

Determination of Aflatoxins in Wheat and Wheat by-products Intended for Human Consumption, Marketed in Rio de Janeiro, Brazil

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Abstract The consumption of wheat bran, whole-wheat grains and other whole-wheat products has grown in recent years in Brazil. These foods are considered more nutritious than the refined ones and have been associated with a reduced risk of some major chronic diseases. On the other hand, other research, carried out in Brazil, has found different groups of fungi toxins, called mycotoxins, contaminating these wheat products. Among these mycotoxins, are the aflatoxins, a group of genotoxic and carcinogenic compounds produced by *Aspergillus* spp. This study aimed to determine the levels of aflatoxins B₁, B₂, G₁ and G₂ in samples of whole-wheat grains and derivatives, intended for human consumption, marketed in the metropolitan region of Rio de Janeiro, Brazil. One hundred and eight samples of whole-wheat grains ($n=35$), wheat bran ($n=32$), whole-wheat flour ($n=26$) and refined wheat flour ($n=15$) marketed in hypermarkets, supermarkets and health food stores were analyzed by High Performance Liquid Chromatography with fluorescence detection (HPLC-FL). Thirty-three samples (30.6%) were positive for at least one aflatoxin and the B₁ form had the highest prevalence in the samples. The overall average was 0.69 µg/kg and the contamination was the highest in the grain samples, followed by bran, whole-flour and refined flour. Just one sample showed total aflatoxins levels (B₁+B₂+G₁+G₂) higher than the limit established by Brazilian legislation (5 µg/kg). The levels found in this study indicated that the presence of aflatoxins in wheat and wheat products consumed in Rio de Janeiro, Brazil, are not a hazard for public health.

Keywords: mycotoxins, AFB₁, wheat bran, whole-wheat products, HPLC

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1. Introduction

Aflatoxins are a group of structurally related toxic compounds produced by the fungi species *Aspergillus* spp, principally *A. flavus* and *A. parasiticus*. These species grow on certain foods or feeds under favorable conditions of temperature and humidity [1]. The major aflatoxins of concern are designated B₁, B₂, G₁, and G₂, and are classified by the International Agency of Research on Cancer (IARC) as carcinogens agents to humans (in Group 1) [2]. They are usually found together in various foods and the B₁ form has the highest carcinogenic potential among the known mycotoxins [3,4]. Since there is no Acceptable Daily Intake (ADI) for aflatoxins, as they are genotoxic and carcinogenic substances, exposure through food should be kept as low as possible [5]. To protect the consumers from this hazard, different countries

have set different limits for the presence of aflatoxins in wheat and wheat products, ranging from 4 µg/kg in the European Union to 30 µg/kg in India [6].

Cereal grains contaminated with aflatoxins represent a public health problem due to the high toxicity of these substances and also because they remain partially stable during the industrial processes when manufacturing derived products [7,8]. The milling of the wheat can minimize mycotoxin concentrations in the fraction used for human consumption as these toxins are redistributed mainly in the bran, which is predominately used for animal feed [9,10]. However, the human consumption of wheat bran as a direct source of dietary fiber has grown in recent years, mainly because it is a cheap product and has a high dietary fiber content [11]. For this reason, the wheat bran intended for human consumption must have low mycotoxin levels in order not to compromise the safety of the final product [12].

The consumption of wheat bran, whole-wheat grains and other whole-wheat products has grown in recent years in Brazil. These foods are considered more nutritious than the refined ones and have been associated with a reduced risk of some major chronic diseases, including cardiovascular diseases and some cancers [12,13]. On the other hand, other research, carried out in the country, has shown that these foods may be contaminated with different groups of mycotoxins [14,15,16,17].

In Brazil, there are few studies concerning the occurrence of aflatoxins in wheat and wheat products. In other countries, such as, Tunisia, Lebanon and Turkey, high levels of aflatoxins have been reported in wheat and derivatives, which represents a health risk to consumers [18,19,20].

This study aimed to determine the levels of aflatoxins B₁, B₂, G₁ and G₂ in samples of whole-wheat grains and derivatives, intended for human consumption, marketed in Rio de Janeiro, Brazil.

2. Material and Methods

2.1. Sampling

During January 2013 to February 2014, samples of whole wheat grain ($n=35$), wheat bran ($n=32$), whole wheat flour ($n=26$) and refined wheat flour ($n=15$) were acquired from different hypermarkets, supermarkets and health food stores in the metropolitan region of Rio de Janeiro, Brazil, totaling 108 samples. Packets of 0.5 to 1 kg were collected and then transported to the laboratory for analysis.

