A Research Note
Determination of Aflatoxins in Bread and Bakery Products

M. T. CUTULI de SIMÓN and G. SUÁREZ FERNÁNDEZ*

Departamento de Microbiologia, Facultad de Veterinaria, Universidad Complutense, Madrid-3, Spain

(Received for publication August 15, 1983)

ABSTRACT

The possible presence of aflatoxins B₁, B₂, G₁ and G₂ was studied on 50 samples of bread and 50 samples of bakery products. The methods used in sample analysis were the following: (a) aflatoxin determination by thin layer chromatography (TLC) with chloroform/acetone solvent system (88/12); and (b) by high pressure liquid chromatography, with the toluene/ethyl acetate/methanol solvent system (68/29/3). With both methods, separations obtained of the different aflatoxins were optimal for accurate identification. Presence of aflatoxins B₁ and G₁ was detected in the extract from a sample of the bakery products group, using both methods mentioned.

One of the most serious consequences of the proliferation of mold on foods is the food poisoning caused by the toxic metabolites produced and spread through the food. We considered it of interest to study the presence of aflatoxins in widely-consumed foods such as bread and bakery products. Presence of aflatoxins in such foods is of obvious concern since aflatoxins are the most active ingested carcinogens known to date. We also offer an updating of the TLC and HPLC determination techniques as adapted to our project.

MATERIALS AND METHODS

Sampling

The study was carried out on: (a) 50 samples of bread; two types were chosen because they are the most common; (i) 25 samples of cottage bread, made of wheat flour, yeast and salt, and (ii) 25 samples of sliced bread in commercial packages composition of the bread was: wheat flour, yeast, salt, sugar, milk solids, fats, emulsifiers and preservatives; and (b) 50 samples of bakery products. Two lots were selected: (i) 25 samples of the product known as “croissant”, made of wheat flour, yeast, oil and sugar, and (ii) 25 samples of the product known as “tea buns”, the composition of which is: wheat flour, yeast, sugar, oil and fresh eggs.

Methods to determine aflatoxins

Thin layer chromatography technique (TLC). This analysis technique was used to test aflatoxins according to the method of Gimeno Ciriano (5), which is based on the analysis system proposed by Stoloff (11). The extraction of aflatoxins was done by adding acetonitrile/potassium chloride 4% (9/1) to the sample (50 g). The extract was cleansed with a separating funnel, washing it four times in 50 ml of iso-octane, followed by another wash in 12.5 ml of distilled water, and changing the pH by adding 1 ml of 1 N HCl. Each sample was analysed twice.

To separate the different aflatoxins, silica gel chromatoplates (SIL G-25HR, Macherey Nagel & Co®) were used, and developed with a chloroform/acetone solvent system. It was observed with U.V. light at 366 nm (Sylvania® F8TS/BLB, USA) with an ultraviolet lens (Camag®). The concentration of aflatoxin was determined by the detection limit method.

High pressure liquid chromatography technique (HPLC). The HPLC for aflatoxin determination was described by Pon (8) and modified by Fernández and Garrido (3). The chromatograph used was a Varian® 5000 and the working conditions used were as follows: (a) wavelength, 365 nm; (b) sensitivity, 0.0038 AUFS; (c) flux speed, 1.0 ml/min; (d) solvent system, toluene/ethyl acetate methanol (68/29/3). Quantification was done by the external standard method.

RESULTS

Thin layer chromatography method (TLC)

Analyses for determining the aflatoxins B₁, B₂, G₁ and G₂ were conducted for each of the 100 samples taken. The results obtained were as follows. Within the group of 50 bread samples, no aflatoxins was detected. Of the bakery products analysed, presence of aflatoxins B₁ and G₁ was determined in a sample from the “croissant” group. This result was confirmed by: (a) modification of the colors of the stains following the pulverization of the chromatoplate with a solution of sulfuric acid/water (1/3). (b) Transformation of aflatoxins B₁ and G₁ by means of trifluoroacetic acid into B₂a and G₂a.

By the limit detection method, 16.32 μg of aflatoxin B₁ and 12.4 μg of aflatoxin G₁ per kg were detected.

High pressure liquid chromatography (HPLC)

According to the HPLC technique used in our study, presence of aflatoxins B₁ and G₁ was determined in the same “croissant” sample that was previously positive by the TLC technique. The retention times obtained and the amounts of aflatoxins identified compared to the external standard method are shown in Table 1. The corresponding graph is shown in Fig. 1.
TABLE 1. Qualitative determination by retention times and quantitative by external standard method obtained with the positive ‘croissant’ sample.

<table>
<thead>
<tr>
<th>Aflatoxins</th>
<th>Standard (min)</th>
<th>Sample (min)</th>
<th>Standard Area (ng)</th>
<th>Sample Area (ng)</th>
<th>Concentration (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>5.81</td>
<td>5.82</td>
<td>430962</td>
<td>2916</td>
<td>67.67</td>
</tr>
<tr>
<td>B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>6.44</td>
<td>nd&lt;sup&gt;a&lt;/sup&gt;</td>
<td>665822</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>G&lt;sub&gt;1&lt;/sub&gt;</td>
<td>7.92</td>
<td>7.91</td>
<td>743795</td>
<td>3475</td>
<td>46.73</td>
</tr>
<tr>
<td>G&lt;sub&gt;2&lt;/sub&gt;</td>
<td>9.11</td>
<td>nd</td>
<td>443560</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

<sup>a</sup>ND = undetected.

DISCUSSION

We would point out the scarce literature found on determination of aflatoxins in the foods we studied. For commercially produced bread, there are studies by Boesenberg (1), in which he only used TLC determination techniques and in which no aflatoxins were detected, by Frank (4), who found only one positive sample, and by Spicher (70), who detected 14 positive samples out of 91 tested.

With regard to the TLC method, acetonitrile/potassium chloride 4% was used, bearing in mind the research by Gimeno (5), Pons (7), Stoloff (11) and Yin (12). Separations we obtained of the different aflatoxins with GHR silica gel chromatoplates and with chloroform/acetone development eluent (88/12) were optimal for accurate identification, in accordance with Egon (2), Gimeno (5), Haggblom (6) and Shotwell (9).

The HPLC was incorporated as an alternative to TLC, because the separations obtained are better and the quantitative determinations more precise. The HPLC suggested by Fernández and Garrido (3) which we used, gave good results in the separation of the aflatoxins and in obtaining retention times comparable to those of other authors, as well as a more sensitive quantification.

REFERENCES

8. Pons, W. A. 1979. High pressure liquid chromatographic determi-

Simón and Fernández, con’t. from p. 628