotoxins. This is the first report in Portugal on natural contamination with DON in wheat-based breakfast cereals.

Natural contamination of wheat with DON has been reported in several different countries (4, 6, 8, 13, 16) (as well as in other countries). Particularly alarming are recent studies of food for human consumption from retail stores in Argentina that showed that 60 wheat samples analyzed, 93.3% were contaminated, with minimum and maximum of 100 and 9,250 µg/kg, respectively (7). Other studies demonstrate that DON is commonly found at high levels in cereals for human and animal consumption (11, 14, 15). The purpose of this survey was to determine the natural occurrence and levels of DON in different kinds of wheat-based breakfast cereals in Portugal.

**MATERIALS AND METHODS**

**Samples.** A total of 88 packaged samples of commercial wheat cereals for breakfast (24 samples of bran, 20 wheat flakes, and 44 of wheat and fruits) were purchased in different supermarkets in Lisbon, Portugal. Each 250-g sample was ground in a blender, and subsamples of 50 g were analyzed.

**DON determination and quantification by liquid chromatography.** DON analysis was carried out following the method described by Cahill et al. (1). A sample of 50 g was extracted in distilled water by blending for 3 min at high speed, filtered through both a fluted and glass microfiber filter paper, and applied to an immunoaffinity column. (DONtest HPLC: VICAM, Watertown, Mass.). Subsequently, the column was washed with distilled water, and the toxin was eluted from the column with methanol, evaporated to dryness in a rotary evaporator, and redissolved in 300 µL of acetonitrile water. Determination of DON was carried out by isocratic reverse-phase liquid chromatography using a LiChrospher 100 RP-18, 5-µm column, 25- by 4.6-mm EcoPack.
TABLE 1. Deoxynivalenol (DON) levels in wheat-based breakfast cereals

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Incidence</th>
<th>ND &lt; 100a (%)</th>
<th>101 to 1,000 (%)</th>
<th>1,001 to 5,000 (%)</th>
<th>&gt;5,001 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bran</td>
<td>16/24</td>
<td>8 (33.3)</td>
<td>8 (33.3)</td>
<td>4 (16.7)</td>
<td>4 (16.7)</td>
</tr>
<tr>
<td>Wheat flakes</td>
<td>16/20</td>
<td>4 (20.0)</td>
<td>14 (70.0)</td>
<td>2 (10.0)</td>
<td>—</td>
</tr>
<tr>
<td>Wheat and fruits</td>
<td>32/44</td>
<td>12 (27.3)</td>
<td>26 (59.1)</td>
<td>6 (13.6)</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>64/88</td>
<td>24 (27.3)</td>
<td>48 (54.5)</td>
<td>12 (13.6)</td>
<td>4 (4.6)</td>
</tr>
</tbody>
</table>

a ND, not detected, <100 µg/kg.

( Merck, Lisbon, Portugal). The mobile phase was acetonitrile water, filtered through a 0.22-µm filter membrane, degassed, and used at a flow rate of 0.6 ml/min. DON was detected using a Merck-Hitachi L7420 ultraviolet detector set to 218 nm. Data were analyzed with a computing integrator (Compaq Deskpro, Merck-Hitachi).

DON was obtained from Sigma-Aldrich (Madrid, Spain), D-0156. Working standards solutions, limit of detection, and percentage recovery were determined according to the method previously referred to herein. The limit of detection was 100 mg/kg. Recovery was determined by spiking DON standards at levels of 100.0, 250.0, and 500.0 µg/kg to wheat samples. Recovery averages were 98.0, 85.0, and 88.0%, respectively.

RESULTS AND DISCUSSION

The analysis of the 88 samples of wheat and bran showed that 72.8% (64 samples) were contaminated with DON. The detected levels ranged between 103 and 6,040 µg/kg. Twenty-four samples (27.2%) did not reveal the presence of toxin (<100 µg/kg).

A summary of the results for bran, wheat flakes, and wheat and fruits is shown in Table 1. Of 24 samples of bran, eight (33.3%) were not contaminated with DON. Eight samples (33.3%) contained levels between 101 and 1,000 µg/kg, four samples (16.7%) were contaminated with levels of 1,001 to 5,000 µg/kg, and four others (16.7%) contained DON at >5,001 µg/kg. The incidence of DON in 20 samples of wheat flakes analyzed showed that four samples (20.0%) were negatives (<100 µg/kg). Fourteen samples (70.0%) were contaminated with levels ranging from 101 to 1,000 µg/kg, and two samples (10.0%) had levels between 1,001 and 5,000 µg/kg. Concerning the 44 wheat and fruit samples, 12 (27.3%) were not contaminated, 26 (59.1%) had levels of 101 up to 1,000 µg/kg, and 6 (13.6%) had contamination between 1,001 and 5,000 µg/kg (Table 1). Dalcero et al. (2) studied DON contamination in wheat from Cordoba, Argentina, during the 1993 to 1994 harvest season. In 40 samples analyzed, they found levels ranging between 300 and 4,500 µg/kg. These results agree with those obtained by our screening. The levels of contamination are important, considering that the United States and Canada have established limits for DON of 2,000 and 1,000 µg/kg, respectively, in wheat and its byproducts for human consumption (3). Reports from the United States (15) have shown that about 40.0% of the 483 wheat samples from the 1993 crop year contained DON levels that were greater than advisory levels of 2,000 µg/kg (3).

The results suggest a risk for consumers of wheat products and the need to monitor final products before consumption.

REFERENCES