2.2. Chemicals and Reagents

Aflatoxins B₁, B₂, G₁ and G₂ standards were purchased from Sigma (St. Louis, MO, USA). HPLC-grade acetonitrile and methanol were purchased from Tedia (São Paulo, SP, Brazil). The other solvents used for extraction were of analytical grade (Vetec, Rio de Janeiro, RJ, Brazil). Deionized water used was obtained from a Milli-Q® purification system (Millipore, MA, USA). Filter papers (No. 1) were purchased from Whatman (Maidstone, UK) and 0.45 µm PVDF membranes (Durapore® 13 mm, Millipore) were used for filtration.

2.3. Preparation of Standard Solutions

The standards of aflatoxins B₁ (5 mg), B₂, G₁ and G₂ (1 mg) were dissolved in methanol. Individual stock solutions (50 µg/mL) and working solutions (2 µg/mL) were prepared by appropriate dilution in methanol and their concentrations were confirmed by UV spectroscopy (Shimadzu UV-1201, Kyoto, Japan), according to the Association of Official Analytical Chemists (AOAC) [22].

2.4. Aflatoxins Extraction and Derivatization

Aflatoxin determinations were carried out in triplicate for each one of the 108 samples. The methodology used for extraction and purification was performed according to the Institute Adolfo Lutz [23], with minor adaptations, as follows. Water (5 mL) at 60 °C was added to 15 g of the grounded sample in an Erlenmeyer flask. Then 50 ml of chloroform was added to the flask and agitated in a Shaker

(Orbit Shaker 3520) for 45 min. The chloroform extract was filtered through filter paper and 25 mL was collected, and then evaporated to dryness in a water bath at 65 °C under a N₂ flow. The dried extract was resuspended with 25 mL of methanol in an ultrasound bath (Thornton T7) for 10 sec and transferred to a separatory funnel containing 25 mL of NaCl aqueous solution (4% w/v). Then 25 mL of hexane was added and the mixture was stirred vigorously for 30 sec, after which the hexane phase (top) was discarded. This step was repeated with another 25 mL of hexane. Subsequently, 25 mL of chloroform was added to the separatory funnel, with vigorous shaking for 30 sec. This step was repeated with another 25 mL of chloroform and then evaporated until dryness in a water bath at 65 °C under a N₂ flow. For the aflatoxin extractions from wheat bran twice the volume of chloroform was used, and for the analysis of the flours water was not added.

Based on the derivatization procedure described by AOAC [24], the extract was resuspended in 0.6 mL of acetonitrile, submitted to an ultrasound bath for 30 sec and 1.2 mL of the derivatizing solution (water: trifluoroacetic acid: glacial acetic acid at 7: 2: 1, v/v/v) was added. The solution was maintained in a water bath at 65 °C for 9 min. Finally, the extract was filtered through a 0.45 µm membrane and injected into the HPLC-FL system.

2.5. HPLC-FL Analysis

The quantification of the aflatoxins was carried out in a High Performance Liquid Chromatography system, using a fluorescence detector (Agilent 1100 Series, Waldbronn, Germany) (excitation at 365 nm and emission at 450 nm), a Rheodyne injector (20 µL), a C₁₈ column (Ace, 250 mm x 4.6 mm, 5 µm) and an isocratic mobile phase, consisting of water: methanol: acetonitrile (7: 2: 1, v/v/v) at a flow rate of 1.0 mL min⁻¹. The results were expressed by the mean of the triplicates, in µg/kg.

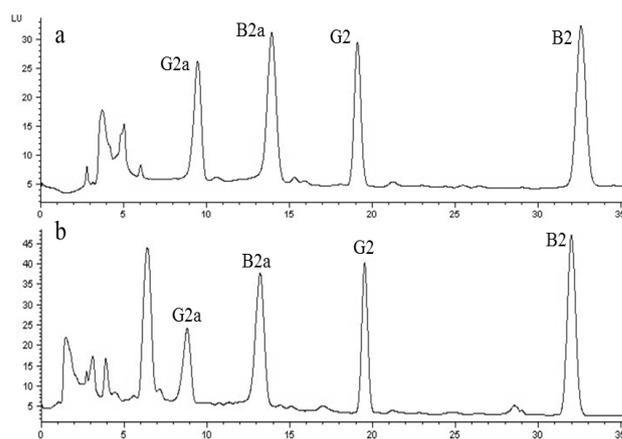


Figure 1. Chromatograms obtained by HPLC-FL in wheat grains (a) and wheat bran samples (b) spiked with approximately 5 µg kg⁻¹ of aflatoxins B₁, B₂, G₁ and G₂. Aflatoxin G_{2a} and B_{2a} are derivatized forms of the G₁ and B₁

The methodology used in this study was previously in-house validated and proved to be suitable for aflatoxin determination in the matrices evaluated (grains, bran and flours), with good levels of recovery (70.2-108.5%) and repeatability (lower than 15%), with limits of detection and quantification corresponding to 0.6 and 1.2 µg/kg, respectively [25]. The retention times were approximately

9, 13, 20 and 32 min for aflatoxin G_{2a} (derivatized form of G₁), B_{2a} (derivatized form of B₁), G₂ and B₂, respectively, as presented in Figure 1.

