Research Note

Determination of Aflatoxin B1 in Food and Feedstuffs in Cuba (1990 through 1996) Using an Immunoenzymatic Reagent Kit (Aflacen)

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ABSTRACT

The presence of aflatoxin B1 was analyzed in imported food and feedstuffs of national production in the period of 1990 through 1996, destined to animal and human consumption using an immunoenzymatic reagent kit (Aflacen, Ckure, la Habana, Cuba) with a detection limit of 0.3 μg/kg. It was found that the 17.04% of a total of 4,594 analyzed samples presented aflatoxin B1, and the biggest percentages were in sorghum and peanut with an 83.3 and 40.4%, respectively. The corn, oat, wheat, and soy are fundamental raw ingredients in the elaboration of concentrates. Percentages of contamination with aflatoxin B1 of 23.3, 10.7, 25, and 4.6 were found in corn, oat, wheat, and soy, respectively. Other analyzed foods like rice, beans, and peas presented percentages of contamination with aflatoxin B1 inferior to 5% of the analyzed samples. It was found that more than 455 samples surpassed the value of 10 μg/kg. Corn and peanut products present a high demand in population showing levels of contamination superior to 50 μg/kg. The 11.3% of the samples contaminated with aflatoxin B1 have values between 1 and 20 μg/kg, where peanut and concentrates show the highest percentages (21.9 and 18.7), respectively. These results show levels of aflatoxin B1 in the population that constitute a great risk for human and animal health.

Aflatoxins are toxic substances produced by secondary metabolism in some species of microscopic fungi. They appear as natural contaminants in food when climatic conditions are favorable and where aflatoxin B1 (AFB1) is considered as one of the most potent environmental carcinogens (14, 16).

The Food and Agricultural Organization (FAO) estimates that the 20 or 25% of the total production of cereals is contaminated with a kind of mycotoxin, where aflatoxins, fumonisins, and deoxynivalenol are those that appear more frequently in food (13, 19).

Cuba annually imports around 391,000 millions of tons of cereals that are destined to human and animal consumption (11). The presence or not of AFB1 in food and feedstuffs is controlled through the program for prevention and control of this substance in food. In Cuba, there is a maximum permitted level (MPL) for this substance of 5 and 50 μg/kg, respectively, for human and animal consumption (10).

The objective of this work was to determine the presence of AFB1 in food and feedstuffs using an immunoenzymatic reagent kit developed in Cuba.

MATERIALS AND METHODS

Reagents. Methanol, analytical grade, was purchased from Fluka (Sigma Aldrich Co., Buchs, Switzerland) the remaining reagents are part of an enzyme-linked immunosorbent assay (ELISA) kit (Aflacen).

Samples. A total of 4,594 samples were analyzed during the 1990s. The analyzed samples were peanut, corn, wheat, rice, cocoa, soy, oat, sorghum, sunflower flour, beans, peas, and concentrates for animals.

Procedure. Determinations were made using the instructions from the reagent kit (Aflacen), which is based on an indirect competitive assay (9). The assay is briefly described below.

Extraction. Twenty grams of the pulverized sample was passed through a no. 20 sieve, weighed, and 40 ml of a methanol: water mixture (65:35 vol/vol) was added and agitated for 30 min in a mechanical shaker at high speed. For those substrates that absorb significant water it was necessary to add 60 ml of the mixture instead of 40 ml. The extract was gravity-filtered through filter paper (Whatman 1, Whatman International, Kent, UK), collected in a 20-ml amber-colored flask, and stored at 4°C until analysis (for no more than 16 h). An aliquot of filtrate was diluted 10 times with phosphate-buffered saline (R6).

Assay. In a 96-well plate (R1), 100 μl of AFB1 for each point of the curve (R2) and the sample were added in triplicate and duplicate, respectively, and subsequently 100 μl of the specific antibodies were added (R3) to each well. The plate was incubated at 37°C in a humid chamber for 60 min. It was washed five times with a solution of phosphate-buffered saline-Tween, and 200 μl of conjugated anti-IgG peroxidase (R4) was added to each well, and the plate was further incubated in a humid chamber at 37°C for 30 min. It was washed again, and 200 μl of substrate
solution (R5) was added and the plate was incubated for 20 min in a dark chamber at room temperature. After 20 min, the reaction was stopped with 50 μl of 1 M H₂SO₄ (R8). Absorbance at 492 nm was measured in an ELISA reader (MCC plus Labsystem, Manchester, UK).

RESULTS AND DISCUSSION

Table 1 shows the percentage of contaminated samples and the range of concentration of AFB1 in each food, analyzed by the reagent kit (Aflacen). The detection limit of the reagent kit was 0.3 ± 0.05 ng/ml (30 pg per well), detecting 5 ng/ml to 50% inhibition. The limit of quantification was 10 μg/kg, being linear between 0.2 and 500 ng/ml with a recovery higher than 95%, and an intra- and interassay precision between 8 to 14% and 10 to 35%, respectively (8).

The previously mentioned characteristic of the reagent kit (Aflacen) fulfills the quality requirements that an immunoenzymatic kit should have for mycotoxin determination as recognized by the Association of Official Analytical Chemists (6). It also allows for superior detection of concentrations of AFB1 of 5 μg/kg in food, where the MPL in Cuba for any food of this type is 5 μg/kg.

As observed in Table 1, peanut is one substrate that is most commonly contaminated with aflatoxin (40.4%), because it is one of the most susceptible foods to contamination by toxigenic fungi producing aflatoxins. In a report presented by the Food and Drug Administration, the aflatoxin levels in peanut butter and roasted peanuts with shells were increased in 1989 (24). In an international symposium held in San José, Costa Rica in 1991 and supported by the FAO and Organización Panamericana de Salud (OPS), 49.9% of the peanut samples analyzed were observed to be contaminated with AFB1 (21). Another report also showed that peanut butter samples are susceptible to contamination with aflatoxin (71%) (23). These results confirm that this food type is a very susceptible substrate to contamination by fungi producing aflatoxins. For this reason, the application of good practices in crop transfer and storage to avoid contamination are very important (12).

Other products like sorghum and sunflower flour presented high percentages of contamination with aflatoxins, although the number of samples analyzed was small. The values of contamination of all the analyzed sorghum samples surpassed the MPL for animal consumption. In contrast to other reports, the values for aflatoxin concentrations on these substrates are less than 3 μg/kg (15, 23). Céspedes and Díaz (5) found that 24% of the sorghum samples analyzed had AFB1 values greater than 1 μg/kg.

The samples of sunflower flour had a high percentage of contamination in a single sample that surpassed the MPL for animal consumption. It appears that sunflower flour is a substrate not very susceptible to contamination by fungi producing aflatoxin, which is not true for Altenaria alternata (Fr) Keissler that is isolated with high frequency in sunflower fruits in the field and in storage (7).

Corn is another substrate in which the presence of mycotoxins is controlled worldwide, and the percentages of aflatoxin contamination can oscillate up to 80%, depending
on the climatic conditions of each country and the conditions of manipulation. A study made by Mora and Lacey (17) in Costa Rica reported that more than 80% of the samples analyzed presented AFB1 in concentrations greater than 20 μg/kg, with differences among the areas studied and among the different stages of the production process. Other studies carried out in Latin America report a percentage of contamination with aflatoxin that oscillates between 12 and 25% (5, 19, 20, 22).

The rest of the products analyzed presented aflatoxins in percentages less than 7%. These results are in correspondence with other studies where the percentages of contamination with aflatoxin in wheat, rice, and soy do not surpass 10% of the samples analyzed (22).

In the peculiar case of concentrates, 20.2% were found to be contaminated with AFB1. This is logical if the starting material is considered contaminated with this toxic substance and if more than 90% of the concentrates earmarked for animal consumption are produced in the country of origin.

As shown in Table 1, 455 samples surpassed values of 10 μg/kg, for a total of 9.99% of the samples analyzed. These results are better than the MPL for AFB1 in Cuban foods which is 5 μg/kg.

As previously mentioned, peanut is one of the most susceptible products to contamination, and at the same time, it is also a product like rice that is in high demand in the population. The presence of AFB1 in these foods in concentrations over the MPL means a great risk to the health of consumers.

Studies carried out by Alvarez and collaborators in two municipalities in the capital have found an AFB1 prevalence in adults of 7.32%, with a tendency to accumulate AFB1 during the last 48 h of exposure (4). Later studies in patients with hepatic diseases showed that more than 42% of the sample analyzed had acquired AFB1 (1, 3). Other studies have reported the presence of aflatoxin in serum of adults with levels of 25 pg of lysine-aflatoxin equivalent to 1 mg albumin (2).

Although these studies have not been correlated with the diets that patients have consumed, the levels of aflatoxin found in serum and urine indicate significant levels of this substance in the population that could be a health risk if the high carcinogenic power of AFB1 is considered.

The application of the reagent kit (Aflacen) allowed extension of the System of Alimentary and Nutritional Surveillance with regard to AFB1 to other provinces that have ELISA readers, thereby diminishing the costs of analysis of this substance with regard to the analyses carried out by thin-layer chromatography.

Another important aspect of the application of the reagent kit (Aflacen) is that it allows results to be obtained in a short time. Thus, contaminated products may be prevented from reaching the population and the measures monitored by the Health Ministry and the Department of Agriculture can be applied. In this way, protection for consumers is guaranteed.

REFERENCES