2.6. Statistical Analysis

Statistical analyses were performed using Sisvar® 5.3 Build 77 (UFLA, Brazil). A probability value of 0.05 was used to determine the statistical significance in ANOVA and in the Tukey test.

3. Results and Discussion

One hundred and eight wheat and wheat by-products, marketed in Rio de Janeiro, Brazil, were analyzed for the presence of aflatoxins B₁, B₂, G₁ and G₂. Thirty-three samples (30.6%) were positive for at least one aflatoxin and the B₁ form had the highest prevalence in the samples. It was detected in 27.8%, with the highest level found in a grain sample, corresponded to 4.2 µg/kg. The G₁ and G₂ were detected in only 10.2% and 0.9% of the samples, respectively. The B₂ form was not detected in any sample (Figure 1).

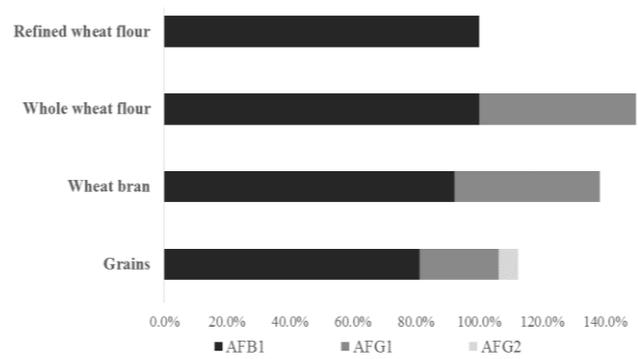


Figure 2. Occurrence of aflatoxins B₁, G₁ and G₂ in positive samples. The B₂ form was not detected in any sample

Just one sample (wheat grain with 6.2 µg/kg) showed total aflatoxins (B₁+B₂+G₁+G₂) higher than the limit established by Brazilian legislation for cereals in general (5 µg/kg). The overall average was 0.69 µg/kg and the contamination was the highest in the grain samples, followed by bran, whole-flour and refined flour, as presented in Table 1. The levels of total aflatoxins in positive samples did not differ ($p > 0.05$) between the analyzed foods.

Table 1. Aflatoxin levels in samples of wheat grains, wheat bran and wheat flours, marketed in Rio de Janeiro, Brazil

Food	n	Positive samples	Mean in positive samples ¹ (µg/kg)	Highest value found (µg/kg)	MPL (µg/kg)
Wheat grains	35	45.7%	2.2 ^a	6.2	5
Wheat bran	32	40.6%	2.4 ^a	4.8	5
Whole wheat flour	26	7.7%	2.3 ^a	3.4	5
Refined wheat flour	15	6.7%	1.2 ^a	1.2	5
Total	108	30.6%	2.2 ^a	6.2	5

¹Means followed by the same letter in the column are not different ($P > 0.05$) by Tukey test. n - Number of samples analyzed. MPL - Maximum Permitted Level adopted by Brazilian regulations [12].

Considering the European Union limit for the presence of aflatoxins in wheat (4 µg/kg), one sample of wheat bran containing 4.79 µg/kg would also be considered unfit for human consumption [26]. The current legislation about mycotoxins prevailing in Brazil came into force in 2011 [12]. After this date, the present study is the first report giving data on levels of aflatoxins in wheat and wheat products intended for human consumption marketed in Brazil. However, more studies evaluating the occurrence of aflatoxins and other groups of mycotoxins in wheat and derivatives should be carried out in different regions of the Brazil, as these foods are an important source of nutrients for the Brazilian population diet.

4. Conclusion

From a total of 180 samples analyzed, 33 (30.5%) were positive for at least one aflatoxin and just one sample (wheat grain) showed levels higher than the limit established by Brazilian legislation (5 µg/kg). The contamination was the highest in the grain samples, followed by bran, whole-flour and refined flour. The levels found in this study indicate that the presence of aflatoxins in wheat and wheat products consumed in Rio de Janeiro, Brazil, is not hazard for public health.

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